THE UTILITY OF THE AUDITORY BRAINSTEM RESPONSE IN CHILDREN WITH ATYPICAL SACCADIC EYE MOVEMENTS

By

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Abstract

Lesions in the brainstem result in widespread damage to a number of sensorimotor systems including oculomotor and auditory neural circuits. Although these systems are spatially separate and highly specialised, they are also co-located. This thesis, investigates whether lesions in the oculomotor system will also cause co-morbid dysfunction in the auditory pathways. Specifically, we investigated the usefulness of the Auditory Brainstem Response (ABR) in two oculomotor conditions: slow saccades in Gaucher disease (GD) and opsoclonus in Dancing Eye Syndrome (DES).

We present four empirical studies. In our first study we systematically investigated the ABR in GD. We found that multimodal testing can better delineate underlying neurological deficits in neuronopathic GD (nGD) and distinguish between phenotypes. In the second study we examined the ABR's utility as a longitudinal, objective marker of disease burden and in a randomised clinical control trial. ABRs continued to deteriorate regardless of treatment. In our third study we assessed audiological function in DES. We found that at least 43% of DES patients have hyperacusis. We also found subtle abnormalities in the auditory brainstem, as shown by the ABR. Our final study explored the onset-offset response in the ABR and assessed its utility as a clinical marker. Overall, this thesis provides new evidence that auditory pathways are also affected in diseases which are traditionally assumed to be ‘oculomotor’ in nature. We believe that there is sufficient evidence to warrant the inclusion of audiological testing, such as the ABR, as part of the standard assessment of newly diagnosed GD patients and that they undergo these tests prior to commencing treatment. These tests may also have a wider application as longitudinal

outcome measures for use in clinical trials or as markers of neurological burden in GD and we believe may be useful in other metabolic diseases; we found that current therapies for GD have low efficacy. Understanding the underlying neurological deficits in these debilitating illnesses can only help to improve treatments and the long-term outlook for these patients.

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ACKNOWLEDGEMENTS

This is the one page that I have dreamt of writing – and if I am entirely honest, this page is up there with my 'if I won the lottery' day-dream. You know the one - when ….well… you spend millions in your head taking care of everyone that is important. Anyway here we go.

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DEDICATION

I would like to dedicate this thesis to my beautiful family – my three R's (Rich, Ruby and Riley). I would particularly like to dedicate this work to my daughter who has taught me what it means to enjoy life's beauty. It is a privilege to be your mum.

To smile is to acknowledge life……

AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.
Relevant scientific seminars and conferences were regularly attended at which work was often presented; external institutions were visited for consultation purposes and several papers prepared for publication.

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Campbell PE, Harris CM, Vellodi A (2004) Deterioration of the auditory brainstem response in children with type 3 Gaucher disease *Neurology*; 63: 385-387

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Abbreviations

ABR	Auditory brainstem response
APD	Auditory processing disorder
ART	Acoustic reflex threshold
AVCN	Anteroventral cochlear nucleus
BMT	Bone marrow transplant
CIC	Central area of the inferior colliculus
CN	Cochlear nucleus
CNS	Central nervous system
CPA	Cerebellopontine angle
DCN	Dorsal cochlear nucleus
DES	DES
DR	Defense reflex
DLPN	Dorsolateral pontine nucleus
EBN	Excitatory burst neurons
EEG	Electroencephalogram
ERT	Enzyme replacement therapy
FEF	Frontal eye fields
GD	GD
GD1	GD type 1 phenotype
GD2	GD type 2 phenotype
GD3	GD type 3 phenotype

GSL	Glycosphingolipidoses
hSIF	Horizontal saccade initiation failure
IBN	Inhibitory burst neurons
IC	Inferior colliculus
IHC	Inner hair cell
INC	Interstitial nucleus of cajal
IQ	Intelligence quotient
LL	Lateral lemniscus
LSD	Lysosomal storage disorders
LSO	Lateral nucleus of the superior olive
MEMR	Middle ear muscle reflex
MGB	Medial geniculate body
MLF	Medial longitudinal fasciculus
MOCS	Medial olivocochlear suppression
MRF	Mesencephalic reticular formation
MRI	Magnetic resonance imaging
MSO	Medial nucleus of the superior olive
MTNB	Medial nucleus of the trapezoid body
NGD	Neuronopathic GD
NLL	Nucleus of the lateral lemniscus
NPH	Nucleus prepositus hypoglossi
OAEs	Otoacoustic emissions
OCB	Olivocochlear bundle

OHC	Outer hair cell
OKN	Optokinetic nystagmus
OMA	Ocular motor apraxia
OPN	Omnipause neurons
OR	Orienting response
PAC	Primary auditory cortex
PD	Parkinson's disease
PME	Progressive myoclonic epilepsy
PPC	Posterior parietal cortex
PPRF	Paramedian pontine reticular formation
PTA	Pure tone audiometry
PV	Peak velocity
PVCN	Posteroventral cochlear nucleus
riMLF	Rostral interstitial nucleus of the medial longitudinal fasciculus
SB	Synkinetic blinking
SC	Superior colliculus
SEP	Somatosensory evoked potentials
SGC	Spiral ganglion cells
SIF	Saccade initiation failure
SOC	Superior olivary complex
SRT	Substrate reduction therapy
TN	Tonic neurons
VCN	Ventral cochlear nucleus

VOR Vestibulo-ocular reflex

VRA Visual reinforcement audiometry

vSIF Vertical saccade initiation failure

Chapter 1
General Introduction

1.1 The normal brainstem

"Up until then I had never even heard of the brainstem. I've since learned that it is an essential component of our internal computer, the inseparable link between the brain and the spinal cord. I was brutally introduced to this vital piece of anatomy……. In my case, blinking my left eyelid is my only means of communication" (Bauby)

The brainstem is a highly organised, intricate network. Neuroscientists often refer to the brainstem as the *neural gateway* between the brain and the body. With the exception of olfaction, all sensory information that is sent to the brain, from the body is obliged to travel through this remarkable system. As such, the brainstem plays a central role in a number of life-supporting functions including sensorimotor control, respiration, alertness and consciousness, to name a few.

Despite the high clinical significance of the brainstem, this phylogenetically older part of the nervous system is often dismissed as comparatively primitive – incapable of demonstrating the same degree of neural plasticity that is evident in higher cortical structures. However, recent studies in the auditory brainstem complex are now challenging this prevailing idea (Tzounopoulos and Kraus, 2009).

These studies have clearly shown that the auditory brainstem is not a 'passive' structure (Kraus and Chandrasekaran, 2010). This multifaceted system does not simply extract core acoustical features, through a series of successive 'bottom-up' processes as sound travels from the outer ear to the auditory cortex. It has a *dynamic* role, actively interacting with other higher cortical processes, influencing important non-sensory factors such as linguistic experience (Krishnan et al., 2005), musical expertise (Musacchia et al., 2007, Wong et al., 2007, Musacchia et al., 2008) and attention (Galbraith et al., 1998,

Galbraith et al., 2003). Moreover the output of this intricate sub-cortical network has been shown to change with auditory training, reflecting a real-time transformation (Song et al., 2008b). These converging lines of evidence clearly show that the brainstem demonstrates neural plasticity and is not 'primitive' at all; these facts have re-ignited scientific interest in studying this area of the brain.

But *how* do we study the brainstem? One well established approach is to compare healthy brain activity with brains that have, either deliberately or through injury or neurological disease, been made dysfunctional and where normal interrelating pathways are partial or absent (Bear et al., 2001). This lesion-based approach provides us with a powerful method of revealing the brain's internal structures and identifying the essential pathways responsible for their various functional activities.

Lesion studies are typically the result of two possibilities: experimentally induced, e.g. through chemical or surgical ablation, or they can occur as the result of naturally occurring disease processes (Bear et al., 2001). These studies are based on the assumption that a measurable change in behaviour – as indexed by clinical sign or symptom – can help identify *where* the brain is damaged and also allow us to learn about normal function through dysfunction, i.e. brainstem deficits provide a unique window to observe the relationship between brain and behaviour (Griffiths et al., 1997, Griffiths, 1999).

One major drawback to the use of lesion studies is that they fail to show how co-located anatomical areas, *such as the oculomotor and auditory brainstem regions*, are functionally integrated. This is because the effects of human lesions, as a result of disease, are difficult to estimate. For example, isolated lesions involving only the auditory

brainstem are rare. Because the auditory pathway is closely related to key motor and sensory tracts, co-morbid dysfunction is often observed in other sensory modalities and as a result of these shared neural substrates, a typical brainstem lesion affecting the auditory pathway will also involve multiple areas and levels of the brain (Griffiths, 1999).

The challenge is *how* to capture any deficits that may arise as the result of a brainstem lesion in the auditory brainstem. Of particular interest to this thesis, is how do we undertake this in the developing brain of a young child?

1.2 Abnormalities and brainstem dysfunction: signs and symptoms

Brainstem dysfunction often results in a wide range of signs and symptoms including visual disturbances, hearing difficulties, altered sensation, muscle weakness, vertigo, swallowing and speech difficulty, voice change, and co-ordination problems.

Although there are innumerable signs of brainstem abnormalities, in this thesis we are concerned only with eye movement and auditory signs as the neural substrates that control them are co-located in the brainstem. The literature is replete with case reports and small scale studies of patients with established brainstem lesions that exhibit *both* eye movement and auditory abnormalities. For example, abnormal auditory brainstem and atypical visuomotor signs have been reported in demyelinating diseases such as Pelizaeus-Merzbacher disease (Nezu, 1995), Fisher's syndrome (Minoda et al., 1999), the leukodystrophies (Inagaki et al., 2006), degenerative diseases such as the spinocerebellar ataxias (Rub et al., 2008, Schols et al., 2008), metabolic diseases such as Gaucher disease (Bamiou et al., 2001), Tay-Sachs disease (Bembi et al., 2006), Niemann-Pick disease

(Patterson et al., 2007), neurodevelopmental disorders such as Cornelia de Lange syndrome (Harris et al., 1996) and autism (Rosenhall et al., 1988); this list is not exhaustive. Although many of these clinical conditions are known to affect both eye movement circuits in the brainstem and subcortical auditory structures, few systematic studies have actually examined this issue.

1.2.1 Eye movement signs

The primary aim of all eye movement systems is to maintain an optimal retinal image on the fovea – a small area located centrally on the retina – which has the greatest density of photoreceptors. Six eye movement sub-systems have been identified: fixation, the vestibulo-ocular reflex (VOR), the optokinetic reflex (OKR), saccades, smooth pursuit and vergence eye movements (Leigh and Kennard, 2004). The saccadic, smooth pursuit and vergence eye movement systems are important in 'acquiring' the target of interest. The VOR and OKR sub-systems are activated during head movement. These reflexes play a substantial role in stabilising a moving target, by ensuring that the eyes are directed on the target during head movement. We will provide some background about each of these subsystems in the following chapter.

At this juncture we elaborate on our core concern – saccadic eye movements in the context of brainstem disease. Saccades are highly stereotyped eye movements that enable the visual system to shift gaze from one object, in the line of sight, to another point of interest (Leigh and Zee, 1999a). Saccadic movements have evolved a very elegant strategy of foveal compromise – balancing the need for a large visual field and the requirement for

high visual acuity. This has been described as *"cutting up the visual continuum into a series of stills*" thus allowing the world to appear stable (Ings, 2007).

The study of saccades has become an increasingly popular method to probe brainstem function (Sparks, 2002, Leigh and Kennard, 2004), for a variety of reasons. Firstly, there is a wealth of data from lesion and unit recording studies in nonhuman primates, using oculomotor paradigms, which have identified the neural circuitry responsible for the generation of saccadic behaviours (Strassman et al., 1987, Ohtsuka and Noda, 1995, Colby et al., 1996, Kaneko, 1996, Munoz and Istvan, 1998, Takagi et al., 1998, Buttner-Ennever et al., 1999, Everling and Munoz, 2000, Hanes et al., 2005). Secondly, saccades depend on very circumscribed populations of neurons and are highly sensitive to brainstem disease (Zee, 1986, Noda, 1991, Munoz et al., 2000, Sparks, 2002, Leigh and Kennard, 2004, McDowell et al., 2008, Linzenbold et al., 2011). Finally, saccades can be measured accurately, across a wide age spectrum, using eye-movement tasks that typically require very little instruction (Cassidy et al., 2000a). This means that such tasks can be used with young children and in difficult-to-test clinical populations – providing precise information about the temporal dynamics of information processing.

Saccadic abnormalities can be identified by measuring three robust characteristics. These include changes in *velocity* (e.g. saccades may deteriorate and become either too slow or too fast, or demonstrate different velocities between each eye in terms of magnitude or direction), *latency* (e.g. prolongation, or shortening or asymmetry between eyes) and *accuracy* (e.g. saccades can overshoot or undershoot the desired target). In brainstem disease, many of these well defined parameters are abnormal.

In this thesis, we will examine two types of saccadic disturbance: slow saccades and, at the other end of the velocity spectrum, opsoclonus (see Section 2.2 for a more detailed overview). We have chosen these abnormalities as they represent the two extremes of saccadic eye movements.

1.2.2 Auditory signs

The brainstem also has a very important function in the auditory system. It is at the level of the brainstem that monaural pathways converge, first in the region of the pons and then again in the midbrain, resulting in binaural processing. This type of processing involves a fine-grained comparison of intensity and phase cues that originate independently at each ear. Lesions in the auditory brainstem have been shown to cause disruption in this processing; resulting in weakened representations of temporal, spectral and spatial auditory patterns (van der Poel et al., 1988, Furst and Algom, 1995, Furst et al., 1995, Griffiths et al., 1997, Griffiths et al., 1998). An example of disruption in binaural processing has been clearly documented in a patient with a well-circumscribed, central pontine lesion involving the trapezoid body (Griffiths et al., 1997). The patient reported difficulties with listening to speech during background noise, but also goes on to describe:

> *"….a difficulty with sound localisation such that she had become unable to detect which of three well separated telephones in her office was ringing. …and also had difficulty with the perception of moving sounds. For example, she was unable to detect which way a train was travelling when she was standing on a platform, on the basis of sound alone"* (Griffiths et al., 1997, p522).

As mentioned earlier, lesion-based approaches have been used extensively to study the auditory brainstem (van der Poel et al., 1988, Furst and Algom, 1995, Furst et al., 1995,

Griffiths et al., 1997, Griffiths et al., 1998). A number of different techniques (e.g. psychophysics; brain imaging, electrophysiological measures) in human adult studies have been used to make inferences about the role of the auditory brainstem and the effect of any disturbances on auditory performance. However these studies are usually undertaken in adults and provide limited information about the function of the developing brain. In this thesis, rather than concentrating on the study of disorders solely in adults, which is most commonly the case, we study disorders in children. By measuring the developing brainstem early we are able to construct a better picture of how different phenotypes of brainstem disorder may unfold.

1.2.3 Multimodal approach to studying the brainstem

We have mentioned above that eye movements and audiological functions are both compromised early on in a number of brainstem disorders, although systematic studies in a host of neurologic conditions is lacking. Often eye movement and auditory signs are still considered in isolation. Why is this the case and why are there only a relatively small group of investigators working at this juncture of disciplines? A partial explanation may be found in the differing research methods and 'scientific jargon' used across the two fields. Another key issue is the length and depth of the necessary training required in each field (i.e. eye movements or audiology) to develop the required expertise for undertaking measurements.

Others have strongly argued that administering multimodal tests is beyond the scope of clinical practice in Audiology. For example,

*"Do we, as audiologists, have sufficient training in these related areas to do this? Should we be trained in these areas, considering our unique role as specialists in auditory and vestibular system function and the access to other professionals whose scopes of practice and areas of expertise encompass these areas?"(*Musiek et al., 2005, p131).

We argue that such an assertion is inaccurate as saccadic eye movements are regularly assessed in Audiology clinics as a part of routine vestibular investigations (Hall, 2007).

The argument in favour of studying the brain using a multimodal approach remains a highly contentious issue in auditory neuroscience (McFarland and Cacace, 1997, Cacace and McFarland, 1998, 2005, Katz and Tillery, 2005, Musiek et al., 2005, Rosen, 2005).

Cacace and McFarland (2005) have called for modality specific testing as a *'means of differentiating auditory processing problems from more generalized supramodal dysfunction'* (Cacace and McFarland, 2005, p 117). Other researchers have raised objections to this viewpoint, arguing that this approach is 'unrealistically narrow' and 'exclusive' (Salvi et al., 2002, Poremba et al., 2003, Musiek et al., 2005, Rosen, 2005).

In this thesis, we will present new data to show that the application of auditory testing in conjunction with eye movement studies, has an even wider application, for example, in baseline diagnostic assessment, longitudinal monitoring and in clinical drug trials.

1.3 Why study auditory signs and symptoms in saccadic eye movement disorders?

In section 1.2.1 we presented reasons for the use of eye movements in identifying brainstem dysfunction. In this section we will present the reasons why are we advocating

the additional use of auditory tools in this thesis and account for why eye movement tests are not sufficient.

Eye movement studies have a number of serious drawbacks, which auditory tests may overcome. Firstly, we mentioned that the instructions for most saccadic tasks are relatively uncomplicated, with a low cognitive load. Simple instructions, however, should not imply that the task is easy. Eye movement studies require considerable co-operation on the part of the patient – this is particularly so in the nonverbal patient (Cassidy et al., 2000a). The advantage of the auditory measures, for example, the Auditory Brainstem Response (ABR) is self-evident. This non-invasive, electrophysiological measure is routinely used to assess the integrity of brainstem pathways requires no overt response and is typically best measured while the subject is sleeping (Hall, 2007). We will discuss the ABR in some detail in the following chapters. (see Chapters 2 and 3).

Secondly, the success of eye movement recordings are critically dependent on the visibility of the target and/or the ability to fixate on that target[1] whereas the ABR makes no demands on verbal or motor abilities (Hall, 2007). Thirdly, an important consideration is neural maturation. The saccadic system is not fully developed until the age of 1 year and can be absent at birth (Cassidy et al., 2000a), however, the ABR can be reliably recorded in infants from birth (Moore, 1987a, Musiek et al., 1988, Moore et al., 1995, Moore et al., 1996, Ponton et al., 1996, Moore and Linthicum, 2001, Hall, 2007, Moore and Linthicum,

[1] Typically, during saccadic eye movement testing, the subject is tasked to 'follow a target with your eyes'. Although the cognitive load of these instructions is low, there is always the question whether (a) the subject can understand these simple instructions and (b) whether the subject is able to actually generate the appropriate eye movement to fixate on the target. There is also the inherent assumption that the subject is able to see the target. Indeed, Cassidy et al., (2000) make the point that children who cannot move their eyes may appear blind because of saccade initiation failure.

2007). Fourthly, saccades can be difficult to interpret and require considerable expertise to both administer the tests and analyse the results (Leigh and Zee, 1999a).

Given these reasons, we argue in this thesis that the development of a non-invasive, inexpensive, marker of early brainstem damage, such as the ABR would be valuable addition in informing and planning the management of younger patients.

We have chosen to focus our attention on two clinical conditions: Gaucher disease (GD) and Dancing Eye Syndrome (DES). We have selected these conditions for three reasons. First, there are widespread anecdotal patient reports of auditory difficulties in children with these two conditions. Secondly, there is literature, albeit limited (and incomplete in many cases) that suggest that children with GD and DES may also have abnormal ABRs. Finally, given the co-location of the auditory and eye movement centres in the brainstem, it might also be expected that auditory pathways would also be affected.

1.3.1 Why study auditory signs and symptoms in Gaucher disease (GD)?

An autosomal recessively inherited lysosomal storage disorder, GD is characterised by deficient activity of the lysosomal enzyme *ß*-glucosidase, resulting in the accumulation of its substrate, glucosylceramide. This leads to multi-system disease involving enlargement and dysfunction of the spleen and liver, bone destruction and in severe cases, pulmonary infiltration. In some patients, there is also widespread involvement of the brainstem (Beutler and Grabowski, 1995, Scriver, 2001)[2]. Among the well-described

[2] GD is traditionally subdivided into the following groups: GD1 (non-neuronopathic) in which there is an absence of CNS involvement; GD2 (acute infantile) in which there is a rapid neurological progression leading

primary neurological signs, eye movement abnormalities[3] are considered to be the universal indicator of the neuronopathic GD-subtypes (nGD).

Audiological abnormalities have also been described in nGD (Dreborg et al., 1980, Bamiou et al., 2001, Grasso et al., 2006), although there is still no agreement as to whether audiological investigations could be considered reliable tests of neurological involvement in GD. Clearly further studies examining the potential role of auditory studies, particularly those that test the integrity of brainstem pathways are required.

We have chosen to study GD because it remains one of the most attractive candidates, among the inherited lysosomal storage diseases, for developing effective therapeutic interventions (Butters et al., 2003, Platt and Jeyakumar, 2008). As such, novel insights in this area may be directly applicable to other disease conditions.

1.3.2 Why study auditory signs and symptoms in Dancing Eye Syndrome (DES)?

Dancing eye syndrome (DES)[4] is a rare disease which is characterised by a jerky ataxia, shivering movements, and bursts of multi-directional conjugate eye movements along both horizontal and vertical axes (opsoclonus)[5] (Mezey and Harris, 2002). These initial symptoms often appear to resolve after a few days, or weeks, making diagnosis of

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to death, usually by 2 years of age; GD3 (subacute neuronopathic) in which there is a slower and more variable neurological progression.

[3] Eye movement abnormalities include horizontal saccade initiation failure (SIF) (also known as 'ocular motor apraxia'), horizontal saccade slowing, vertical saccade initiation failure (especially downward), 6th nerve paresis (in some cases) and in rare cases decreased gain of the vestibulo-ocular reflex. We will consider each of these later in Chapters 2 and 4.

[4] DES is known by many names including opsoclonus - myoclonus, opsoclonus ataxia, Dancing Eye and Dancing Feet, myoclonic encephalopathy or Kinsbourne's disease.

[5] These are the primary features of DES; however there are a number of other associated signs and symptoms which we will discuss later in chapter 6.

DES extremely difficult. The disease is usually seen in young children (aged typically between 10 months and three years) with no preceding history of neurological disease (Dale, 2003). There is little clear evidence as to the cause of DES and treatment options are limited (Pang et al., 2010).

The inability to accurately identify the site of lesion in DES is a major gap in our clinical knowledge. Eye movement studies have implicated the brainstem (Harris, 1997, Mezey and Harris, 2002) and the cerebellum (Shawkat et al., 1993). Auditory studies in DES are scant, although there is a suggestion that hyper-excitability is present within the brainstem pathways (Maeoka and Maegaki, 1998). Whatever the underlying cause of DES, it is crucial that we identify which parts of the brain are affected by the disease.

The more we understand the neurobiological basis of impairment, the easier it becomes to develop tools to capture a clearer picture of the intricate relationships that exist between brain and behaviour. Furthermore, the presence of a documented auditory deficit leads to a diagnosis of auditory dysfunction that may be amenable to auditory rehabilitation.

We have chosen to concentrate our efforts on the utility of the ABR in both of these conditions because we believe this test could translate successfully from laboratory to clinic. The ABR test is relatively straightforward, easy to administer, score, and interpret. It uses equipment that is commonly available in clinical practice. We will further develop this aspect of the thesis in the next chapter, where we will also locate it within the emerging literature. We now provide an overview of the thesis before summarising the contributions that it makes to the field.

1.4 Thesis overview

The questions that motivated this thesis are:

(1) Do children diagnosed with GD and DES with documented saccadic eye movement abnormalities, originating in the brainstem, also have auditory problems? and

(2) How can the application of the auditory brainstem response be used to complement eye movement studies?

In the next chapter, we present the relevant background literature to the thesis. This is followed by a description of the general methods and procedures that were used throughout the thesis (Chapter 3). The next four chapters focus on the experimental work undertaken. Chapters 4, 5 and 6 concentrate on developing an audiological profile in children with atypical saccadic eye movements; Chapter 7 examines the ABR when offset stimuli are employed. We now briefly summarise the content and main results of the thesis.

1.4.1 Chapter 2: Background

We begin by outlining the saccadic eye movement and auditory brainstem systems and draw attention to the neural substrates most relevant to the thesis. We then present an overview of current eye movement and audiological techniques that are used to examine the brainstem. This will set the scene for our discussion in later chapters.

We then proceed to examine the established literature concerning eye movement studies and audiology signs, specifically, those that are concerned with slow saccades and

opsoclonus. Our goal here is to examine whether there is a documented association between auditory deficits in children with these atypical eye movements.

1.4.2 Chapter 3: General Methodology

We explain in detail all of the general methods that we have employed during our studies that are described in Chapters 4, 5 and 6. We have chosen to use audiological investigations that test overlapping efferent and afferent pathways in the peripheral and central auditory nervous system, in an attempt to evaluate the extent and degree of brainstem lesions. We will also provide detailed justifications for each of the assessments used.

1.4.3 Chapter 4: Audiological profile of Gaucher Disease

We begin by providing a detailed clinical description of GD, paying particular attention to the abnormal eye movements which are considered a hallmark of nGD (Harris et al., 1996, Harris et al., 1999). We then outline what is known experimentally about the use of eye movement and audiological techniques from the published literature relating to the diagnosis of GD, and draw attention to those results most relevant to the thesis. Finally, we will present our first set of results, constructing an 'audiological profile' of GD by carrying out investigations on a wide range of Gaucher patients, with known oculomotor status.

Our aims in this study are twofold. Firstly we wish to investigate whether audiological tests can detect subtle brainstem involvement and whether they point to focal lesions consistent with saccadic eye movement disturbances, or whether there is more

widespread degeneration. Secondly, we wish to examine whether there is a similar continuum between neuronopathic and non-neuronopathic disease by using combined eye movement and auditory investigations.

In this chapter, we show that when audiological tests are coupled with eye movement studies, they can be used to provide reliable subclinical and pre-symptomatic tests of neurological involvement in GD.

1.4.4 Chapter 5: The use of audiological assessments in the longitudinal monitoring of GD

We build on the results presented in Chapter 4 by asking whether these combined techniques can be used to measure the disease burden for those patients on treatment. Two key questions arise:

(1) *Can the ABR be used for long term monitoring of any neurological progression in GD?* Little is known about the fluctuations in the ABR over time and if we hope to exploit the full potential of the ABR for longitudinal monitoring, then it is imperative that we address this issue.

(2) If the ABR is shown to be a sensitive and reliable longitudinal measure of subclinical neurological disease burden, then *can the ABR be used to capture any drug efficacy over the limited time frame of clinical trials?* Such work is important. Our ability to test the neurological efficacy of new therapies is very limited, if not absent, for the young or cognitively impaired child.

We present two experimental studies. Our first study is concerned with our question of whether the ABR can be used to reliably measure the disease burden in the neuronopathic subtypes of GD. We present data, for the first time, of serial ABR recordings in type 2 and type 3 GD. A preliminary account of this work has appeared in two papers (Campbell et al., 2003, Campbell et al., 2004).

In our second study, we take an in-depth look at an emerging and promising treatment, i.e., substrate reduction therapy (SRT), an alternative approach to the current enzyme replacement therapy. We then present our data on use of the ABR as a secondary outcome measure in the substrate restriction therapy GD3 trial.

1.4.5 Chapter 6: Audiological profile of Dancing Eye Syndrome

We begin this chapter by presenting a synopsis of the signs and symptoms that are seen in DES and a summary of previous eye movement and auditory studies that are pertinent to this thesis. This is then followed by two experimental studies. In our first study, we present our preliminary findings from a parental questionnaire that was administered to families of DES throughout the UK. Our core goal in this experiment is to determine whether the hyper-excitability expressed in the eye movement system, as opsoclonus, is mirrored in the auditory system, i.e. *do children with DES have hyperacusis?*

In our second study, we present for the first time a systematic audiological evaluation of DES. Here, we are chiefly interested in identifying whether children with DES have auditory deficits, and if so, whether there is any evidence of longer-term brainstem disease, particularly in the absence of any overt eye movement abnormalities.

In this chapter, we show that *children with DES do have auditory problems*, including an abnormal sensitivity to ordinary environmental sounds. Furthermore, *these deficits can be reliably measured by the ABR*. We also show that auditory tests, such as the ABR, in addition to eye movement studies, can result in an improved understanding of the pathophysiology underlying DES.

1.4.6 Chapter 7: The 'offset ABR' in saccadic eye movement disorders

In the final experimental chapter we are concerned with the first stages of understanding the importance of the "offset" of sound using the ABR. The offset of sound is an important acoustic cue in consonant identification (Pind, 1998), discriminating sound duration (Schlauch et al., 2001) and in the acoustic startle reflex (Ison and Allen, 2003). Despite the obvious importance of these phenomena, there has been relatively little study of the offset response. The paucity of work undertaken in this area may be a reflection of the technical challenges to accurately evaluate and record the offset response.

One technique that has been used sporadically, to address this problem, is the use of the 'offset ABR' (Kodera et al., 1977b, Van Campen et al., 1997). In this chapter, we begin by presenting an in-depth review of all 'offset-ABR' studies undertaken in the last 30 years. We then present offset-ABR data from a series of different experiments, employing a wide range of frequencies (and rise-fall times) and polarity (rarefaction, condensation) in normal hearing participants. This work replicates and extends previous studies (Van Campen et al., 1997). Finally, we present, for the first time, a clinical application of the offset ABR. This

application involves recording the offset ABR in our two eye movement disorders (GD and DES) that have been the subject of our previous chapters.

1.4.7 Chapter 8: General Discussion and Conclusions

In this concluding chapter, we summarise our main findings in this thesis. Although the technique introduced in Chapter 7 requires more research, the emerging message from our thesis is unambiguous; *there is ample evidence that the use of audiological assessment in children with aberrant saccadic eye movements would contribute to clinical practice*.

Finally, we suggest possible directions for future work, and ways in which the ABR could be used to complement other studies. This may lead to improved early detection and may allow for new, meaningful rehabilitative intervention.

1.4.8 Main contributions of the thesis

These are as follows:

1. Our ABR investigations, using onset and offset stimuli, have clearly shown that there are aberrant electrophysiological characteristics in two diverse eye movement disorders (GD and DES). These findings have not previously been documented.
2. Objective measurement of neurological dysfunction remains a major challenge when testing new drugs, especially in young children. Nowhere is this more obvious than when testing the new drug therapies that are emerging for the treatment of metabolic diseases. Although it is unlikely that one single outcome measure can

capture all aspects of the disease process, we present new data highlighting the potential for the ABR as a clinical trial biomarker.

3. A novel use of the ‘offset’ clinical ABR is presented for the first time. The atypical ‘offset’ ABR response to stimulus cessation may account for some of the persistent learning difficulties that are reported in some children with atypical saccadic eye movements.

Chapter 2
Background

2.1 Introduction

The eye is not a lonely miracle. It is a sense organ, similar in application to an ear, or a nose. Of course there are differences between seeing and hearing and smelling but there are common features too, and these are important. (Ings, 2007, p15)

The brainstem is a small but extremely significant part of the brain. Sensory and motor nerve connections, from the main part of the brain to the rest of the body, pass through the brainstem. Located posteriorly, the brainstem is structurally continuous with the spinal cord, providing the main sensorimotor innervation via the corticospinal tract, the spinothalamic tract and the posterior column-medial lemniscus pathway. The brainstem is a continuously adapting structure which rapidly modulates and shapes incoming messages, controlling the way that information, particularly sensory data, is transformed, encoded and routed.

In this chapter we will focus on two experimental approaches by which the brainstem has been investigated: namely the study of eye movements and auditory circuits. We begin this chapter by providing an overview of the brainstem, followed by a detailed discussion of the neurology of the saccadic and auditory systems; the neural substrates most relevant to this thesis. Here, we will confine our review to saccadic eye movements. The reason we have chosen to focus on saccadic abnormalities is that they are the most stereotyped eye movements and the ones that are least influenced by the external stimuli. Our emphasis in this chapter is on two abnormal oculomotor developments: slow saccades and opsoclonus.

We will then present a brief overview of current eye movement and audiological techniques that are used to examine the brainstem. This will set the scene for our discussion in later chapters. We then proceed to briefly review eye movement studies, specifically, those investigating slow saccades and saccadic intrusions (i.e. opsoclonus). Our goal here is to examine whether there is a documented association between auditory deficits in children with these atypical eye movements.

2.2 A gross anatomical description of the brainstem

The brainstem is composed of three major structures: the medulla oblongata, pons and the midbrain. Each of these areas within the brainstem, incorporate a number of important, highly specialised regions of the auditory and oculomotor systems. Because of the relevance of these structures to our thesis, we now provide a brief outline of the major brainstem subdivisions (for a more comprehensive treatment see e.g., Standring et al., 2008). These structures and their spatial relationships are shown in Figure 2.1. An understanding of these structural relationships is critical for our later discussion, where we examine whether the auditory system is also implicated in different eye movement disorders.

The *medulla oblongata* is the most caudally located part of the brainstem. It extends from the inferior pontine sulcus to the spinal cord. The boundary between them is the region where the lateral walls of the fourth ventricle converge in a V shape at the midline obex, at the level of the foramen magnum. The ventral surface of the medulla displays the

anterior midline fissure, bordered on each side by the pyramids and crossed by the pyramidal decussations, connecting the right and left pyramids to each other. Lateral to each pyramid are the olivary bodies – olive pit-shaped swellings. These paired structures can be divided into the inferior olivary nucleus, which is involved in cerebellar motor-learning and function, and the superior olivary nucleus, a critical part of the auditory system that has an important role in sound localisation. We will discuss the functional significance of this region later in this chapter (see section 2.3.4.5).

Figure 2-1 Gross anatomy of the human brain

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As can be seen in Figure 2.1, above, the cerebellum overlies and hides the dorsal aspect of the brainstem, but its ventral aspect, *the pons*, is clearly evident. Rostrally, the superior pontine sulcus acts as the boundary between the pons and the midbrain and the inferior pontine sulcus as the boundary between the pons and the medulla. Part of the floor of the fourth ventricle is formed by the dorsal aspect of the pons. This is known as the pontine tegmentum, a structure that incorporates the nuclei of the trigeminal (V), abducen (VI), facial (VII), and vestibulocochlear (VIII) cranial nerves. Three of these cranial nerves have significant functional importance in the oculomotor and auditory systems. The abducens nerve is important in the control of the lateral rectus muscle (see section 2.3.2.2) and the facial and vestibulocochlear nerves are important structures involved in the

ascending and descending subcortical auditory brainstem. These three nerves exit the brainstem at the level of the inferior pontine sulcus, whereas the trigeminal nerve exits the brainstem through the middle cerebellar peduncle.

The *midbrain* is the smallest segment of the brainstem. It is a relatively narrow structure that extends from the thalamus to the pons, close to the cerebral aqueduct. The midbrain can be subdivided into two regions: the *midbrain tegmentum*, an area of the midbrain below the cerebral aqueduct, and the *tectum*, which houses the paired superior and inferior colliculi. These structures are closely associated with the lateral and medial geniculate bodies, respectively, and as we will show later in this chapter, they collectively play an important role in visual and auditory functions. The trochlear nerve (CN IV) controls one of the extraocular muscles (the superior oblique muscle) of the eye. This cranial nerve is the only one to exit the midbrain tectum, just below the inferior colliculus. The other cranial nerves exit the ventral aspect of the brainstem.

The brainstem is connected to the cerebral hemispheres by two large fibre tracts – the cerebral peduncles *(cerebral crus)*. The depression between the peduncles (the interpeduncular fossa) is the site of origin of the oculomotor nerve (CN III) which is responsible for eye movement control, pupil constriction and maintaining an open eyelid. We discuss the mechanics of eye movement control later in this chapter (see section 2.3.2.2).

2.3 The neurology of oculomotor and auditory systems

In our previous section, we briefly described the neuroanatomical spatial relationships of the auditory and eye movement centres within the brainstem. In the following sections, we describe in more detail the neural substrates of each of these systems. We begin with an introduction to the study of eye movements.

2.3.1 An introduction to the oculomotor system

Eye movements have evolved in order to maintain a stable image of the world. However, the human brain is not able to support a visual system that is capable of maintaining a high resolution over an entire field of vision. These competing requirements have resulted in the human oculomotor system evolving a highly sensitive 'compensatory' strategy. Objects of interest are targeted on the fovea, an area on the retina where visual acuity is optimised, due to the high density of photoreceptors.

In order to achieve this *'foveal compromise'* the oculomotor system has evolved six specialist subsystems. These can be functionally subdivided into two groups: (a) eye movements that are responsible for maintaining a steady target on the retina, including fixation, vestibular (VOR) and the optokinetic systems and (b) subsystems that direct the fovea onto an object of interest which include saccades, smooth pursuit and vergence systems. Each of these eye movements are characterised by a unique set of properties and involve different parts of the brain which we have summarised below in Table 2.1.

Table 2-1 Functional classification of the six eye movement systems
Adapted from Leigh and Zee (1999)

Function	Eye movement system	Physiological function
Hold images steady on the retina	Fixation	Responsible for holding the image of a stationary object on the fovea when the head is not moving
	Vestibular	Maintains a stable retinal image during brief head movements
	Optokinetic	Maintains a stable retinal image during sustained head movements
Directs the fovea to an object of interest	Saccades	Ensure that the image of an object of interest is brought rapidly into view on the fovea.
	Smooth pursuit	Maintains the image of a small moving target on the fovea
	Vergence	Moves the eyes in an opposite direction (i.e. convergence or divergence) so that images of a single object are held simultaneously on both foveae.

Since this thesis investigates whether children with atypical saccades also have associated abnormalities in the auditory brainstem, we will concern ourselves only with the saccadic eye movement (SEM) system. For a comprehensive review of the other five eye movements see Leigh and Zee, 1999 or Carpenter, 1981.

In the next section, we will outline the functions and characteristics of saccades. We then describe the anatomy and physiology of the brainstem circuits that are responsible for generating horizontal, vertical and torsional saccades. Finally, we briefly discuss the higher level neural control of the saccadic system.

2.3.2 Organisation of the saccadic system

Saccades are rapid conjugate eye movements that move the line of sight between successive points of fixation. It is estimated that we make in excess of 100 000 saccades per day (Leigh and Zee, 1999b), the majority of these are made unconsciously and

spontaneously. Their highly stereotyped behaviour and well defined temporal properties have resulted in the saccadic system being the most intensively studied oculomotor subsystem (Sparks, 2002). Moreover, saccades are diverse – they are not only triggered by visual targets, but to auditory targets as well as memorised targets. The most common SEM are summarised in Table 2.2.

Table 2-2 Classification of different types of saccades
Adapted from Leigh and Zee, 1999)

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In order to help us understand the importance of the saccadic eye system, the analogy of the eye as a 'camera with a slow shutter speed' is a useful one. Shutter speed is related to the length of time that we actually visualise or 'see' the scene that we are interested in capturing. Slow shutter speeds on a camera, result in a fuzzy photo. In the *absence of a normal functioning saccadic system*, our view of the world would be significantly distorted.

2.3.2.1 Functions and characteristics of saccades

Saccades are characterised by a unique set of properties that can be reliably measured and our ability to accurately quantify these behaviours – velocity, latency and accuracy – has significantly contributed to our understanding about what constitutes normal and abnormal brainstem function (Leigh and Zee, 1999a, Sparks, 2002, Leigh and Kennard, 2004). Here, we consider each of these unique properties in more detail.

2.3.2.1.1 Velocity

Saccades are fast. Not only are they the fastest of the oculomotor subsystems, but these remarkable movements are the quickest generated by the human body. Some studies have report peak velocity measures in a normal adult subject in excess of 650 degrees/sec[6]. A core reason for using saccades to examine the brain is the striking consistency shown in their relationship between magnitude, speed and duration. The size of a saccade is closely linked to velocity and duration, thus "*the bigger the saccade, the greater its peak velocity and the longer its duration*[7]*"* (Leigh and Kennard, 2004, p463). This relationship – between amplitude and peak velocity (and sometimes amplitude and duration) – is referred to as the main sequence. Examples of the main sequence relationships between peak velocity, duration and amplitude are shown in Figure 2.2.

Deviation from the main sequence allows clinicians to be able to determine whether (a) a saccade is actually made and (b) whether the velocity of a saccade falls within the normal range or demonstrates abnormal characteristics (e.g. too fast or too slow). Abnormal velocity is the hallmark of a number of brainstem disorders. For example, the slow

[6] Peak velocity is reported in a number of studies as 30 – 700 degrees/sec for amplitudes varying over 0.5-40 degrees. Saccades can vary depending on a number of subject factors (e.g. age, alertness) and technical factors (e.g level of illumination, sequence of target presentation). Saccadic velocity also depends upon the direction of the movement, and the initial and final eye position. These factors require consideration when comparing saccadic behaviour in patients with normal subjects.

[7] Saccadic duration is another quantitative measure of saccadic performance that is sometimes reported. Studies have shown that the relationship between amplitude and duration is nearly linear and it varies from 30 – 100ms, for amplitudes of 0.5 to 40 degrees.

horizontal saccades which are observed in patients with neuronopathic Gaucher disease (Figure 2.2).

2.3.2.1.2 Latency

Another measure commonly used to analyse saccades is latency. This is defined as the interval between stimulus onset and the start of saccadic execution (Leigh and Zee, 1999a). Latency is extremely variable – in normal adults, the latency for medium amplitude saccades (5°-10°) is usually around 200ms. However, it can range from as low as 100ms, or as high as 350ms, depending upon the paradigm that has been used to elicit the response (e.g. luminance, cognitive load of the task) and the arousal level of the subject. For example, attention or active fixation on a target increases the latency of a saccade (Hoffman and Subramaniam, 1995)[8].

Figure 2-2The main sequence of horizontal saccades in Gaucher disease
A) The relationship between duration and amplitude of saccadic eye movements. The green symbols show the main sequence for a child with GD1 and shows a normal relationship. The red symbols are the abnormal main sequence recorded from a child with GD3 shows markedly longer duration saccades. B) The relationship between peak velocity and amplitude. The relationship for the GD1 patient is normal, but the GD3 patient shows markedly abnormally slow saccades. Figure reproduced from Harris et al., (2003).

Figure has been removed due to copyright restrictions

Study of saccadic latency has also been used extensively to examine other aspects of saccadic programming such as visual processing and target selection (Kowler, 2011). This is supported by recent clinical studies which have shown that saccadic latency is

[8] There is an emerging literature about the role of attention and top-down processing on saccadic eye movements-for a recent review of this topic see Noudoost et al., (2010).

abnormal in disorders, particularly those affecting cortical areas concerned with vision, cognitive function and eye movements (Perneczky et al., 2011).

2.3.2.1.3 Accuracy

The ability to 'accurately' execute a saccade is another highly stereotyped behaviour of the saccadic system. Accuracy is generally subdivided in one of the three following categories: (a) orthometria – in which the saccade is executed without any errors; (b) hypometria – in which the saccade falls short or 'undershoots' the initial target and requires additional corrective saccades so that the image of interest is on the fovea or (c) hypermetria – in which the saccade overshoots the initial target and secondary, corrective saccades are needed. Saccadic hypermetria, when conjugate is considered is typically associated with disease affecting the cerebellum, especially the fastigial nucleus or its projections (Selhorst et al., 1976, Noda, 1991).

It is not uncommon for normal subjects to display a small degree of saccadic hypometria, especially when a larger movement (>10°) is generated but as Harris (1997) clearly points out, in healthy adults and children, the initial saccade is usually within 90-100% of the target distance. This 'marginal error' becomes more prominent with increased age, fatigue and inattention. Hypometria has been described in association with ocular motor apraxia (Harris et al., 1996) and in basal ganglia disease (Armstrong et al., 2002).

2.3.2.2 The mechanical properties of the oculomotor system

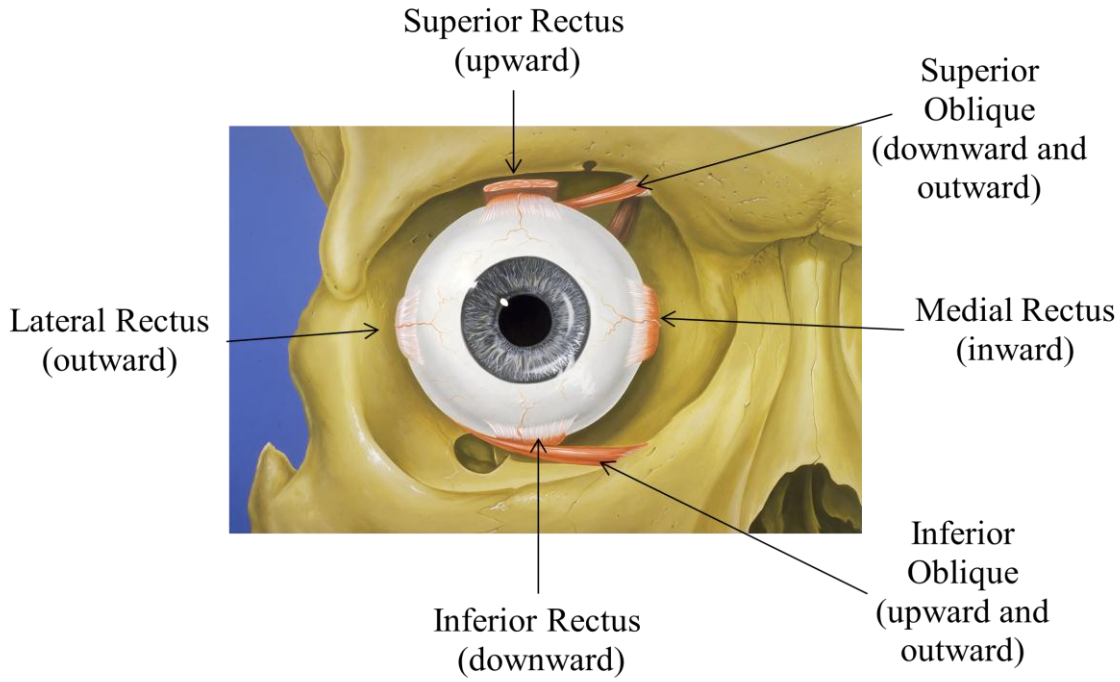
In our previous sections, we outlined the characteristics that have made the measurement of SEM so popular in Neuroscience. In this section, we describe the mechanics or the *process* by which a saccade is generated. In order for us to explain this successfully, we present a brief overview of the anatomical structures that enable the eye to move. Due to practical constraints, we cannot provide a comprehensive review of the anatomy of the eye and we limit our discussion here to a brief anatomical overview of the motor apparatus (i.e. the structures that allow the eye to move[9]).

The motor apparatus of the eye includes six eye muscles arranged into three reciprocally activated pairs. These are controlled by three cranial nerves – the oculomotor nerve (CN III), the trochlear nerve (CN IV) and the abducens nerve (CN VI) (Angelaki and Hess, 2004). Each agonist-antagonist pair is responsible for the rotation of the eye in different directions – horizontal, vertical and torsional (i.e. around the line of sight) rotations (Angelaki and Hess, 2004, Demer, 2006). Figure 2.3 shows the different eye muscles, rotation and relationship to the respective cranial nerve(s).

In addition to the eye muscles, passive tissue structures such as the fascia bulbi, exert elastic and viscous forces on the eye, affecting the dynamics of eye movements and the steady-state muscle activity required to keep the eye at a given orientation (Demer, 2006, 2007).

[9] The study of ocular kinematics is steadily growing in popularity following recognition that this system involves a series of highly complex behaviours see Demer (2006, 2007); Angelaki and Hess (2004). For a complete description of the sensory apparatus (i.e. anatomical structures at the front end of the visual system such as the retina and lens) see Standring et al., (2008).

Figure 2-3 Anterior view of the right eye and associated eye muscles.
The Oculomotor nerve (CN III) innervates four of the six eye muscles: the superior rectus musle, the medial rectus muscle, the inferior rectus muscle and the inferior oblique muscle. The superior oblique muscle is innervated by the Trochlear nerve (CN IV). The lateral rectus muscle is innervated by the Abducens nerve (CN VI). Rotation of the eye is described in brackets. Image reproduced with permission from Patrick J. Lynch, medical illustrator; C. Carl Jaffe, MD, cardiologist under the creative commons license (2006).



2.3.2.3 Pulse-step of innervations for Saccadic eye movement

Robinson (1973) postulated that saccades are the product of two commands: an eye velocity command, called the pulse, and an eye position command, termed the step. The pulse is a high-frequency *burst* of phasic activity in the motor neurons of the agonist extraocular muscles. This allows the eye to move rapidly from one position to another

against the viscous drag of the orbit. Once the eye is in the new position, agonist motor neurons operate at a new level of *tonic* innervation, higher than the original resting level. This sustained innervation is the step component and it allows the eye to stay in its new position against orbital elastic recoiling forces (Buttner and Buttner-Ennever, 2006).

The saccadic step, an eye position command, is created from the pulse (an eye velocity command) by a neural network (the neural integrator or NI) that mathematically integrates these commands into the appropriate position-coded information for the oculomotor neurons. For horizontal movements, the putative NI consists of the medial vestibular nucleus and the nucleus prepositus hypoglossi in the brainstem. The NI for vertical and torsional movements is localised to the interstitial nucleus of Cajal, in the midbrain (Harris, 1997, Buttner and Buttner-Ennever, 2006).

The pulse-step innervation applies to all types of eye movements although the demand for speed, as required by the saccadic system means that the phasic component is naturally greater than that seen in other eye movement circuits. The pulse-step relationship is illustrated in Figure 2.4.

Understanding the relationship between the pulse and step has allowed saccadic disorders to be modelled in terms of pathophysiological mechanism. These models have been developed in an attempt to explain how the brain specifies a command for a saccade before it starts. Early models were originally based on the premise that saccades were ballistic, and simply 'played out' during the movement (Ramat et al., 2007). However, it is clear that saccades are not pre-programmed and a number of very sophisticated models

describing saccadic behaviour have been described. Further, these models have been applied to explain clinical disorders. A discussion of these models is beyond the scope of this thesis but this topic has been extensively reviewed (see e.g. (Leigh and Zee, 1999a, Sparks, 2002, Leigh and Kennard, 2004, Ramat et al., 2005, 2007, Chen et al., 2011). Figures 2.4 and 2.5 show two models of saccadic control.

Figure 2-4 A. Early model of saccadic control. B. The pulse-step relationship and neural generators. Image reproduced from (Ramat et al., 2007)

Figure has been removed due to copyright restrictions

2.3.2.4 Brainstem saccade generation

Saccadic pathways are widespread and complex and the relative role of each pathway in generating and suppressing various types of saccades is still unclear. The essential machinery required for generating saccades resides in the brainstem. Imaging studies and animal models have identified two distinct neural circuits – neurons located within the *pons* are mostly concerned with the horizontal component of saccades, whereas the *midbrain* controls vertical and torsional components (Sparks, 2002, Buttner and Buttner-Ennever, 2006, Linzenbold et al., 2011).

Figure 2-5 The neural control of saccades

EBN generate a pulse, which is integrated to create a new eye position command (the step) by the velocity to position NI. The pulse-step of innervation is sent to the motor neurons to move and maintain the eye in a new position (Wong, 2007).

EBN also activates IBN, which inhibit antagonist motor neurons and OPN during the saccade. When the eye position matches the target, BN cease firing, the OPN resume their tonic activity and the saccade stops.

Figure has been removed due to copyright restrictions

Abbreviations: III = oculomotor nerve; INC = interstitial nucleus of Cajal; io = inferior olive; MLF = medial longitudinal fasciculus; nIII = oculomotor nucleus; nIV = trochlear nerve; nVI = abducens nucleus; PC = posterior commissure; PN = pontine nuclei; NPH = nucleus prepositus hypoglossi; PPRF = paramedian pontine reticular formation; ri = rostral interstitial nucleus of the MLF; SC = superior colliculus; VN = vestibular nuclear complex. Other areas shown: EW = Edinger-Westphal nucleus; mb = mammillary body; nD = nucleus of Darkschewitsch; PTc = pyramidal tract decussation; rn = red nucleus. Adapted from Büttner-Ennever and Büttner, 1992.

Two types of neurons have been identified – burst neurons and omnipause neurons Burst neurons can be divided into excitatory or inhibitory types. Excitatory burst neurons (EBN) can be further subdivided into medium-lead burst neurons and long-lead burst neurons (Figure 2.5). We will consider each of these in more detail in the following sections.

2.3.2.4.1 Burst neurons

Medium-lead burst neurons (premotor burst neurons) activate the motor neurons at a high frequency, innervating agonist muscles approximately 8-15 ms before and during saccades. The characteristics of medium lead burst neurons are summarised in Table 2.3 (Ramat et al., 2007).

These neurons are inactive during fixation, pursuit, and vestibular and optokinetic eye movements. These neurons are located in the brainstem – in the nucleus reticularis pontis caudalis (nRPC) situated within the paramedian pontine reticular formation (PPRF) for horizontal saccades. The BN responsible for generating vertical and torsional saccades have been localised in the rostral interstitial nucleus of the medial longitudinal fasciculs (riMLF). Figure 2.5 shows a sagittal view of the human brainstem showing the location of structures important in the control of saccades.

Long-lead burst neurons activate medium-lead EBN and inhibit OPN to release their tonic inhibition on EBN. They discharge at irregular intervals – up to 100 msec prior to the onset of a saccade. They are located predominantly in the rostral PPRF and the mesencephalic reticular formation. The characteristics of long-lead burst neurons are described in further detail below in Table 2.4 (Ramat et al., 2007).

Table 2-3 Characteristics of medium-lead burst neurons

Text has been removed because of copyright restrictions

Table adapted from (Ramat et al., 2007)

Burst neurons can also be inhibitory (IBN). These neurons inhibit motor neurons to antagonist muscles – discharging just before and during saccadic activity. They are located in the nucleus paragigantocellularis dorsalis in the PPRF, for horizontal saccades. For

vertical and torsional saccades, IBN are located in the interstitial nucleus of Cajal (INC) and possibly within the riMLF.

Table 2-4 Characteristics of long-lead burst neurons

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Table adapted from (Ramat et al., 2007)

2.3.2.4.2 Omnipause neurons

Omnipause neurons (OPN), also called pause neurons, are glycinergic neurons that have been shown to fire tonically in animal models (Strassman et al., 1987, Horn et al., 1994). In primates, OPNs are located near the midline within the nucleus raphe interpositus (rip) in the PPRF (Buttner-Ennever et al., 1999). They are thought to inhibit medium-lead burst neurons bilaterally in the pons and midbrain however, their exact role is still poorly defined – particularly in disorders of saccadic triggering (Harris, 1997).

Table 2-5 Characteristics of omnipause neurons

Text has been removed because of copyright restrictions

Table adapted from (Ramat et al., 2007)

OPN have been shown to stop discharging or to 'pause' 12-18 ms before and during saccades in the cat model (Yoshida et al., 1999) and 12-25 ms in primates (Keller, 1974). This initial observation led Robinson, (1975) to suggest that their function was simply to act as an 'inhibitory gate' for the driving system in saccades. However, experimental studies involving chemical lesions damaging the RIP in the rhesus macaque resulted in slower saccades with normal latency and accuracy – not the saccadic oscillations as predicted by Robinson's model (Robinson, 1975, Kaneko, 1996). The data suggests that OPNs must contribute, in some way, to the drive signal for saccade generation, possibly by reducing the firing rate of EBN. Computational models have also suggested that OPNs have a complex neuromodulatory role (Miura and Optican, 2006).

2.3.2.5 Brainstem generation of horizontal saccades

The brainstem horizontal saccadic centres are ipsilateral to the eye and consist of a variety of units in the pontine tegmentums that have saccade-related burst activity. Horizontal saccades are generated by EBN and IBN units and OPN. The OPN fire tonically and inhibit EBNs and their activity inhibits the triggering of saccades. The EBNs project directly to the ipsilateral sixth nucleus, and to interneurons that relay the premotor signal via the contralateral medial longitudinal fasciculus (MLF) to the contralateral third nucleus. EBNs also drive IBNs which inhibit the contralateral sixth motorneurones and interneurones (Leigh and Zee, 1999a, Ramat et al., 2007).

2.3.2.6 Brainstem generation of vertical and torsional saccades

The premotor circuitry for vertical saccades resides in the midbrain, particularly the rostral interstitial nucleus of the MLF (riMLF), where upward and downward control are separated – upward control is more medial than downward control. The posterior commissure is an important link in the generation of upward saccades. Vertical circuitry does not have its own pause neurones, and depends to some extent on the integrity of the lower brainstem horizontal saccade structures. However, the vertical system appears to be more independent of the horizontal saccade system in humans than in the monkey (Leigh and Zee, 1999a).

2.3.2.7 Superior colliculus

The superior colliculus (SC) is a paired structure, located within the midbrain. The SC is multi-layered, with neurons laid out in a topographic map of the visual system (retinographic map). Cells located within the superficial layer have been shown to have well-defined receptive fields and these neurons are activated only in relation to visual events. The intermediate and deeper layers of the SC contain two major types of neurons that are active in response to visuomotor events (Munoz et al., 2000).

The role of the SC in the generation of saccades remained the subject of considerable debate until the mid 1990s. Early SC lesion studies in the monkey were shown to cause deficits in the generation of saccadic eye movements. This lead to the widespread

assumption, that the SC was important in the control of saccadic amplitude, direction and trajectory. This view was subsequently challenged by Schiller et al., (1980) who pointed out that the differences observed between early studies was likely to reflect the "*variety, size and configuration"* in SC lesions (Schiller et al., 1980, p1188).

The current consensus is that the SC is important in the target selection and target initiation. Lesion studies have also shown that the SC also contributes to the speed of a saccade but not the accuracy of the saccade (Hanes et al., 2005).

2.3.2.8 Cerebellar control of saccades

The importance of the cerebellum, particularly in determining saccadic accuracy is well documented (Jenkinson and Miall, Ron and Robinson, 1973, Optican and Robinson, 1980, Zee, 1986, Noda, 1991, Ohtsuka and Noda, 1995, Takagi et al., 1998, Robinson and Fuchs, 2001, Helmchen et al., 2003c, Leigh and Kennard, 2004). Several areas within the cerebellum have been shown to have an important strategic role in modifying saccades. These include the vermis (and paravermis) and the underlying deep cerebellar nuclei (fastigial, interpositus and dentate).

The area of the cerebellum that has been most thoroughly studied is the vermis: a narrow structure divided into nine lobules and located between the hemispheres of the cerebellum. Studies have shown that electrical stimulation of lobule V in an alert monkey evokes upward to horizontal saccades, while stimulation of lobule VI evokes saccades that range from horizontal to downward (Ron and Robinson, 1973).

Within lobules VI and VII is another area that has important relevance to the saccadic system and is called the dorsal '*oculomotor'* vermis (DV)[10] (Noda, 1991). Neurophysiological and lesion studies in primates, as well as neuroimaging studies in humans, that have clearly shown that the DV is critical for saccadic adaptation and plasticity (Ron and Robinson, 1973, Optican and Robinson, 1980, Robinson and Fuchs, 2001). These cells have been shown to discharge 15ms before saccades in a preferred direction (Ohtsuka and Noda, 1995). Lesions of the DV, which spare the deep nuclei structures, impair the ability to rapidly adapt the amplitude of saccades. After such lesions, both leftward and rightward saccades were between 20-30% hypometric, with gains that were twice as variable as the baseline measured pre-lesion (Takagi et al., 1998, Barash et al., 1999). Clinical studies have also shown that damage to this area, results in dysmetric and slow saccades (Leigh and Zee, 1999a, Leigh and Kennard, 2004).

Purkinje cells (P-cells) located within the DV inhibit neurones in the caudal part of the fastigial nuclei (FN). The FN is an ellipsoidal region which is important in the control of saccade accuracy and consistency. Projections of the FN decussate within the cerebellum to reach the brainstem, where they terminate onto BN, OPN and the rostral pole of the SC.

Other important areas that contribute to the continuous adaptive control of saccades include the flocculus (Fl) and paraflocculus (PFl). These regions are crucial in 'repairing' saccadic inaccuracies. The flocculus and paraflocculus are important for the adaptation of

[10] There is a large literature on the role of the DV and the modification of saccades (see Robinson et al., 2001 for a review).

the pulse and pulse-step mismatch for saccades. Lesions of the flocculus and paraflocculus results in post-saccadic drift because adaptation to pulse-step mismatch of saccades is lost.

The flocculus receives inputs through mossy fibres arising in numerous sources in the brain stem and through climbing fibres from the inferior olive (Noda, 1991, Robinson and Fuchs, 2001, Buttner and Buttner-Ennever, 2006). Climbing fibres from the inferior olive extend into the cerebellar cortex. The role of the climbing fibres is still not entirely clear but all relevant signals (e.g. eye position and velocity, target position and velocity) needed to control saccades arrive in the cerebellar cortex on mossy fibres.

2.3.2.9 Cortex and basal ganglia

Multiple cortical and subcortical areas have been identified as important in saccadic programming. These include the frontal eye field (FEF); the supplementary eye field (SEF), the pre-SEF, the parietal eye field (PEF); the dorsolateral prefrontal cortex (DLPC) and the posterior parietal cortex (PPC). These pathways and connections between the brainstem, basal ganglia and cerebellum are shown in Figure 2.6.

Frontal and parietal cortical areas project directly to the superior colliculus and frontal areas project indirectly through a basal ganglia pathway. The frontal areas also project, via pontine nuclei such as NRTP to the DV and FN of the cerebellum (Leigh and Kennard, 2004).

Leigh and Kennard (2004) have hypothesised that there is an unexplored functional dichotomy between the neurons in the cerebral cortex and basal ganglia and those in the

brainstem, and cerebellar saccadic circuits. They argue that the generation of saccades by the premotor burst neurons and the control of the size of saccades by the FN are both performed by units whose discharge is related temporally to the eye movement. Thus, the maximum discharge rate of burst neurons correlates with the saccade's top speed and the timing of discharge of FN neurons correlates with saccade start and end. This contrasts with spatial coding of saccade-related neurons in cortical areas. The concept of a 'spatial–temporal transformation of sensory-to-motor signals' offers an interesting framework for further scientific study, further discussion regarding of the function of these higher centres is beyond the scope of this thesis – for a review see (Leigh and Kennard, 2004).

Figure 2-6 Areas of cerebral cortex and their projections that contribute to generation of saccades. Cortical areas important for eye movements in human brain. (B) Major structures that project to the brainstem saccade generator Abbreviations: IML = intramedullary lamina of thalamus; MST = medial superior temporal visual area; MT (V5) = middle temporal visual area V5; NRTP = nucleus reticularis tegmenti pontis; STN = subthalamic nucleus; V1 = primary visual cortex.*Image reproduced from Leigh and Zee (1999)

2.3.3 An introduction to the auditory system

The auditory system is a highly sophisticated system and is able to process complex sound patterns across a number of dimensions – frequency, time, amplitude and space (Griffiths, 1999). In order to make sense of the acoustic landscape, the human auditory system is tasked with three objectives. Firstly, it must deliver sound to the end receptors in the auditory periphery. Secondly, it must faithfully transduce the stimulus from an air-filled medium into a fluid-filled medium – converting these 'vibrations' into an electrical signal. Finally, it must transmit these neural signals to downstream sub-cortical and cortical stations for further (serial and parallel) processing, in order that our brain can extract meaningful information such as speech.

How the auditory system accomplishes each of these tasks is the subject of much of the next section – an understanding of these central concepts is essential for much of our later discussion in this thesis. In the following sections we will *briefly* overview the anatomy and physiology of the ascending 'classical' auditory pathway – from the auditory periphery, brainstem up to the primary auditory cortex – focusing on the sub-cortical auditory brainstem network which is our primary concern in this thesis.

The accounts of both structure and function given here are necessarily simplified; more detailed reviews on the anatomical and physiological characteristics of each of these structures can be found in a number of extensive reviews, see e.g., Hackett (2011); Pickles (2008).

2.3.3.1 The peripheral auditory system

The peripheral hearing mechanism is generally divided into three distinct components: the outer, middle and inner ear, according to both anatomical position and function. Figure 2.7 shows a cross-sectional view of the auditory periphery. The primary role of the outer ear is to collect impinging sound waves and, acting as a passive sound amplifier, to 'funnel' the resulting eardrum vibrations towards the middle ear. Two ears provide us with the means for capturing two samples of sounds, allowing for a direct comparison in time and intensity – fundamental cues for sound localisation (Gelfand, 2004).

Figure 2-7 General anatomy of the peripheral auditory mechanism.

Figure has been removed due to copyright restrictions

The outer ear is composed of the pinna *(or auricle)*; external ear canal *(or external auditory meatus)* and the eardrum *(or tympanic membrane)*. It performs two different physiological functions: auditory (acoustic) and non-auditory functions (Gelfand, 2004). The main components of the middle ear system are: the tympanic cavity *(tympanum)*; the ossicles *(malleus, incus and stapes);* the middle ear muscles *(tensor tympani and stapedius)*; ligaments and the eustachian tube. The middle ear system has four functions: to protect the inner ear; to conduct, transduce and to amplify the incoming acoustic signal (Pickles, 2008). The inner ear is composed of the semicircular canals, the vestibule and the cochlea. Image reproduced from www.blockedear.net

The middle ear (Figure 2.7), acts as a mechanical transformer, modifying the acoustic spectrum of sounds by transforming air-borne sounds into pressure waves in the fluid-filled compartments of the inner ear (Jahn and Santos-Sacchi, 2001, Gelfand, 2004, Pickles, 2008). The inner ear is composed of the semicircular canals, the vestibule and the cochlea (Figure 2.7). However, it is the cochlea which houses the 'organ of hearing' and which we shall consider in more detail in our next section.

2.3.3.2 The cochlea

The basic structure of the cochlea is similar across a number of mammalian species. The cochlear canal is divided into three chambers: the *scala vestibule* and *scala tympani* – which are filled with perilymph; these areas are able to communicate via the helicotrema. The *scala media* is separated by Reissner's membrane and is filled with endolymph – a substance similar in composition to intracellular fluid. The essential components of the

organ of Corti, which is located on the basilar membrane (BM) within the cochlea, is shown in a cross-sectional view below in Figure 2.8.

Figure 2-8 Schematic of the organ of Corti

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The BM separates the scala tympani and scala media. The organ of Corti, which is attached to the BM, consists of hair cells, supporting cells and neurons that innervate these cells. OHCs have a cylindrical form, with 50-150 stereocilia arranged in 'V' or 'W' patterns. The OHC stereocilia are in contact with the Tect membrane (shown incorrectly here in this figure) and are in contact with both efferent and afferent nerve endings. Image reproduced from (Oghalai, 2004)

Displacement waves are produced by the incoming 'acoustic' vibrations and travel along on BM. As it travels, the wave – *commonly referred to as the travelling wave* – increases in amplitude, peaking at a maximum displacement and then dying out. The location of maximum BM motion is significant.

Figure 2-9 Transduction and cochlea mechanics

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The cochlea is shown here uncoiled (normally coiled structured as in the inset). Different frequencies excite different regions along the cochlea. This is shown in kHz and in humans the dynamic range is from 0.1 to 20 kHz. Images reproduced from (Fettiplace and Hackney, 2006)

(a) In the human auditory system, there are two types of sensory cells: one row of IHC and 3 rows of OHCs. IHC are flask shaped with approximately 60 stereocilia. The IHCs are the dominant transducer of sensory input, as they are innervated by a high proportion of cochlear afferents.

Incoming acoustic stimuli cause the BM to vibrate, which results in a movement of the hair cells relative to the gelatinous membrane (tectorial membrane).

(b) The number of SGC varies significantly among species but in humans is thought to number between 25000 and 30000 (Gelfand, 2004). The majority of SGC are large bipolar, myelinated (type I) neurons that synapse directly with IHCs. Approximately 20 afferent fibres innervate each IHC such that there is a 'many-to-one' connection. This is in direct contrast to the 5-10% smaller, unmyelinated (type II) neurons which make dendritic contact with 10-20 clustered OHC. The function of the type II cells is still unknown (Pickles, 2008).

Because of the unique properties of the BM, the location of the maximal amplitude displacement is a function of stimulus frequency and each cochlear site has a characteristic frequency (CF), to which it responds maximally. In essence, the cochlea performs a frequency decomposition of the incoming signal, with the result that information is sent to the brain via frequency-specific neural channels. Thus high frequency stimulus elements selectively vibrate the basal end of the membrane (near the stapes), while low frequency

acoustic energy selectively vibrates the basilar membrane at its apical end. This is shown above in Figure 2.9.

Hair cells located along the organ of Corti (Figure 2.9) are responsible for transducing these signals from a mechanical to an electrical signal (executed by IHCs) and actively altering the dynamics of the BM (performed by OHCs). Auditory nerve fibres that innervate the IHCs transmit information to the brain about the amplitude and temporal cues within only its own narrow frequency range.

A further discussion of cochlear structure and mechanics is beyond the scope of this thesis but several review papers and texts have dealt with this topic in depth (e.g. Cochlear structure and mechanics (Oghalai, 2004, Fettiplace and Hackney, 2006); for a general overview of the cochlea (Geisler, 1998, Jahn and Santos-Sacchi, 2001, Gelfand, 2004, Pickles, 2008).

2.3.3.3 The auditory nerve

The sole input pathway of auditory information from the cochlea to the CANS is via the auditory nerve (VIII CN). This is composed of three discrete groups of axons including the afferent axons of (a) the spiral ganglion cells (SGC) and (b) vestibular neurons and (c) the efferent axons of the olivocochlear bundle (Palmer, 1987, Pickles, 2008).

The afferent fibres leave the inner ear through the internal auditory canal located on the posterior surface of the petrous part of the temporal bone. They enter the brainstem at the level of the cerebellopontine angle (CPA) and terminate in the cochlear nucleus (CN).

The cell bodies of the efferent fibres are located in the SOC (in the periolivary and preolivary nuclei) (Hall, 2007). Most of these fibres synapse with outer hair cells (OHCs). A small percentage synapse with the dendrites of the afferent fibres beneath the IHCs (Nadol et al., 1990, Pickles, 2008).

The tonotopic arrangement of the cochlea is preserved in the cochlear nerve – the high frequencies are near the periphery (surface) of the nerve while the low frequencies are located deeper – thus the frequency-to-place conversion seen in the cochlea is maintained in the auditory nerve. Moreover, it is this precise temporal locking of the excitatory response to sound, particularly the onset response, which provides the neural synchrony which allows us to record far-field (from the scalp) auditory evoked potentials.

Two theories dominate the way in which the auditory system encodes the frequency: the frequency code and the place code (Gelfand, 2004). There is strong evidence to suggests that the frequency code is maximally efficient for frequencies <50 Hz whereas the place code operates at frequencies above 1000 Hz (Palmer, 1987, Geisler, 1998)[11]. There are also two main theories regarding how the auditory system encodes intensity information: the firing rates of neurons and the number of neurons that fire (Gelfand, 2004, Fettiplace and Hackney, 2006). For a comprehensive treatment of how the auditory system encodes simple and complex sound see (Pickles, 2008; Geisler, 1998; Palmer, 1987).

2.3.4 Organisation of the auditory brainstem system

"The human soundscape is characterized by complex sounds with rich harmonic structures, dynamic amplitude modulations, and rapid spectrotemporal fluctuations.

[11] Both of these mechanisms appear to play a role for frequencies between 50-1000 Hz.

This complexity is represented by an exceptionally precise temporal and spectral neural code within the auditory brain stem, an ensemble of nuclei belonging to the efferent and afferent auditory systems" Skoe et al., (2010).

The auditory brainstem consists of a series of spatially separate nuclei which include the cochlear nuclei (CN), the superior olivary complex (SOC), the lateral lemniscus (LL) and the inferior colliculus (IC).

Figure 2-10 The major auditory nuclei and pathways in the human brainstem.
Image reproduced from Moore et al., (1987).

Figure has been removed due to copyright restrictions

Figure 2.10 shows the major nuclei of the human auditory brainstem. In the following sections, we will consider the unique properties of each of these 'relay' stations and how they interact with each other.

2.3.4.1 Cochlear Nucleus

The cochlear nucleus (CN) is the first obligatory 'relay station' in the ascending auditory pathway – *all* fibres from the auditory nerve terminate here. The CN is functionally divided into three nuclei: the anteroventral CN (AVCN), posteroventral CN (PVCN) and the dorsal CN (DCN). Afferent fibres that enter CN bifurcate, with the ascending branch terminating in the AVCN and the descending branch in the PVCN and DCN. These two pathways are thought to represent different processing streams: the ventral ('where') stream and the dorsal ('what') stream. For example, the projection from the

rostral AVCN has been shown to preserves temporal data necessary for sound localisation (Palmer, 1987).

The output from the DCN is strongly influenced by spectro-temporal inhibitory interactions, which are important for identifying which (or 'what') aspects of complex sounds are important in our understanding of speech (Arnott et al., 2004). Thus the tonotopical organization that started in cochlea is well preserved in the CN.

Our understanding of the function of the CN is far from complete. The CN contains a number of principal cells which are *"characterised by their diversity"* (Palmer, 2007, p221). More than 20 cell types have been identified but the significance of these remains unclear. Examples of some of these cells are shown in Figure 2.11.

Recent cellular electrophysiological studies in the DCN have,

"revealed synaptic plasticity at synapses between parallel fibers and their targets: the principal cells (fusiform) and feedforward inhibitory interneurons (cartwheel cells) located in the molecular layer" (Tzounopoulos and Kraus, 2009, p465).

These studies are an exciting development in auditory neuroscience – challenging traditionally held views of a 'passive' auditory brainstem.

Figure 2-11 Auditory brainstem circuitry and function

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Projections to the SOC derive mainly from the AVCN and terminate in the medial superior olive (MSO) bilaterally, in the ipsilateral lateral superior olive (LSO) and in the contralateral medial nucleus of the trapezoid body. Some neurons of the superior olive send their myelinated axons via the efferent olivo-cochlear bundle (OCB) of the 8th nerve mostly, but not exclusively, to the outer hair cells (OHCs) of both cochleas, with a considerably larger contralateral than ipsilateral projection. Activation of the OCB inhibits the OHCs inhibiting their activity. Olivocochlear axons do not make direct contact with the IHCs, only with the dendrites of the type I spiral ganglion cells that synapse with the IHCs. Image reproduced from (Tzounopoulos and Kraus, 2009)

Projection axons from the DCN form the dorsal acoustic stria of Monakow. They join the contralateral lateral lemniscus (LL) and terminate in the inferior colliculus (IC). Projections from the PVCN form the intermediate acoustic stria of Held. They project bilaterally to the superior olivary complex (SOC) and to the nuclei of the LL. A few PVCN axons travel through the ventral stria (trapezoid body) and project to the contralateral IC. Projection axons from the AVCN form the ventral stria and project to the ipsilateral lateral nucleus of the SOC (LSO), to the ipsilateral and contralateral medial nucleus of the SOC

(MSO) and to the contralateral nucleus of the trapezoid body, which in turn projects ipsilaterally to the LSO (Palmer, 1987, Pickles, 2008).

2.3.4.2 Superior olivary complex

The SOC consists of several nuclei including the LSO, the MSO, the medial nucleus of the trapezoid body (MNTB) and the periolivary and preolivary nuclei. These are shown schematically in Figure 2.11. The functions of many of these nuclei are still unclear (Guinan, 2006), although the SOC is the first major convergence site for binaural information. There is strong evidence to show that the SOC has a very important role in localisation of sound by exploiting differences based on timing and intensity cues. Here we are restricted to a relatively cursory treatment of the role of the SOC nuclei in sound localisation, but detailed reviews are available see e.g. Guinan et al., (2006) and Pickles, (2008). The LSO is the largest nucleus within the SOC and plays an important role in comparing interaural level (intensity) differences (ILD) from both ears. Several distinct cell types have been identified in the LSO (see Pickles, 2008 for an overview). These cells receive excitatory input from small spherical bushy cells in the ipsilateral VCN and inhibitory input from the MNTB on the same side. The MNTB receives excitatory input from the bushy cells of the contralateral CN. Because of a number of structural features of this pathway, for example, large synaptic endings and thick axons, ensure that information arriving will coincide with arrival from the ipsilateral pathway.

There is significant evidence from cellular recordings – single cell and population studies – that the LSO responds preferentially to high-frequencies, although the lateral part

of the LSO has been shown to respond to low frequency input (Gifford and Guinan, 1983, Backus and Guinan, 2006, Guinan, 2006, 2010). For a detailed discussion of the mechanisms involved in ILD see Pickles et al., (2008).

The functions of the LSO are complemented by the functions of the MSO, a thin sheet of cells that lie medial to the LSO. The MSO is involved in detecting the interaural time differences (ITD), which is particularly useful for locating low frequency sounds. The MSO has two principal cell types which provide (mainly) ipsilateral excitatory input to the central nucleus of the IC and the dorsal nucleus of the lateral lemniscus. A model for interaural time delay (ITD) was proposed by Jeffress (1948) suggested that cells within the MSO performed coincidence detection between incoming excitatory inputs from each side. He suggested that input to the coincidence detector was delayed with respect to the other side which is supported by biological evidence to show that principal cells in the MSO lack sensitivity to ILD or the onset time delay (Palmer, 1987). Although a number of aspects of the Jeffress model are well established, activity in the MSO is difficult to study and our knowledge of this structure is limited to a small number of studies.

2.3.4.3 Lateral lemniscus

The output from the SOC joins fibres ascending from the CN to the IC to form the lateral lemniscus (LL) tract. The LL is located within the rostral pons – where the CN and pontine reticular formation (PRF) crossover – is composed of three nuclei: the dorsal (DnLL), ventral (VnLL) and intermediate (InLL) nuclei.

The VnLL and the InLL comprises part of the 'monaural' sound identification stream (Batra and Fitzpatrick, 2002). Comparative studies show that this area is poorly developed in humans (see (Moore, 1987b) for a review). The VnLL receives input from all cell types from contralateral VCN. The function of the vnLL is not entirely clear but several studies have shown that cells are sensitive to timing and amplitude fluctuations. There is no input from the LSO or MSO so it is unlikely that the vnLL is involved in sound localisation. Other studies have also shown that VnLL is thought to have an important role in the primary startle reflex pathway (Kofler et al., 2001).

The DnLL receives input from the ipsilateral MSO; ipsilateral and contralateral LSO and the contralateral CN (Stabler et al., 1996, Pickles, 2008). The DnLL is thought to constitute part of the sound localisation stream; however the exact role is unclear but is thought to be important in coding ITD. Human data is limited (Levine et al., 1993, Furst et al., 2000, Cho et al., 2005). Lesion studies have typically shown that the damage to the LL in humans has resulted in dysfunction in ITD to low-frequency stimuli (Levine et al., 1993). Corroborating evidence from animal models supports the idea that the DnLL is important in sound localisation. These studies have shown that damage to cells in the DnLL in unanesthetised rabbits resulted in difficulty localising sound especially in the horizontal plane (Kuwada et al., 2006).

The right and left dorsal nuclei of the LL are joined by the commissure of Probst. The axons of the dorsal nucleus are primarily inhibitory (GABAergic) and terminate in the ipsilateral or the contralateral IC via the commissure of Probst. Other fibres project to the contralateral dorsal nLL.

2.3.4.4 Inferior Colliculus

The majority of all ascending fibres from the brainstem synapse in the midbrain at the level of the IC. Thus the IC is thought to represent a major site of 're-convergence' whereby all information from ascending and descending brainstem structures are integrated in parallel.

The IC is composed of several distinct regions including a central area (CIC) surrounded by a belt area. The right and left CIC are connected by the commissure of the IC. The CIC receives a contralateral projection from each of the subdivisions of the CN complex; bilateral projections from the LSO and from the dorsal and intermediate nuclei of the LL and an ipsilateral projection from the MSO, the MNTB and the ventral nucleus of the LL. Binaural interaction therefore occurs almost simultaneously at several levels of the brainstem.

The belt receives afferent projections from the DnLL and the VnLL and from the VCN and DCN. Axons from the IC regroup and form the brachium of the IC. Most cells will send their axons in the ipsilateral branch of the brachium IC but a few may send it to the contralateral branch through the commissure of the IC. Figure 2.12 shows the projections from the IC to the auditory cortex in two mammals (macaque monkey and cat).

The neurons from the IC exhibit several emergent properties including sensitivity to dynamic localisation cues (sound motion) and responses that appear to be a result of convergent input from processors of localisation cues at lower levels. The sensitivity of the

IC to many complex sounds can be understood by examining the processing that takes place at lower levels of the auditory brainstem.

Figure 2-12 Projections from the IC to the auditory cortex in the macaque monkey (left) and cat (right).

A number of projections link core regions with the CIC. Image reproduced from Hackett et al., (2011).

Figure has been removed due to copyright restrictions

For example, the stellate cells of the VCN may convey directly to the IC the rate-coded spectra of complex sounds including speech. Onset cells, within the CN are specialised to signal transients in complex sounds, including cues that are related to pitch. These project to the IC, via the vnLL, which suggests a role in temporal processing. The principal cells of the DCN are sensitive to sharp spectral notches and appear to be involved in pinna-related localisation cues, which are again relayed directly to the IC, where the sign of the response is inverted. There is a large literature concerning the anatomy and physiology of the IC (for a more detailed review of this topic see Pickles (2008).

2.3.5 Medial Geniculate Nucleus

The medial geniculate nucleus (MGN), located in the thalamus is the final sub-cortical station before the auditory signals reach the primary auditory cortex (Suga and Ma,

2003, Pickles, 2008). The MGB consists of several cytoarchitecturally distinct regions which are typically subdivided into three main anatomical divisions: the ventral MGB (vMGB), dorsal MGB (dMGB) and the medial MGB (mMGB) (Schreiner and Winer, 2007, Suta et al., 2008). Figure 2.12 shows projections from the MGB to the AC in the macaque and cat models.

Cells in the MGB show a wide variety of response types; however, electrophysiological studies have shown that cells in the vMGB are primarily responsive to acoustic stimuli; as such this division is often considered to represent the 'true' part of the auditory relay station (Winer and Lee, 2007). The tonotopic relationship established in the IC, is preserved in the vMGB, thus low frequencies are presented laterally and high frequencies are represented medially. Cells in the other two divisions receive multiple inputs and are typically classed as part of the extra-lemniscal pathway with 'diffuse' and non-specific auditory responses (Schreiner and Winer, 2007). These nuclei project to the secondary areas of the auditory cortex.

2.3.6 Primary Auditory Cortex

Following a number of years of confused nomenclature, recent anatomical studies have re-structured the primary auditory cortex (PAC) into a single cohesive framework based on a core-belt-parabelt network (Kaas and Hackett, 1999, Kaas et al., 1999, Romanski and Averbeck, 2009). Auditory stimuli are thought to be analysed first in the core areas and subsequently in the belt and parabelt areas. Although there are significant

differences across species, it is believed that there are three core areas and up to as many as eight separate areas in the surrounding belt regions (Kaas and Hackett, 1999, Kaas et al., 1999, Griffiths and Warren, 2004, Griffiths et al., 2004, Pickles, 2008, Hackett, 2011). Figure 2.13 shows a schematic view of the primary auditory cortex and its association areas.

Detailed anatomical studies have investigated the functional relationships in the primate model. These studies have resulted in a functional, auditory-cortical organization analogous to the information flow in the visual system (Kaas and Hackett, 1999, Kaas et al., 1999, Romanski and Averbeck, 2009, Recanzone and Cohen, 2010). This model proposes that auditory information is processed in two parallel and hierarchical streams: one stream responsible for processing spatially-related ('where') information carried in the dorsal pathway and a non-spatial processing ('what') stream (Pickles, 2008, Recanzone and Cohen, 2010).

Figure 2-13 A human model of the auditory cortical organisation
Darkest shading indicates 'core' field including PAC which is tonotopically organised from high frequencies (H) to low (L). The core receives direct input from the auditory thalamus and

Figure has been removed due to copyright restrictions

relays that to association areas Image reproduced from Pickles (2008).

Abbreviations: AS – arcuate sulcus; CS – central sulcus; HG – Heschl's gyrus; LS – lateral sulcus; STG – superior temporal gyrus, STS – superior temporal sulcus,

Scientific evidence based on physiological studies (Recanzone, 2002) and functional-imaging data (Lewald et al., 2008) has provided strong support for the 'where' pathway. However, there is conflicting evidence for the 'what' pathway (Recanzone and Cohen, 2010). This may be a direct reflection of the degree of complexity that the original research question poses (i.e. how do we define 'what'?) or it may simply reflect differences in information processing across species, e.g. primate vs human processing (Recanzone and Cohen, 2010).

The concept of a 'dual-stream hypothesis' clearly offers a valuable framework for further study, however more detailed discussion regarding the function of these higher centres is beyond the scope of this thesis. A number of review papers have appeared over the years which have addressed this topic in detail (e.g. Recanzone and Cohen (2010); Romanski and Averbeck (2009); Smiley and Falchier (2009); Kaas et al., (1999)[12].

2.4 Measurement of the oculomotor and auditory brainstem systems

2.4.1 Examination of Saccades

[12] We have not included a discussion about the interaction pathways within auditory cortices or other sensory areas. For an excellent review of this topic see (e.g. Hackett et al., 2011).

Earlier in this chapter, we discussed the different parameters (accuracy, velocity and latency) that need to be considered when analyzing SEM (i.e. whether they are full, free and without impediment). There are many ways of assessing eye movements. Some of these are based on clinical observations and are qualitative (the clinical exam). Other methods are quantitative and objectively assess the range of ocular movements. Here, we provide a brief overview of both approaches that are routinely used to examine saccadic function. A more in-depth treatment of both these topics is available in Cassidy et al., (2000); Harris et al (1997); Leigh and Zee (2006).

2.4.1.1 Clinical examination of saccades

The simplest method to test saccades in a clinical setting or 'at the bedside' is to instruct the patient to look alternatively at two targets. These could be the examiners finger tips or two pens (Leigh and Zee, 1999a). A modified approach for assessing children, advocated by Cassidy et al., (2000) suggests using a large, bright target with the child sat on a parent's lap with their head supported. In both scenarios, the target is then suddenly presented at a known eccentricity. Several studies have shown that the targets are best presented at large angles, as abnormalities are better visualized in larger-amplitude saccades (Harris, 1997, Cassidy et al., 2000a). Table 2.6 presents a summary of routine eye movement subtests that should be undertaken for a comprehensive clinical exam.

Table 2-6 The clinical eye movement examination

1	Establish the range of ocular motility in the horizontal and vertical planes.
2	Look for nystagmus or saccadic intrusions by asking the patient to maintain a stable fixate in a central and eccentric gaze.
3	Examine horizontal and vertical saccades between two fixed visual targets, making note of initiation,

	time, speed and accuracy.
4	Look for "catch-up" saccades by examining horizontal and vertical pursuit of a smoothly moving target.
5	OKN induced with horizontal or vertical motion of a hand-held drum or tape.
6	Ocular alignment during fixation of a distant target and vergence responses to smooth or stepping motion of targets aligned in the patients' sagittal plane.
7	The VOR in response to smooth sinusoidal or sudden, head rotations in horizontal and vertical planes (Looking for corrective saccades that accompany or follow a head rotation)

Table adapted from Leigh and Zee (1999)

The advantages of the clinical exam are self evident: it is inexpensive, non-invasive and can be measured at the bedside. However, the clinical exam has several drawbacks – it can often only detect gross abnormalities and is highly dependent upon the examiner (Harris et al., 1999). For a more sensitive measure, an objective assessment of the oculomotor system is required.

2.4.1.2 Objective measurement of saccadic eye movements

The objective measurement of eye movements requires the use of sensitive equipment that allows accurate and reproducible measurements. This usually involves the detection of a signal which is proportional to the position of the eye in the orbit, and changes when the position of the eye changes in some direct way. Four approaches to recording eye movements have been developed to obtain reliable recordings. These include electro-oculography (EOG), infrared systems, image-based systems or fast frame-rate video-based techniques and magnetic search coil (Leigh and Zee, 1999a, Frens and van der Geest, 2002). We have provided a brief description of each of these techniques below in Table 2.7.

The search coil method was first pioneered by Robinson (1963). It is considered the *gold standard* for capturing the dynamic properties of saccades although it is the most invasive method – with a number of studies reporting that it is poorly tolerated after 30 minutes usage. Furthermore, several studies have shown that inserting a coil in the eye influences SEM kinematics (Frens and van der Geest, 2002, van der Geest and Frens, 2002).

EOG is the most commonly used measure to capture eye movements and when combined with video has been shown to be a very useful measure in children (Harris et al., 1992). However, it is also associated with a number of drawbacks. For example, EOG is sensitive to changes in skin impedances; DC drift in corneo-retinal potentials, electrical disturbances; EEG and EMG disturbances (Eggert, 2007). We have summarised the advantages and limitation of each of these measurements in Table 2.7. For a more in-depth coverage of this topic see (Frens et al., 2002; Eggert, 2007; Leigh and Zee, 2006).

Table 2-7 Summary of the methods used to measure quantitative eye movements.

	Description	Advantages	Disadvantages
EOG	Small voltages are recorded from around the eyes by carefully placed electrodes. Eye position is recorded based on the potential difference between the cornea and the fundus which will change as the eye position varies.	Inexpensive, simple and non-invasive method of recording large eye movements.	Signal can change when there is no eye movement. Dependent on the state of dark adaption. Affected by metabolic changes within the eye. Prone to drift and giving spurious signals
Infra-red systems	Uses photocells positioned over the limbus nasally and temporally to record horizontal eye movements. The photocells detect the amount of infrared light reflected from the surface of the eye.	Not affected by ambient lighting; good spatial resolution (the size of the smallest movement that can reliably be detected) and temporal resolutions of 1ms can be achieved.	Horizontal measurements better than vertical movements. Blinks can be a problem, as not only do the lids cover the surface of the eye, but the eye retracts slightly, altering the amount of light reflected for a short time after the blink.
Image based methods	Typically a video image is combined with computer software to calculate the position of the pupil and its centre.	Allows vertical and horizontal eye movements to be measured.	Lower temporal resolutions achieved than with IR infrared techniques. Spatial resolution can also be limited.
Scleral search coils	Small coils of wire usually copper are embedded in a modified contact lens or annulus. This is inserted into the eye after local anaesthetic has been introduced. When the subject is placed in an AC magnetic field, the position of the eye can be determined from the amplitude of the induction current in the coil.	High temporal and spatial resolution allowing even the smallest types of eye movements to be studied.	Invasive method, requiring something to be placed into the eye. Poor tolerance, other risks if the coil is worn too long include corneal oedema and abrasion. Also introducing an object in the eye, one changes the inertia (by about 5%) and the friction and therefore the force that the eye muscles have to generate to perform an eye movement.

2.4.2 Examination of the auditory brainstem system

Several measures have been used to measure the integrity of the auditory brainstem pathways. The most widely used method is the auditory brainstem response or ABR (Josey et al., 1988, Musiek et al., 1988, Stone et al., 2009). Two other objective techniques that are also commonly used in the evaluation of auditory brainstem function include the acoustic reflex threshold test (Cohen and Prasher, 1988, Cacace et al., 1991, 1992, Cohen and Prasher, 1992, Prasher and Cohen, 1993, Hood et al., 2003, Mukerji et al., 2010) and the medial olivocochlear suppression test (Prasher et al., 1994, Brashears et al., 2003, Brown et al., 2003, Guinan et al., 2003, Backus and Guinan, 2006, Guinan, 2006, Backus and Guinan, 2007, Guinan and Cooper, 2008, Lilaonitkul and Guinan, 2009, Guinan, 2010). These responses are thought to reflect activity from the auditory nerve and lower brainstem pathways. We will discuss each of these in more depth later in Chapter 3.

Our remaining discussion is concerned with the ABR. This non-invasive electrophysiological tool has been used extensively to study the human auditory brainstem system since it was first discovered four decades ago (Jewett et al., 1970, Jewett and Williston, 1971). Here we begin with a review of the important neurophysiological aspects of the ABR including a brief discussion regarding the neural generators that are responsible for the ABR. We then discuss the effect of maturation on the ABR, focusing on the relationship between the ABR and its developmental time course in the early stages of infancy and childhood. Finally, we then present a short discussion to illustrate how auditory neuroscientists are using the ABR to provide evidence for neural plasticity within auditory brainstem circuitry.

2.4.2.1 An overview of the ABR

The auditory brainstem response (ABR) is a robust far-field measurement of neural synchrony. It is evoked by the onset of a brief acoustic stimulus – typically a click stimulus – the resultant waveform is thought to reflect the synchronised activity of *onset-sensitive* units of the auditory nerve and brainstem pathways to the level of the midbrain (Gorga and Thornton, 1989, Van Campen et al., 1997). The ABR is visualised as a series of low amplitude, vertex-positive waves (ranging between 5 and 7 components) that are typically seen within the first 10 ms of the onset of a click stimulus in human adults. These peaks are generally labelled by the Jewett and Williston convention using sequential capital roman numerals although a number of different classification systems have appeared in the literature (Jewett and Williston, 1971, Hall, 2007). Figure 2.14 shows a representative ABR waveform that has been recorded to a click stimulus.

The ABR has a wide clinical application. Because the ABR is unaffected by sleep or sedation it has become a very useful measure to obtain hearing thresholds in young children and difficult to test patient groups (Stein et al., 1983, Cohen and Prasher, 1988, Jiang and Tierney, 1995, Cacace and Pinheiro, 2002, Stone et al., 2009).

The ABR has also been shown to be a sensitive marker of disease burden in the brainstem. Thus it is routinely used to identify lesions (Kaga et al., 1982, Cacace et al., 1984, Odkvist et al., 1992, Levine et al., 1993, Furst et al., 1995, Manganelli et al., 2006), tumours (Stockard et al., 1976, Cohen and Prasher, 1988, Josey et al., 1988, Musiek et al., 1988), and a diverse number of neurological diseases (e.g. excessive drinking that can result in demyelination (Begleiter et al., 1981, Nickel and Riedel, 1987, Cadaveira et al.,

1994) or other conditions that can cause increased intracranial pressure such as hydrocephalus (Kraus et al., 1984). Another application of the ABR is in intra-operative monitoring (Cacace et al., 1994, Rodriguez et al., 1999, Hall, 2007). We will revisit the issue of the pathologic ABR more detail later in this chapter (see Section 2.6).

2.4.2.2 Neural generators of the ABR

Several approaches have been used to determined the neural substrates that generate the ABR. Studies in humans have involved intracranial recordings during surgery (Hashimoto et al., 1981), source localisation studies and lesion studies – relating morphological changes in the ABR with pathologies with circumscribed lesions. There is a general consensus among these studies that the ABR components are evoked by neural activity in structures of the ascending auditory pathways, including the midbrain. Peaks I and II are thought to be generated by the auditory nerve. However, the precise anatomical correlates of the ABR after wave II remain unclear. Figure 2.14 shows a schematic summary of the anatomical location of the neural generators of the ABR.

This is considerable discussion regarding whether ABR activity is based on activity from the nerves and fibre tracts or in nuclei. Wave III is thought to represent activity from the CN although there is evidence that, like wave V, it has multiple generators. There is some evidence to suggest that wave V is generated by activity in the lateral lemniscus tract but because of the complexity of the CANS, it is unlikely that just one structure contributes to this component.

Figure 2-14 Neural generators of the ABR

Panel A. Shows a schematic of the neural substrates that are thought to generate the ABR. A comparison of the ABR from the exposed intracranial portion of the auditory nerve during microvascular decompression surgery showed that the latency of the compound action potential (CAP) corresponds to wave II of the ABR (seen in panel B) when measured concurrently (Hashimoto et al., 1981). Figure reproduced from Hall (2007)

Figure has been removed due to copyright restrictions

In animal studies, the interpretation of the ABR has been based largely on depth electrodes and lesion studies (Moore, 1987a). Comparative studies of the cat and the human brainstem pathways reveal that the auditory nerve appears to generate one main peak. This may reflect a shorter auditory nerve in some species, smaller head size or the absence of the arachnoid space (Fullerton et al., 1987). However, it is generally agreed that peak II in animals corresponds to peak III in humans and that peak IV in animals (including primates) corresponds to peak V in humans (Kraus et al., 1985, Moller and Burgess, 1986). An important caveat to these studies comes from Burkard (2008, p3444) who argues that *"application of animal data to human function is not entirely valid"* and that species specific variation must be accounted for (Burkard, 2008).

2.4.2.3 Recording the ABR

The ABR is a far-field recording, as such it is critically dependent on a number of stimulation and recording parameters such as polarity, rate, intensity level and masking to name but a few (Hecox and Galambos, 1974, Hecox et al., 1976, Kodera et al., 1977a, b, Suzuki and Yamane, 1982, Burkard and Hecox, 1983, Gorga and Thornton, 1989, Purdy et al., 1989, Hall, 2007).

The ABR is also affected by a number of non-pathological issues including age, gender and temperature (Keith and Greville, 1987, Hall et al., 1988, Jerger and Johnson, 1988, Psatta and Matei, 1988, Dehan and Jerger, 1990, Rodriguez et al., 1995, Rodriguez et al., 1999, Hall, 2007). A number of review studies examining these issues in detail have

been published over the last four decades see (e.g. Gorga and Thornton, (1989); Stone et al., 2009). We will revisit this again later in Chapter 3 (see section 3.5).

2.4.2.4 Interpretation of the ABR

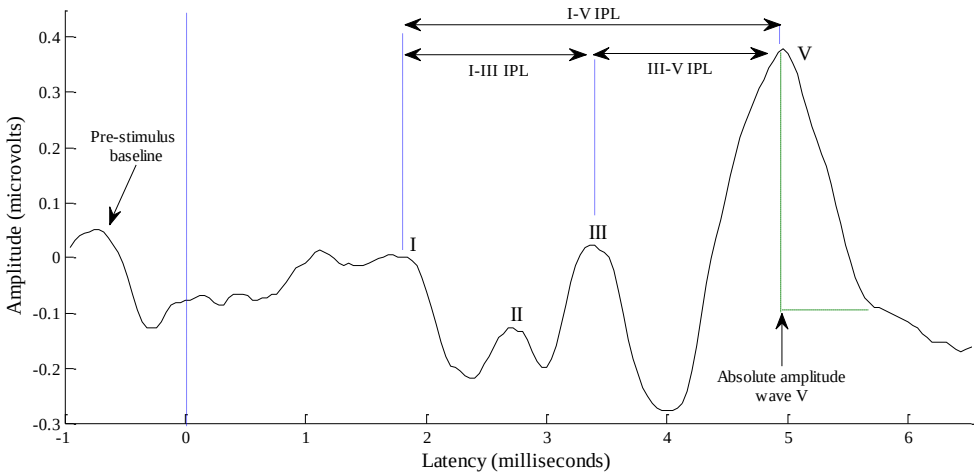
The issue of interpretation of the ABR is complicated by the fact that human auditory centres and their evoked responses are not totally interdependent. Furthermore, the determination of threshold and interpretation of ABR traces tends to rest on the subjective judgment of the tester. While there are no professionally agreed objective criteria against which findings may be judged (Gans et al., 1992, Vidler and Parker, 2004), there is a general consensus regarding the response variables that are useful for clinical and research purposes. These include latency, amplitude and morphology. Figure 2.15 shows a representative ABR waveform illustrating each of these measures and a definition for each variable is presented in Table 2.8.

Latency of different waveforms depends upon the conduction velocity in the VIII nerve, synaptic delay and the time course of excitatory post synaptic potentials in nerve cells including the hair cells. Studies exploring the relationship between pathology and ABR latency have produced heterogeneous responses across disease groups but have shown that ABR latency is a sensitive marker of subclinical damage (Cohen and Prasher, 1988, Josey et al., 1988, Hall, 2007).

Amplitude is another parameter that is used to make inferences about the integrity of the auditory brainstem pathways. Amplitude measures are thought to reflect the degree

of synchronisation and temporal coherence generated by the axons in the CANS. Amplitude is also sensitive to the number of fibres that are activated (Moller and Burgess, 1986, Hall, 2007).

Figure 2-15 Illustration of ABR wave latency and amplitude measurement
The absolute peak latencies of the various ABR peaks are often determined for clinical and research purposes. Waves II, IV and VI or VII are not as reliably present as waves I, III and V so that these latter waves are the most commonly evaluated. Measurement of the absolute wave V amplitude measure is shown in green. Abbreviations: I – absolute latency of wave I; III – absolute latency of wave III; V – absolute latency of wave V; IPL – interpeak latency.



Morphology refers to the subjective appearance, shape and reproducibility of the waveform. This qualitative measure of ABR variability is difficult to evaluate and by its very nature is descriptive, as it refers to the clarity, resolution and definition of the ABR (either in part or overall). It is influenced by a number of factors including stimulus settings (e.g. intensity and polarity); recording settings (electrode montage and the signal-to-noise ratio (Gorga and Thornton, 1989)) and subject factors (e.g. high frequency sensorineural hearing loss and neuropathology (Hall, 2007).

Table 2-8 Definition* of ABR parameters

Text has been removed because of copyright restrictions

2.4.2.5 Maturation and the ABR

During the first 2 years of life, the ABR shows a number of highly reproducible alterations in the I-V ABR complex (Salamy et al., 1975, Salamy and McKean, 1976). These changes are thought to reflect the changes in myelination of the auditory brainstem pathways that are occurring concurrently (Moore et al., 1996, Ponton et al., 1996, Moore and Linthicum, 2007) (Figure 2.16). Animal and human studies have clearly shown that wave I is adult-like (or 'complete') at birth but that later components, such as wave V is not complete until much later into childhood, around 4-5 years of age (Matschke et al., 1994, Jiang and Tierney, 1995).

Interestingly, the progressive myelination of the AC and maturation of later auditory evoked potentials is prolonged compared with the other sensory cortices which mature within the first year of life (Moore and Linthicum, 2007). The reason for this delay in development is not clear but it is well documented that the myelination process of the hierarchical auditory circuits continues well into the second decade of life (Moore, 1987a, Moore and Linthicum, 2007). The delay probably reflects both its anatomical complexity and its changing role in language acquisition.

The delay in cortical development has lead several researchers to suggest that a deficit at the level of the brainstem could adversely alter the processes required for normal cortical processing. For example, researchers have argued that deprivation-driven disruption could cause different types of behavioural deficits, depending on the phase of

development and the system affected (Purdy et al., 2002, Moore and Linthicum, 2007). This is supported by studies in children who have experienced sound deprivation because of late identification of hearing loss and who demonstrate severe difficulties affecting word learning, later in their development (Moore, 2002, Moore and Linthicum, 2007).

The concept of a bottom-up causal relationship between the midbrain and the cortex, has been used to explain how abnormalities detected in ABR studies might adversely influence cortical development in autistic children[13] (Tanguay and Edwards, 1982) and in children with language-based learning difficulties (Banai et al., 2005, Wible et al., 2005). This is further supported by animal studies that have shown that experimentally induced perinatal anoxia in rats causes progressively delayed auditory processing from the brainstem to the auditory cortex (Strata et al., 2005).

While it is tempting to postulate that some of these findings are due to a deficit in brainstem timing, this is not necessarily the case. Other factors (e.g. environmental or genetic factors) leading to abnormal brainstem timing could also cause abnormal cortical function. An alternative explanation is that the brainstem could be malleable to top-down effects[14], e.g. experience-dependent plasticity (Krishnan et al., 2005). This challenges the long held view, as summarised by Tzounopoulos and Kraus (2009), that:

"Mechanisms of plasticity have traditionally been ascribed to higher-order sensory processing areas such as the cortex, whereas early sensory processing centres have been considered largely hard-wired. In agreement with this view, the auditory brainstem has

[13] The 'Whisper of the Bang' theory proposed by Tanguay and Edwards (1982)

[14] The Reverse Hierarchy Theory (RHT) suggests that conscious perception is typically based on the highest possible representation of the stimulus along the perceptual hierarchy. This theory has been used extensively as one conceptual framework to explain the effects of top-down processing on sensory based learning.

been viewed as a nonplastic site, important for preserving temporal information and minimizing transmission delays" (Tzounopoulos and Kraus, 2009, p463)

Contrary to this view is an emerging literature to support the notion that the brainstem is capable of 'learning' (Tzounopoulos and Kraus, 2009).

2.4.2.6 Auditory brainstem plasticity: Evidence from ABR studies

The first set of observations supporting neural plasticity in the auditory brainstem have shown that long-term synaptic (Fujino and Oertel, 2003, Tzounopoulos et al., 2004, Tzounopoulos et al., 2007, Tzounopoulos, 2008) and intrinsic plasticity (Rudy and McBain, 2001, Zhang and Linden, 2003, Steinert et al., 2008) occur in some auditory brainstem nuclei These studies suggest the ability to form 'new memories' and the ability to adapt to changing demands (see Tzounopoulos and Kraus, 2009 for a recent review).

Further support for neural plasticity has come from a plethora of auditory electrophysiological studies in humans using the ABR recorded using a complex stimulus (i.e.) a synthetic speech syllable, /da/ (Chandrasekaran and Kraus, Hornickel et al., Krizman et al., Russo et al., Skoe and Kraus, Skoe and Kraus, Song et al., Strait and Kraus, Strait et al., Wang et al., Koch et al., 1999, Kraus, 1999, Kraus et al., 1999, Cunningham et al., 2000, Kraus et al., 2000, Kraus and Cheour, 2000, Kraus, 2001, King et al., 2002, Hayes et al., 2003, Nicol and Kraus, 2004, Russo et al., 2004, Banai et al., 2005, Johnson et al., 2005, Kraus and Nicol, 2005, Russo et al., 2005, Abrams et al., 2006, Musacchia et al., 2006, Song et al., 2006, Banai et al., 2007, Johnson et al., 2007, Musacchia et al., 2007, Johnson et al., 2008a, Johnson et al., 2008b, Musacchia et al., 2008, Russo et al., 2008,

Song et al., 2008a, Song et al., 2008b, Banai et al., 2009, Dhar et al., 2009, Hornickel et al., 2009a, Hornickel et al., 2009b, Kraus et al., 2009, Lee et al., 2009, Parbery-Clark et al., 2009, Russo et al., 2009, Strait et al., 2009a, b, Tzounopoulos and Kraus, 2009, Wang et al., 2009, Kraus and Chandrasekaran, 2010). These studies have uncovered new forms of learning and behavioural plasticity that are mediated by auditory brainstem structures.

Are these studies simply evidence for 'automatic sound detection'? This is unlikely as multiple lines of evidence from studies on linguistic experience (Krishnan et al., 2005) musical expertise (Musacchia et al., 2007, Wong et al., 2007, Musacchia et al., 2008) and attention (Galbraith et al., 1998, Galbraith et al., 2003) all support the existence of a dynamic brainstem. Moreover this intricate sub-cortical network has been shown to change with auditory training, reflecting a real-time transformation (Song et al., 2008b).

How these top-down influences may interact with developmental factors and brainstem disease is unclear. But other studies suggest that plasticity-induced changes in the auditory brainstem may also cause signs and symptoms of disease (e.g. tinnitus and hyperacusis (Moller, 2007, Tzounopoulos and Kraus, 2009). Taken together, these findings suggest a new role for the auditory brainstem and its modification by experience and pathology.

2.5 Pathophysiologic saccades in brainstem disease

Studies of the ABR have clearly shown that the brainstem is plastic and its importance is underscored by the consequences of disease, this is most profoundly seen in

disorders of oculomotor control. The importance of the brainstem in oculomotor control is underscored by the consequences of brainstem disease that are known to affect their function. Lesions of the pons, medulla and the midbrain can result in a myriad of oculomotor signs and symptoms, depending upon the degree of structural involvement (Harris, 1997, Leigh and Kennard, 2004, Ramat et al., 2005, Chen et al., 2011).

For example, the deficits seen in a focal lesion of the pons such as internuclear ophthalmoplegia (INO), a unilateral lesion of the MLF, can range from a complete lack of adduction of the ipsilateral eye to slow adducting saccades with full range (Harris, 1997, Frohman et al., 2008). For a more extensive review of brainstem disorders that are known to cause abnormal eye movements, see Harris et al., 1997; Leigh and Zee, (2006); Frohman et al., (2008).

In the following section, we focus on two brainstem saccadic abnormalities that are central to our thesis – slow saccades and opsoclonus. These clinically important abnormalities are associated with many conditions and represent two extremes along a continuum of saccadic eye movements.

At the one end of the spectrum is opsoclonus, characterised by a 'flurry of activity' – a striking pattern of back-to-back, high frequency saccades with no inter-saccadic interval. In some cases, excessively fast saccades have been reported in patients with opsoclonus (Bergenius, 1986). Slow saccades, at the other extreme, represent the ultimate inefficiency – decreased saccadic velocity. It has been also been argued that both of these

eye movement deficits arise because of selective cell vulnerability in the pons (Leigh and Zee, 1999b).

2.5.1 Slow saccades

Slow saccades have been described in a varied number of clinical disorders including diseases of the oculomotor muscles; the oculomotor cranial nerves and a diverse group of neurological disorders that directly (and sometimes indirectly) affect a number of highly strategic neural circuits, important in saccadic control. A list of possible causes of slow saccades is shown in Table 2.9 (Leigh and Zee, 1999b).

Slow saccades have also been reported as occurring secondary to therapeutic and toxic levels of a number of pharmacological agents including benzodiazepines, first- and second- generation antipsychotics, anti-cholinergic agents, and anticonvulsant/mood stabilising medications (Reilly et al., 2008). Several studies have also shown that non-pathologic conditions examining alertness (e.g. drowsiness or sleep deprivation) or level of attentiveness also strongly correlates with a decrease in saccadic speed (Goldich et al., 2010, Mazer, 2011).

Slow saccades coupled with limited ocular motility are usually seen in diseases that affect the ocular motor periphery, such as ocular muscle or oculomotor nerve paresis (Brazis, 2009), or a lesion of the MLF (Frohman et al., 2008). Slow saccades occurring when the oculomotor range is unrestricted are typically caused by central neurological disorder (Table 2.9).

Table 2-9 Aetiology of slow saccades*

Causes of slow saccades	Specific examples

Drug Ingestion	Anticonvulsants, Benzodiazepines
Drowsiness or fatigue	
Basal ganglia syndromes	Huntington's chorea, Progressive Supranuclear Palsy (PSP),Wilson's disease, Parkinson's (advanced cases) and related diseases; Lytico-Bodig
Cerebellar syndromes	Spinocerebellar ataxia's especially SCA2, Joseph's disease (SCA-3), Ataxia telangiectasia
Peripheral nerve palsy, diseases affecting the neuromuscular junction and extraocular muscle, restrictive ophthalmopathy	6th and 3rd nerve palsies, Miller Fisher syndrome, Progressive external opthalmoplegia (PEO), Mitochondrial myopathy, Thyroid disorders
Lipid storage disorders	Niemann-Pick disease, Gaucher disease, Tay-Sachs disease
Wilson's disease	
Lesions of the paramedian pontine reticular formation	
In dementia: Alzheimer's disease (stimulus-dependent), and in association with AIDS	
White matter diseases	Adrenoleukodystrophy, Internuclear ophthalmoplegia (common in MS)
Other	Wernicke's ophthalmoplegia, Tetanus, Paraneoplastic syndromes Amyotrophic lateral sclerosis (in some cases); Whipple's disease

*Table adapted from Leigh and Zee (1999)

An important distinction – one that is often difficult to detect clinically unless saccadic slowing is severe – is whether horizontal and vertical saccades are affected differentially. For instance, horizontal saccades are selectively slower in a number of genetic and degenerative disorders such as SCA2 (Geiner et al., 2008, Rodriguez-Labrada et al., 2011), neuronopathic Gaucher disease (Harris et al., 1999, Benko et al., 2011), INO (Frohman et al., 2008, Tilikete et al., 2011), sixth nerve palsy (Brazis, 2009, Agrawal and Singh, 2011), and in disorders of the lateral and medial ocular muscles (Yuksel et al., 2005, Yuksel et al., 2010).

Conversely, examples of disorders which affect vertical saccades (to a greater extent than horizontal saccades) include neurodegenerative disorders affecting the midbrain such

as Niemann-Pick type C disease (Wybar, 1971, Garbutt and Harris, 2000, Patterson et al., 2007), and progressive supranuclear palsy (PSP) (Chen et al., 2011), and thyroid-associated ophthalmology (Schworm et al., 2011) (Table 2.9).

However, the distinction between horizontal and vertical involvement, is not always a useful diagnostic measure. A classic example of this is ocular myasthenia gravis, an auto-immune disorder which can cause weakness in one or all of the ocular muscles, ptosis and diplopia (Barton and Fouladvand, 2000, Haines and Thurtell, 2011).

2.5.1.1 Pathophysiology of slow saccades

Several models have been developed to explain the pathophysiology of slow saccades (Leigh and Zee, 1999a, Leigh and Kennard, 2004, Ramat et al., 2005, 2007). However, the mechanism of saccadic slowing has been attributed to dysfunction in EBN cells directly, or because OPN are lost, indirectly slowing EBN firing (Leigh and Kennard, 2004).

However, it is still unclear why midbrain lesions slow only the vertical component of saccades, whereas circumscribed lesions of the PPRF result in either slow horizontal saccades selectively or a slowing in both horizontal and vertical saccades (Leigh and Zee, 1999a, Ramat et al., 2007). Another potentially confusing issue is that disturbances of higher-level structures, including the SC can also lead to slow saccades (Hikosaka and Wurtz, 1985).

2.5.2 Opsoclonus

Opsoclonus[15] is a highly dramatic oculomotor disorder characterised by chaotic, multidirectional bursts of high-frequency oscillations – these include horizontal, vertical and torsional components. The frequency of the oscillations typically ranges between 5 – 13 Hz (Digre, 1986). Each burst of oscillations consists of a series of back-to-back saccades that lack an intersaccadic interval (Gresty et al., 1980). When these oscillations are confined purely in the horizontal plane, it is known as *ocular flutter* (Leigh and Zee, 1999a). Both of these aberrant eye movements are thought to be saccadic in origin because they conform to the main-sequence relationship (Ramat et al., 2005).

Visual disturbances such as oscillopsia and vertigo have been documented in affected patients (Ramat et al., 2005). Interestingly, one study describes an increased peak velocity in saccadic activity as measured by EOG in 6/8 patients with opsoclonus or ocular flutter. This was observed in saccades in both directions in 5/8 cases (Bergenius, 1986). Other eye movements are usually preserved, although prolonged post-rotatory VN (Shawkat et al., 1993) and abnormal SP have been described (Mitchell et al., 2002). For a more in-depth treatment of this topic see e.g. Pranzatelli (1992); Wong et al., (2007); Harris (1997) and Leigh et al., (1999).

The two most common diseases that are associated with opsoclonus are viral encephalitis (Sheth et al., 1995, Cassidy et al., 2000b, Bartos and Pitha, 2003, Zaganas et al., 2007) and cancer (breast, lung and ovarian) (Anderson et al., 1988, De Luca et al.,

[15] Opsoclonus has also been variously called ‘saccadomania’ or dancing eyes. It is important that it not confused with other eye movement disorders that have been described in the literature including ‘lightning eye movements’, ocular dysmetria, ocular flutter and macro-saccadic oscillations.

2002, Hassan et al., 2008). It is often considered ‘an ominous sign’ when seen in childhood (Morad et al., 2004) as it is closely linked with neuroblastoma (and other neural crest tumours). We have summarised associated clinical conditions of opsoclonus in Table 2.10.

Table 2-10 Aetiology of opsoclonus and ocular flutter*

Causes of opsoclonus and ocular flutter	**Specific Examples**
Paraneoplastic effect of neuroblastoma and other neural crest tumours in children	
Paraneoplastic effect of other tumours (in adults)	
Parainfectious encephalitis	
Multiple sclerosis	
Meningitis	
Intracranial tumours	
Hydrocephalus	
Thalamic haemorrhage	
Associated with systemic disease	AIDS, Coeliac disease, Viral hepatitis, Sarcoidosis
Following allogeneic hematopoietic stem cell transplantation	
Metabolic conditions	Non-ketotic hyperosmolar coma
Toxins	Herbal remedies, Chlordecone, Organophosphates, Strychnine, Thallium, Toluene
Medication effects	Amitriptyline, Haloperidol, Lithium, Phenytoin, Diazepam, Cocaine
Stroke	Thalamic, Pontine haemorrhage, Vertebrobasilar insufficiency
Trauma	Usually assoc with sepsis & hypoxia
Miscellaneous	Transient phenomenon of normal infants Complication of pregnancy

*Table adapted from Leigh and Zee (1999) and Wong (2007)

Acquired opsoclonus is usually seen in combination with myoclonus and ataxia which has been described variously as Dancing Eye Syndrome or Opsoclonus Myoclonus Syndrome (Pranzatelli, 1992, Dale, 2003, Baets et al., 2006, Gorman, 2010, Brunklaus et al., 2011b). We will review this disorder in more detail later in Chapter 6.

Opsoclonus has also been described in a small number of congenital cases including preterm infants (Bienfang, 1974, Hoyt, 1977, Morad et al., 2004). Although these cases are

rare and typically resolve spontaneously (by the age of 6 months), some cases are not always benign (Bienfang, 1974, Cassidy et al., 2000a).

2.5.2.1 Pathophysiology of opsoclonus

The pathophysiology of opsoclonus is uncertain. Acquired opsoclonus is thought to be an immune-mediated disorder but the neural substrates are unknown. Two mechanisms have been proposed to account for eye movement abnormalities in DES: (1) the cerebellar hypothesis; which suggests that disinhibition of the FN in the cerebellum causes the opsoclonus and (2) the 'pontine hypothesis' which suggests that the saccadic system becomes unstable as a direct result of altered synaptic weighting of burst neuron's in the brainstem (Wong, 2007).

The cerebellar hypothesis was initially derived from observations that patients with acquired opsoclonus also typically demonstrate signs of ataxia. This has led to researchers arguing that opsoclonus is the result of damage to the cerebellum. This hypothesis is supported by a number of histopathological and imaging studies. In a recent review, Wong (2007) presented the current evidence to support 'disinhibition' of the FN as the origin of opsoclonus. She presented data to show that damage to Purkinje cells in the DV or their inhibitory projections to the FN may cause opsoclonus by disinhibiting the FN[16].

[16] A number of other studies are presented in this excellent review paper which clearly make a strong case for cerebellar involvement (for a detailed review see Wong (2007).

However, multiple lines of evidence also support a role for the brainstem in opsoclonus. Zee and Robinson (1979) postulated that OPN are targeted in these disease processes. This 'pontine' hypothesis suggests that saccadic oscillations arise because of the changes in the synaptic weighting of saccadic burst neuron circuits in the brainstem due to disease whenever the OPN cells are inhibited (Ramat et al., 2005, 2007).

Presently, there is not enough evidence to discount either model. Further, Harris (1997) cautions that,

"it is erroneous to think of a single site for opsoclonus, but rather that structural or pharmacological 'lesions' in a distributed tecto-pontocerebello-tectal loop can lead to saccadic oscillations" (p96).

2.6 Auditory signs and symptoms in saccadic eye movement disorders?

Current eye movement models that have sought to provide an explanation for saccadic deficits: particularly those that are of key interest to this thesis (i.e.) slow saccades and opsoclonus have implicated the pons – a common neural substrate in both the auditory and oculomotor circuitry – in these atypical SEM disorders (Ramat et al., 2007). The pons is also an important structure in generating the later components of the ABR. One question naturally emerges – *is the ABR abnormal in any of the aetiologies that are associated with slow saccades and opsoclonus? (Hall, 2007).*

We previously argued in Chapter 1, that the auditory pathway is closely related to key motor and sensory tracts. As a result of these shared or co-located neural substrates, it is plausible that a brainstem lesion affecting the one sensory pathway such as abnormal eye

movements could also result in co-morbid dysfunction in other sensory modalities such as the auditory system.

We explored the scientific literature to see whether both signs and symptoms have been reported in conditions. Tables 2.11 and 2.12 present a summary of selected literature for aetiologies which are associated with slow saccades and opsoclonus respectively. Abnormal ABRs have been reported in many cases but there have been no systematic study. Moreover, we found several post-mortem studies to show widespread brainstem degeneration involving oculomotor and auditory centres, but few studies that had examined both modalities.

Clearly there is some evidence of auditory neural involvement in children that also have slow saccades and opsoclonus, but auditory function has not been defined. In summary, there is some evidence to support an association between – clearly has not been explored in any depth. In the following chapters we will examine this issue in two specific conditions: Gaucher disease and Dancing Eye Syndrome and explore the utility of audiological measures in these conditions.

Table 2-11 Summary of selected studies of the ABR in clinical disorders which are also associated with slow saccades

Aetiology of Slow saccades		Study	n	ABR findings		Comments	Multimodal Study?
				Peak	IPL		
Basal Ganglia Syndromes	Huntington's chorea	(Ehle et al., 1984)	12	N	N	-	–
		(Bollen et al., 1986)	20	N	N	Abnormal blink reflex	–
		(Lin et al., 2011)	19	V	V	ABR abnormal consistent with peripheral hearing loss. Authors concluded that hearing loss thus appears to be an symptom of HD	–
	Progressive supranuclear palsy	(Pakalnis et al., 1992)	8	A	A	Minor abnormalities in ABR	–
		(Laffont et al., 1991)	17	V	V	9/17 patients had normal ABR; 4/17 patients showed an abnormal III wave; 3/17 patients showed an abnormal V wave. One patient poor ABR morphology	–
	Parkinson's (advanced cases) and related diseases; Lytico-Bodig	(Yylmaz et al., 2009)	20	A	A	PTA results were significantly elevated for PD patients at 4 and 8 kHz. PD patients also showed significantly increased latencies in wave V and I-V interpeak latencies	–
		(Al-Bunyan, 2000)	54	A	N	Prolonged wave I and V in the PD group, normal IPLs	–
Cerebellar syndromes	Spinocerebellar disease SCA17	(Manganelli et al., 2006)	9	A	A	Generally dysmorphic waveforms	–
	SCA6	-	10	A	N	Delayed wave I consistent with peripheral impairment in the auditory pathway	–
	SCA3 (Machado-Joseph disease)	Kondo et al. (1990) (hall p397	13	A	A	ABR was abnormal in 8 cases; only wave I present in 5 cases	?

Aetiology of Slow saccades		Study	n	ABR findings		Comments	Multimodal Study?
				Peak	IPL		
	Ataxia telangiectasia	(Scarpini et al., 1996)	6	V	V	Abnormal ABR in 2/4 cases	–
Peripheral oculomotor nerve or muscle weakness	Miller Fisher syndrome	(Ogawara et al., 2002)	1	A	N	Prolonged wave I	+
		(Durand et al., 2001)	10	N	N	Normal ABR in all cases – authors found no evidence to support any brainstem abnormality in any MF case.	+
Metabolic disease	Niemann-Pick disease	(Aisen et al., 1985, Pikus, 1991, Higgins et al., 1992, Lossos et al., 1997)	8	A	A	Diverse number of abnormalities	+
	Gaucher disease	-		A	A	We review the ABR in GD in detail later in Chapter 4	–
	Tay-Sachs disease	(Bembi et al., 2006)					–
	Wilson's disease	(Das et al., 2007)	18	A	A	Abnormal ABR in 61.5% cases	+
		(Hsu et al., 2003)	30	A	A	Prolonged interpeak latencies of brainstem auditory evoked potentials III-V, I-V in all of the neurological cases (n=12); but only 16.6% abnormal ABRs in non-neuroolgical cases	
		(Fujita et al., 1981)	6	V	V	Abnormal ABR in 3 symptomatic cases. ABR was normal in asymptomatic cases	
		(Chu and Yang, 1987)	20	V	V	Statistically significant prolonged I-V in WD group	
Lesions of the paramedian pontine reticular formation	One and a half syndrome	Koyama et al. 1998 (10078040)	1	A	A	ABR findings indicated abnormalities in the pontine tegmentum	+
	'Vertical' one and a half syndrome	Gulyas et al. 2006 (16786714)	1	N	N	Normal ABR	+
	One-and-a-half syndrome ,	Andree' et al. 1989	1	A	A	Prolonged ABR latency	+

Aetiology of Slow saccades		Study	n	ABR findings		Comments	Multimodal Study?
				Peak	IPL		
	possibly secondary to multiple sclerosis	(2619617)					
Dementia	Alzheimer's disease (stimulus-dependent),	Gates et al. 2010 (21150347)	313	?	?	'central presbyacusis'	–
		Gimeno-Vilar and Cervera-Paz (2010) (20112213)	14	?	?	ABR normal when corrected for peripheral hearing loss. But HL sig worse in AD group than controls	–
		O'Mahoney et al. (1994) (from Hall p408)	35	NS	A	Significant prolongation in I-V in AD group	–
White matter diseases	Adrenoleukodystrophy,	(Schmidt et al., 2001)	8	A	A	Study group was 8 female carriers for X-link ALD - all cases had abnormal IPL	
Miscellaneous	Wernicke's ophthalmoplegia	(Haas and Nickel, 1991)	21	A	A	Abnormal ABR waveforms were frequent, impossible either to quantify them or correlate them with the level of the clinical brainstem symptoms. However, IPL prolongations were a very reliable diagnostic criterion. WE cases diagnosed by the characteristic clinical syndrome with signs of disturbances of the oculomotor system, delayed IPLs, particularly a delayed CCT, were found as typical signs.	+
	Amyotrophic lateral sclerosis (in some cases)	(Radtke et al., 1986)	17	V	V	Only 2 cases showed evidence of prolonged I-V	–
		(Matheson et al., 1986)	32	V	V	Four cases showed mild ABR abnormalities	–
	Whipple's disease	(Scheurer et al., 2010)	1	NT	NT	Presented with hearing loss	–
	Tetanus	(Kagoya et al., 2011)	1	N	N	Normal ABR. Head thrust test was abnormal, with refixation saccades noted	+

Aetiology of Slow saccades		Study	n	ABR findings		Comments	Multimodal Study?
				Peak	IPL		
						with head movements both to R and L.	

Table 2-12 Summary of selected studies of the ABR in clinical disorders which are also associated with opsoclonus and ocular flutter

Aetiology of opsoclonus and ocular flutter	Clinical disorder	Study	n	ABR findings		Comments	Multimodal Study?
				Peak	IPL		
Paraneoplastic effect of neuroblastoma and other neural crest tumours in children	Dancing eye syndrome	-	-	-	-	We review this in Chapter 6	–
Thalamic haemorrhage	-	-		NR	NR	No auditory investigations	–
Associated with systemic disease	AIDS	(Matas et al., 2010)	73	V	V	Diverse ABR abnormalities reported in children and adults with HIV	–
	Coeliac disease,	(Deconinck et al., 2006)	1	N	N		+
	Sarcoidosis	(Souliere et al., 1991)	2	A	A	CPA granuloma in one case. Both cases presented with sudden onset HL. Abnormal ABR even when corrected for HL	–
Following allogeneic hematopoietic stem cell transplantation		Strober et al., (2009) Bishton et al., (2005)	1 1	NR	NR	No auditory investigations	
Metabolic conditions	Non-ketotic hyperosmolar coma	Noda et al., (1985) and Weissman et al., (1989)		NR	NR	Two single case reports - no mention of any auditory	–
Toxins	Herbal remedies	–	–	NR	NR	Animal studies only	–
	Chlordecone (Kepone)	–	–	NR	NR	Animal studies only	–
	Organophosphates	(Jayasinghe and Pathirana, 2011)	70	N	N	Serial ABRs showed no alteration in IPL over a 6 week interval	–
	Strychnine	–		NR	NR	Animal studies only	–

	Thallium	(Pelclova et al., 2009)	2	A	A	Prolonged I-V IPLwhich improved after two years; mild hearing loss in one case	–
	Toluene	(Poungvarin, 1991)	1	A	A	Sniffed lacquer polish for 5 years produced abnormal ABR inter-peak latencies	–
		(Abbate et al., 1993)	300	A	A	Occupational exposure to toluene – the ABR showed a latency delay at two different stimulation rates (11/s and 90/s)	–
Medication effects	Amitriptyline	(Rumpl et al., 1988)	2	NR	A	Two cases of amitriptyline overdose showed prolonged IPLs	–
	Haloperidol	–	–	NR	NR	Animal studies only	–
	Lithium,	–	–	NR	NR	Animal studies only	–
	Phenytoin	(Rysz and Gajkowski, 1996)	21	V	A	Serial ABRs showed prolongation of IPLs with long-term treatment	–
		(Enoki et al., 1996)	7	V	V	1/7 had prolonged IPLs	–
	Diazepam	(Adams et al., 1985)	NR	NR	A	Small but significant increase in IPL I-V	–
	Cocaine	(Tan-Laxa et al., 2004)	4	A	A	Prolonged absolute peak and IPL in prenatally exposed infants	–
		(Elkardoudi-Pijnenburg and Van Vliet, 1996)	1	N	N	Normal ABR reported – no details available.	+
Stroke	Vertebrobasilar insufficiency	(Factor and Dentinger, 1987)	8	A	A	Initial ABRs showed absence of waveforms, prolonged IPLs, and/or amplitude reduction. 5/6 patients showed reversal of ABR changes to normal; the remaining patient returned to near normal.	–
		(Lee and Baloh, 2005)	364	V	V	29 patients with VBI were found to have various abnormalities on the ABR – mainly due to sudden cochlear HL	–
	Pontine haemorrhage	(Nakane et al., 2006)	1	A	A	All waves absent except for wave I; sudden onset hearing loss also described which showed some improvement	–

Abbreviations: A=abnormal; N=normal; NR = not reported; NS = not specific; V=various; * in excess of >100 studies using the ABR; + studies investigating oculomotor and auditory findings; – no multimodal reports

Chapter 3
General Methodology

3.1 Introduction

One of the primary aims of audiological assessments is to identify hearing loss. On the surface of it, this would appear to be a relatively simple undertaking. Indeed, the suggestion of having a hearing test often evokes vague childhood memories of being told to *'press a button when a beeping sound is heard'*. While there is significant merit in employing a psychoacoustic test to characterise how well we can hear simple tonal stimuli, this simplistic view of testing hearing, *per se*, is misleading as it fails to reflect the complexity of the system we are interested in exploring. Hearing is not a unitary skill and capturing how well one hears, or more importantly what is perceived, is exceptionally difficult.

There are several approaches that have attempted to capture this information. Tests can be functionally divided on the basis of their anatomical location within the auditory pathways (see Chapter 2, section 2.3.3). These include tests of the *peripheral* auditory pathways (e.g. tympanometry measures for middle ear function and otoacoustic emissions for measures of the cochlea) and *central* auditory pathways (e.g. electrophysiological tests). More sophisticated tests assess the centrifugal (descending, efferent) auditory pathways; e.g. medial olivocochlear suppression testing is used to test the integrity of the efferent pathway in the auditory brainstem. A summary of tests that are commonly used in clinical practice, and the anatomical areas of the auditory system they are thought to index, are shown in Figure 3.1.

Another useful framework when considering the measurement of the auditory system is to distinguish between tests on the basis of their desired response. For example, a

measure requiring an overt response would be considered subjective (also referred to as behavioural), whilst an objective test would elicit an output that was independent of a behavioural response. Examples of objective physiological tests include otoacoustic emissions (OAEs) and auditory evoked potentials (AEPs); both of these are used routinely to evaluate the integrity of auditory function from the cochlea to the auditory cortex.

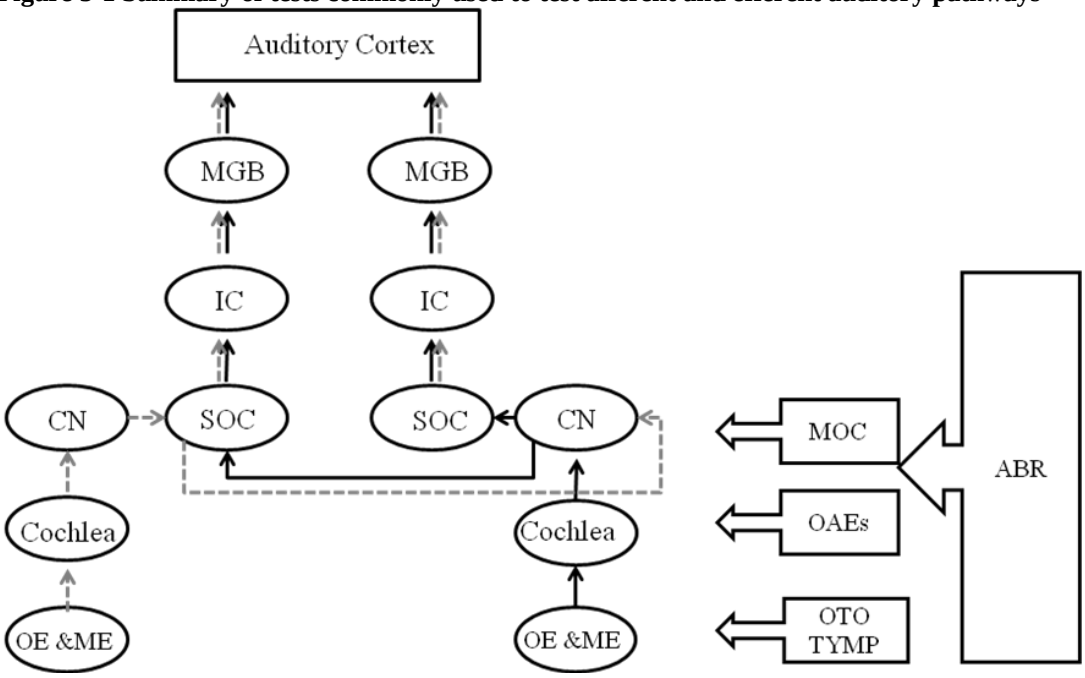
The advantages of objective measures are self evident: they are non-invasive and as we have previously described in Chapter 2, they can be used to detect a number of neurological disorders. The application of objective measures is also advantageous when measuring the auditory function in young children or difficult to test populations – particularly those that are unable to provide a response on behavioural tests. The use of combining objective and subjective tests provides a powerful method of validating or 'cross checking' results (Jerger and Hayes, 1976, Turner, 2003).

In order to assess and evaluate the extent of brainstem lesions and functionality in participants with atypical eye movements, we implemented a wide range of audiological investigations. These included both objective and subjective measures and tested overlapping, but not identical, efferent and afferent pathways and brainstem structures. The tests are described in detail below.

In the following sections, we outline the general methods that we have employed in our core experimental chapters 4, 5 and 6. We have organised this chapter into three sections: self-assessment questionnaires, subjective measures and objective measures. We

provide a brief overview of each technique and follow this with a detailed description of the procedure.

Figure 3-1 Summary of tests commonly used to test afferent and efferent auditory pathways



Abbreviations: ABR – auditory brainstem response; CN – cochlea nucleus; IC – inferior colliculus; ME – middle ear; MGB – medial geniculate body; MOCS – medial olivocochlear suppression; OAEs – otoacoustic emissions OE – outer ear; OTO – otoscopy; SOC – superior olivary complex; TYMP – tympanometry

3.2 Self assessment measures

Rosenberg (1978) describes the comprehensive case history as the 'first test' in the auditory battery of assessments. A reliable case history can often provide invaluable information about the probable site and nature of the disorder. However, due to the inherently unreliable nature of self-reporting, a number of checklists and questionnaires have been developed, to guide the patient (and interviewer) in obtaining the important details in the case history. The ability to complete the case history was dictated by the age of the participant. Older children and adults were asked to complete a face-to-face

questionnaire directly. Details about younger children were obtained directly from their parents (or guardians). The full case history form and modified listening profile questionnaire are shown in Appendix 3.

All participants were asked about their history of ear disease, neurological disease, occupational exposure to noise and organic solvents, hypertension, diabetes, history of language/learning disabilities, difficulties in understanding speech in the presence of background noise or any other hearing-related difficulty. Additional information was also sought regarding prenatal, perinatal and postnatal history in each case.

Detailed information was sought regarding each presenting symptom or symptoms. This included questions about the onset of the symptom(s); whether the symptom was constantly present or intermittent; the duration and severity of the symptom(s); did the symptom show signs of progression (i.e. did it improve or deteriorate?); was the symptom bilateral or unilateral? Finally, did the symptom(s) have any effect on the quality of life of the participant?

3.3 Behavioural audiometric measures

Earlier in section 3.1, we mentioned that one approach to differentiate between measures of auditory function is to subdivide tests on the basis of participant co-operation; i.e. is a test subjective or objective? (Turner, 2003). The different subjective, or 'behavioural', and objective measures are highlighted in Figure 3.2. Behavioural measures were focused on assessing hearing threshold and physical condition of the outer ear.

A double-walled soundproofed room (Industrial Acoustics Corporation, Staines, UK), meeting specifications for permissible ambient noise, served as the test environment (ISO8253-1, 1998). Behavioural procedures were obtained from all participants in the same session in which objective tests were performed.

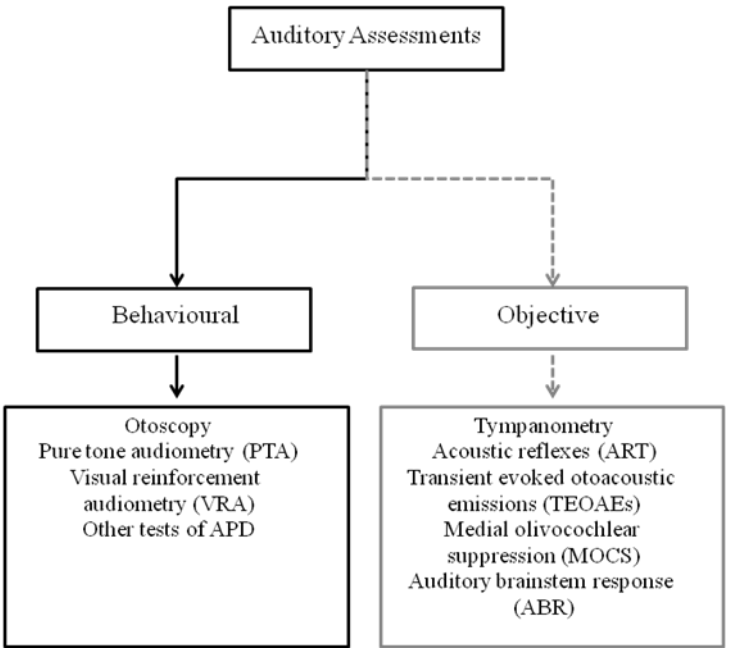


Figure 3-2 Flowchart showing the behavioural and objective measures used in this thesis

3.3.1 Otoscopy

Otoscopy is a visual examination of the outer ear. Otoscopy involves inspecting the pinna, ear canal *(external auditory meatus)* and the ear drum (tympanic membrane), to ensure that there are no pathologic alterations to any of these anatomical landmarks. For example, that there is no wax blocking the tympanic membrane which may result in an 'artificial' conductive hearing loss. Otoscopy was undertaken in all participants prior to commencement of any audiological test, following the British Society of Audiology recommended procedures (BSA, 2010).

3.3.2 Establishing hearing thresholds

The cornerstone of every audiological investigation is the psychophysical method of limits assessment, *pure tone audiometry*. This test is used to obtain minimal audible hearing thresholds across a wide frequency range, typically 0.25 – 8 kHz. Participants are asked to detect signals presented with two different transducers: 1) headphones (or insert earphones) to measure air conduction (AC) thresholds and 2) bone conductor placed on the mastoid bone, to measure bone conduction (BC) thresholds.

Data from AC testing provides valuable information about the entire auditory pathway, whereas BC bypasses the conductive mechanism (i.e.) the outer and middle ear. Comparison of the AC and BC thresholds allows for a measure of the 'type' of hearing loss (whether the hearing loss is conductive, sensorineural or mixed). These data are then plotted on an *audiogram* – a graphical representation of auditory sensitivity as a function of frequency. An example audiogram is shown in Figure 3.3.

The severity or degree of hearing loss is calculated from the AC thresholds and is generally classed as normal, mild, moderate, severe or profound (BSA, 2004) (for a more detailed discussion of the interpretation of the audiogram (see Section 3.3.2.4).

Behavioural data suggests that in younger children, responses in conventional audiometry are significantly influenced by age, gender and ear; this has implications for assessing the accuracy of pure tone audiometry. For example, several studies have shown that hearing as assessed by conventional audiometry improves with age (Orchik and Rintelmann, 1978, Rahko and Karma, 1989, Buren et al., 1992). This difference has been attributed to the consequence of both physical changes and changes in perceptibility and

reactivity of a child. (Trehub et al., 1989, Anonymous, 2000). Current international standards for hearing thresholds for air conduction audiometry in the conventional range of 0.125-8 kHz as a function of age and sex for otologically healthy persons have been published only for adults (Standardization, 1984). For age groups below 18 years no standard exists (Buren et al., 1992).

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Figure 3-3 An audiogram showing unmasked air conduction and bone conduction thresholds.
By convention, increasing intensity (which indicates a hearing loss), is plotted on the y-axis and frequency is plotted along the x-axis.
Symbols: circles indicate air conduction thresholds on the right ear, crosses = air conduction thresholds on the left ear; shaded triangles = masked bone conduction thresholds.

Therefore in this thesis, we have measured hearing sensitivity using two age-appropriate methods. For children younger than three years old, we used visual reinforcement audiometry (VRA) and for older participants (age > 3 years), pure-tone audiometry (PTA) was used to measure conventional hearing threshold levels.

3.3.2.1 Pure tone audiometry

Pure tone audiometry was measured using an audiometer (model GSI 61; Grason-Stadler, Milford, NH) recently calibrated with earphones equipped with TDH-49 cushions.

Test equipment was calibrated for frequency accuracy and sound pressure levels in the coupler by an authorised audiometric service (EMIS).

AC thresholds were measured using a modified Hughson-Westlake ascending method of threshold estimation. Participants were initially presented with a suprathreshold tone at 1 kHz in the better ear. Short duration tones (1-3 seconds) were randomly presented using an up 5dB-down 10dB technique. Briefly, the presentation tone is decreased in steps of 10 dB following a positive response, until a hearing threshold level is reached at which the subject fails to respond. Then, the tone is increased (by 5 dB), if the subject hears this increment; the tone is reduced by 10 dB. Threshold is defined as the quietest tone that a participant can hear, 50% or more of the time. Inter-stimulus intervals were also randomised throughout testing. Frequencies were tested monaurally at 0.25, 0.5, 1, 2, 4, and 8 kHz, with testing repeated at 1 kHz following the procedure recommended by the British Society of Audiology (2004). Testing began with the better ear in all participants.

3.3.2.2 Visual reinforcement audiometry

Insert earphones connected to a Grason-Stadler (GSI 10) audiometer were used to present a series of warble tone stimuli across the frequency range of 250 to 4000 Hz. Two loudspeakers were positioned at an equal distance to the right and left of the child, with the child seated on an adult's lap. Reinforcer puppets were situated above each loudspeaker. Figure 3.4 shows the two-speaker and two reinforcement unit arrangement.

Figure has been removed due to copyright restrictions

Figure 3-4 Test room arrangements used when undertaking VRA testing

Abbreviations:
L – loudspeaker,
R – reinforcer cabinet,
S – participant,
T1 – tester 1,
T2 - Tester

Before commencing VRA, the procedure was clearly explained to the parent(s) with suitable cautions about cueing the child to the presence of an auditory stimulus, and the need to minimize distracting noise. In order to obtain reliable responses, a 1 kHz warble tone was initially presented at a supra-threshold level of 60 dB HL. Visual reinforcement was presented when a clear head-turn response was observed. This is referred to as 'conditioning'.

Once two consecutive correct responses were observed, Tester 1 commenced with the test trials. Intensity was decreased in steps of 20 dB as long as responses are still observed. At the estimated threshold a '10dB down, 5dB up rule' was adopted. The criteria for accepting a threshold was 2 out of 3 responses at any level. The minimum response level at one frequency was defined before moving to another frequency.

3.3.2.3 Interpretation of hearing thresholds

Hearing was considered normal when thresholds were better than 20 dB HL in both ears (BSA, 2004). The audiometric type and degree of hearing loss were further analyzed according to guidelines published by the British Society of Audiology (BSA) and are described in Table 3.1.

Table 3-1 Description* of audiometry analysis used throughout the thesis

Audiogram	Terminology	Description
Type	Sensorineural hearing loss	Average air-bone gap of less than 15 dB for 0.5, 1 and 2 kHz
	Conductive hearing loss	Normal BC thresholds and average air-bone gap of 15 dB or more for 0.5, 1, and 2 kHz
	Mixed hearing loss	BC threshold greater than 20 dB HL in combination with averaged air-bone gap 15 dB or more for 0.5, 1, and 2 kHz
Degree	Normal	Thresholds were better than 20 dB HL in both ears
	Mild	Thresholds were measured between 20-40 dB HL in both ears
	Moderate	Thresholds were measured between 40170 dB HL in both ears
	Severe	Thresholds were measured between 71-90 dB HL in both ears
	Profound	Thresholds were > 90 dB HL in both ears

*These definitions are derived from BSA (2004) document

3.4 Objective audiometric measures

3.4.1 Clinical measurement of acoustic immittance

In order to determine how efficiently the middle ear transmits acoustical energy, a number of *indirect* measurements are made based on how efficiently a low frequency tone is transmitted. This is called acoustic immittance. The resultant data provides information about the 'likelihood' of a specific condition or can be used as part of a differential

diagnosis. Collectively this data can be used to provide invaluable information about the middle ear, cochlea, VIIIth CN, brainstem and facial (VIIth) nerve integrity.

The clinical measurement of acoustic immittance is based on three components: static compliance, tympanometry and stapedial reflex thresholds [17]. Because static compliance has limited clinical value[18], only tympanometry and stapedial reflex thresholds are described, below.

Both of these measures were recorded using the same equipment: a v2 GSI-33 Middle Ear Analyzer (Guymark, UK). Using the manufacturer supplied test cavity, the calibration of the ml/mmho meter and graphic display at 0.5cc, 2.0cc and 5.0cc was performed at the start of each day. A qualitative check of each test mode using the ipsilateral and contralateral probe was also completed daily. The middle ear analyser was calibrated annually by an independent contractor (EMIS).

3.4.1.1 Tympanometry

Tympanometry is an objective technique for determining how the compliance of the middle ear system changes in variation to the air pressure on the tympanic membrane. A probe tone is introduced into a hermetically sealed ear canal by means of a loudspeaker located with the probe box. The probe tone selected is age-dependent – in subjects older than 6 months a low probe tone of 226 Hz is selected because the normal middle ear system

[17] In the literature, the acoustic reflex threshold (ART) has also been called the middle ear muscle reflex, or the stapedial reflex threshold. In this thesis we will refer to it as the acoustic reflex threshold (ART).

[18] Static compliance is considered a weak component of the immittance test battery because of the wide range of variability shown in normal individuals.

is a ‘stiffness dominated’ system (Meyer et al., 1997). The intensity of the probe tone is monitored via a microphone, also located within the probe box.

The measurement is carried out by introducing controlled degrees of both positive and negative air pressure into the closed cavity of the external ear canal. As the pressure within the ear canal is varied, the tympanic membrane is subjected to varying degrees of stress which alters its mobility. Maximum mobility occurs when the pressure on both sides of the tympanic membrane are equal. Changes in tympanic membrane mobility (e.g. otitis media or ‘glue ear’) will result in a change in the probe tone level within the ear canal.

The tympanogram is a graphical representation of the relationship of air pressure in the ear canal to impedance (resistance to movement) of the ear drum and middle ear system. This is plotted on calibrated x- and y-axes; where the x-axis represents the change in differential pressure across the tympanic membrane and the y-axis represents the mobility or admittance of the middle ear system. The shape of a tympanogram is altered in some predictable ways by various ME abnormalities, which are summarised in Table 3.3.

Tympanograms are classified in one of three ways (Table 3.2). Firstly, it can be described according to the shape of the function – whether it is ‘peaked, flat, rounded etc. Secondly, where the location of the compliance peak is obtained and finally according to the difference in compliance between the value at +200 (daPa) (or mmH_20) and that at the peak (static compliance).

Table 3-2 Tympanogram interpretation

Jerger Pattern	Characteristics	Pattern associated with known pathologies
A	Clear peak that occurs at 0 daPa ± 50 daPa with a base-peak compliance difference in the range of 0.3-1.6 ml	None – normal middle ear status
As	Clear peak that occurs at 0 daPa ± 50 daPa with a base-peak compliance difference in the range of < 0.3 ml	Thickened TM Adhesions Ossicular fixation
Ad	Clear peak that occurs at 0 daPa ± 50 daPa with a base-peak compliance difference in the range of > 1.6 ml	Ossicular discontinuity Hypermobile TM (usually due to widespread atrophic scarring on the TM surface)
B	No clear peak (flat)	A number of pathological conditions and technical errors can cause this pattern so need to consider the shape and the equivalent volume. Pathologies that can result in a flat tympanogram include: iimpacted cerumen, foreign bodies, middle ear effusion, tympanic membrane perforation and in-situ and patent grommets Technical errors include incorrect canal wall placement of the probe tip.
C	Clear peak that occurs at > 100 daPa, with a base-peak compliance difference in the range of 0.3-1.6 ml	Eustachian tube dysfunction

Tympanograms were recorded using a 226-Hz probe tone and a positive (+200 daPa) to negative (–400 daPa) air-pressure sweep using a slow (50 daPa/second) test speed. The first test ear was selected at random. The test was repeated if the curve required confirmation. Tympanograms were classified according to the British Society of Audiology (1992) recommendations (BSA, 1992).

3.4.1.2 Acoustic Reflex Thresholds

In a normal healthy ear, middle ear admittance will show a sharp reduction in response to a loud acoustic stimulus. This is due to a bilateral contraction of the stapedial reflex muscle[19] which results in an increase in the impedance of the ossicular chain and tympanic membrane (Mukerji et al., 2010). The acoustic reflex response depends on adequate functioning of the entire reflex arc, including the sensory receptors (cochlea), afferent neurons (NVIII), inter-neurons (brainstem – superior olivary complex), efferent neurons (NVII) and effector organ (stapedius muscle). The function of the stapedial reflex is still unclear but it is thought that it may confer some (nominal) protection of the inner ear from loud sound and have a role in identifying stimulus in continuous background noise. For a more in-depth review of this topic see Mukerji et al., (2010)

Figure 3.5 shows a block diagram of the ipsilateral (same side) and contralateral (opposite side) pathways that are thought to generate the response. It is important to note that no response is usually observed when there is a conductive loss or in cases of severe or profound sensorineural hearing loss. The known influence of peripheral hearing loss clearly underscores the need to accurately measure hearing sensitivity when interpreting the ART.

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Figure 3-5 Block diagram of the stapedial reflex arc

[19] Two small muscles are attached to the ossicles in the middle ear: the tensor tympani and the stapedius muscle. The tensor tympani is attached to the malleus near the tympanic membrane and anchored in the wall of the Eustachian tube. It is innervated by the trigeminal (fifth) cranial nerve. The stapedius muscle is attached to the posterior side of the neck of the stapes and anchored in the wall of the middle ear cavity. It is innervated by the facial (seventh) cranial nerve. In humans, only the stapedius muscle contracts to acoustic stimuli; the tensor tympani contracts to non-acoustic stimuli such as touch and pressure.

Reflex contraction following a loud acoustic stimulus causes the stapes footplate to swing outward and backward from the oval window. This limits the motion of the stapes footplate, therefore reducing the fluid motion in the inner ear. This results in an increase in resistance with which acoustic energy can be conducted through the auditory pathway. Figure adapted from Katz (1994).

The acoustic reflex response has a relatively small magnitude, typically resulting in a decrease in mobility of >0.02ml when a low frequency probe tone has been used (e.g. 226 Hz). Because the stapedial muscle contracts bilaterally, stapedial reflex measurements can be obtained either *ipsilaterally* (i.e. the stimulus is presented to the same ear where the measurements are made) or *contralaterally* (i.e. the measurements are made with the probe in one ear while the stimulus is presented to the opposite ear through a transducer).

In this thesis, the acoustic reflex threshold was measured both ipsilateral and contralateral to the stimulated ear, with pure-tone stimuli from 500 Hz to 4000 Hz and using the GSI33 Middle Ear Analyser (Guymark, UK). For ipsilateral measurements, a pulsed tone was used and a steady tone was used for contralateral measurements (Mellott, 1992).

ARTs were elicited with pure tones in the following order: 1, 2, 4 and 0.5 kHz. Intensity was started at 70 dB and increased in 5 dB steps until a reflex threshold was

observed. The intensity of the tone was increased by a further 5 dB in order to observe growth of the reflex and then decreased in steps of 5 dB until the reflex disappeared. The acoustic threshold was taken as lowest level reflex observed on descending run. The reflex threshold was judged subjectively by shape and looking for growth, although a reduction in compliance of 0.02ml or more was usually observed (BSA, 1992).

The reflex was considered elevated for responses > 100 dB HL or abnormal if they exceeded 105 dB HL at two or more frequencies or if the inter-aural threshold difference exceeded 10 dB on at least two frequencies (Cohen and Prasher, 1988). Acoustic reflexes at 4kHz were not considered as they are frequently absent. The patterns interpreted as indicating brainstem lesions were *vertical* (abnormal ART by stimulation of one ear only), *horizontal* (ART abnormal by contralateral stimulation of both ears), *inverted L* (combined vertical and horizontal) and *'full house'* (all ipsilateral and contralateral reflexes abnormal) (Cohen and Prasher, 1988).

3.4.2 Otoacoustic emissions

Otoacoustic emissions are low-amplitude acoustic responses associated with normally functioning cochlear outer hair cells (OHCs) (Kemp et al., 1990) (see Chapter 2). As a result of the active non-linear mechanical feedback, acoustic energy is returned from the cochlea into the ear canal which can be recorded if a sufficiently sensitive microphone is placed in the ear canal. The exact mechanism for the generation of otoacoustic emissions is still not clear but there is overwhelming evidence linking the presence of OAEs to the

normal functioning of the OHCs[20]. Furthermore, it seems that the efferent system allows for some regulation of this mechanism by the CANS (see e.g. Guinan et al., 2010 and Pickles, 2008 for a review).

Otoacoustic emissions can be spontaneous or evoked. Spontaneous otoacoustic emissions (SOAEs) occur without external stimulation, whilst evoked otoacoustic emissions (EOAEs) can be evoked and recorded depending on the stimulus that is selected. For example, transient evoked OAEs (TEOAEs) are evoked using a click (broad frequency range) or with a brief duration tone burst stimulus. The click stimulus evokes a wide frequency range from a healthy cochlea. Distortion product OAEs (DPOAEs) are evoked using a pair of primary tones f_1 and f_2 with particular intensity and ratio (f_1: f_2) (Robinette and Glattke, 2007).

We chose to employ TEOAEs over DPOAEs for several reasons, namely: sensitivity, frequency resolution and speed. Although DPOAE technology has the advantage of superior detection of high frequency OAEs, there are several disadvantages in using it. Firstly, it suffers from lower frequency resolution and lower noise immunity at low frequencies, relative to TEOAEs. This is an important consideration when measuring OAEs in children. A more serious practical drawback is the dependence of DPOAE on the precise stimulus configuration (frequency and level ratios); for further discussion and a review of this topic see Kemp et al., 1990 or Robinette and Glattke, 2007.

[20] Evidence for this includes (1) OAEs still recorded even when VIIIth nerve is severed or blocked chemically, (2) OAEs reverse in polarity along with the stimulus (unlike neural responses) and are affected by stimulus rate (3) OAEs are vulnerable to acoustic trauma, hypoxia, and ototoxic medication which cause hearing loss and assoc damage to OHCs

3.4.2.1 Transient Evoked Otoacoustic Emissions

The ILO v6 otoacoustic emission analyser (Otodynamics Ltd) was used to generate test stimuli and record the TEOAE responses. The ILO v6 is a dual channel machine which allows the presentation of two independent stimuli. Channel A provides for a stimulus output and a response input while channel B provides stimulus output only. Standard TEOAE probes containing a loudspeaker and a microphone were used in each channel.

Transient evoked otoacoustic emissions (TEOAEs) were recorded and analysed using the method proposed by Kemp and colleagues (Kemp et al., 1990). During the initial insertion of the probe (pre-collection mode), a series of stimuli were presented, and the stimulus spectrum was derived. If there was substantive ringing in the waveform, stimulus spectrum amplitude was low or uneven across the frequency range, or if the OAE analyser warned of poor probe fit, then the probe tip was repositioned or changed prior to retesting (Norton and Widen, 1990). The probe was calibrated daily using the manufacturer supplied 1 cm^3 calibration cavity and software.

The emission data were obtained using the preset mode with a low-cut filter and a stimulus band width of 500 to 5000 Hz. The stimulus intensity across ears ranges from 75 to 85 dB peak SPL with 50 clicks per second. This type of click stimulus is ideal for clinical investigations and capturing low frequency OAEs. The IL0 v6 system was set in nonlinear click mode, in which responses to sets of four clicks are subaveraged and alternately sent to two different buffers. The non-linear stimulation and signal processing

help eliminate stimulus artifacts from the TEOAE response. After 260 subaverages were collected on each buffer, the test was complete and was stopped automatically (Kemp et al., 1990). During testing, the stimulus is repeatedly presented to the ear. Recordings of this type of OAEs use a system of time-synchronous averaging. As a stimulus is presented to the ear, the sound in the ear canal is sampled and with continued presentations, new samples are averaged with previous samples so that each OAE response is the same. The rationale for this system is to reduce any noise or artefacts which are not related to the ear and improve the signal to noise ratio.

The presence of normal OAEs in the 2.5 to 20ms post stimulus period was determined by an overall response amplitude signal-to-noise ratio of at least 3dB and waveform reproducibility in at least three octave bands of >75% (Hurley and Musiek, 1994). At least three of the frequency bands were required to be robust to pass this criterion.

3.4.2.2 Medial olivocochlear suppression test (MOCS)

Medial olivocochlear suppression (MOCS) testing provides a non-invasive window to allow the study of the centrifugal pathways in the auditory brainstem (Guinan, 2010). Suppression occurs as a result of activity of the medial efferent pathways descending from the olivocochlear bundle to the OHCs (Chapter 2). Figure 3.6 is a simplified representation of the pathways that are implicated in the MOCS response.

Figure 3-6 Block diagram showing the olivocochlear reflexes to one frequency region in the right cochlea*.

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Abbreviations: Dashed lines indicate pathways that have not been conclusively identified or might contain additional unidentified interneurons. Some MOC and LOC collaterals are not shown. OHCs, outer hair cells; IHC, inner hair cell; AN, auditory nerve; LOC, lateral efferents; MOC, medial efferents.* Figure reproduced from Guinan et al., (2006).

MOCS is measured clinically by comparing OAEs obtained with and without introduction of a suppressor stimulus. The suppressor stimulus can be presented ipsilaterally, binaurally or more commonly as shown in Figure 3.7, in the ear contralateral to the OAE stimulus.

The suppression of OAEs refers to the reduction of amplitude of acoustic output measured at the tympanic membrane (Figure 3.7). The reduction in amplitude is typically small – usually between 1-4 dB SPL (Ceranic et al., 1998). It is usually seen soon after introduction of the suppressing stimulus and will last as long as the stimulus is present, disappearing with the removal of the suppressor.

Figure 3-7 A simplified representation of MOCS application and analysis*
In order to accurately measure MOCS, hearing must be greater than 30-35 dB HL with normal ME function.*Figure reproduced from www.otoacoustic emissions.com

Figure has been removed due to copyright restrictions

The functional role and clinical relevance of the efferent auditory system remains largely unknown, but current theories include: enhancement of frequency-resolving capacity and the vowel discrimination, especially in a background of noisy environment and the optimization of interaural intensity differences for high frequency stimuli (Pickles, 2008).

The MOCS is not used routinely in a clinical setting but a number of studies have applied it in clinical populations and have found that it is abnormal in auditory neuropathy (Starr et al., 1991, Starr et al., 1996, Starr et al., 2001, Hood et al., 2003); e.g.,. in cases of acoustic neuroma (Prasher et al., 1994), vestibular neurectomy (Williams et al., 1994), in

children diagnosed with auditory processing disorders (Kumar and Vanaja, 2004) and tinnitus (Ceranic et al., 1998). Of particular interest to this thesis, is recent work in children with hyperacusis that has shown an increased or 'hyperactive' MOCS response (Veuillet et al., 1999, Attias et al., 2008). A similar finding has also been shown in neurodevelopmental disorders such as autism (Khalfa et al., 2001, Hitoglou et al., 2010) and musicians (Brashears et al., 2003).

We recorded and analysed the MOCS using the method proposed by (Ceranic et al., 1998). Briefly, a dual channel otoacoustic emission analyser (Otodynamics) was used, one channel (A) for ipsilateral and the other (B) for contralateral acoustic stimulation. For ipsilateral stimulation, a linear click at 60 (SD 3 dB) SPL intensity, and for the contralateral, broad band noise (0.50-6 kHz) at 40 dB sensation level (SL), were used, applying an alternating technique, a "difference B on/off" mode, from the ILO92 software. This allows alternating recording of TEOAE responses with and without contralateral stimulation. A total of 600 sweeps were recorded, in 10 groups of 60 sweeps. The average responses were directly computed and the difference, obtained by their subtraction, represented the suppression effect.

3.5 Click evoked Auditory Brainstem Response Measurement

3.5.1 The generic ABR

Earlier in Chapter 2, we introduced the ABR and provided a detailed background regarding the neural substrates that generate each of the waveform components (Chapter 2).

We considered at some length the effects of maturation on the ABR waveform and then we systematically reviewed the scientific literature to explore the relationship between the ABR and two atypical eye movement disorders: slow saccades and opsoclonus.

However, the ABR is strongly dependent on a wide range of stimulus and acquisition manipulations, and non-pathological (subject) factors. To accurately measure and interpret the ABR depends on an appreciation of how each of these factors interacts (for more in-depth treatments of each of these factors see (Suzuki and Yamane, 1982, Gorga and Thornton, 1989, Don et al., 1996, Hall, 2007).

3.5.1.1 Stimulus factors

In order to successfully generate an ABR, a suitable acoustic stimulus must be selected. Several acoustic stimuli have been used to record the ABR including clicks, chirps, tone bursts and more recently syllable's (Dhar et al., 2009, Hornickel et al., 2009a). Here we focus our attention on the most commonly used stimulus for ABR measurement – the click stimulus.

Traditionally, the ABR is considered to be an 'onset' response. As such it is best generated when recorded with a very brief acoustic (transient) stimulus (i.e. a stimulus with a fast rise and fall time). This rapid onset ensures neural synchronicity. A click stimulus is produced by passing a brief square wave through an earphone. This results in a broad frequency spectrum with null value at frequencies that are dependent on the duration of the square wave. The flatness and upper frequency cut-off of the spectrum are determined by the transfer function of the transducer.

A myriad of studies have shown that the properties of the click stimulus – such as frequency, duration, intensity, rate and polarity – exert a profound and often inter-related effect on the ABR. A summary of each of these factors and their effects on the ABR is presented in Table 3.3.

For the majority of our experimental work (see Chapters 4-6), the ABR data was collected using an electrical click of about 100μS; with alternating polarity to minimise stimulus artefact (the effects of stimulus polarity on the ABR recording are considered in more depth in Chapter 7). Stimuli were presented at rate of 11.1 per second. This rate of stimulus presentation was selected for two reasons. Firstly, to ensure clarity of the waveform, which is paramount in our interpretation of the ABR. Secondly, because the waveform definition in our study population may be compromised by the presence of brainstem disease; a slower rate helps to recruit the greatest aggregate of VIIIth nerve fibres, improving neural synchrony.

Click stimuli were generated by 100μs rectangular voltage pulses delivered monaurally through an insert earphone (EAR-3A). The duration of this stimulus was selected because some studies have shown that stimuli with shorter durations can result in a reduction in intensity, by as much as 13 dB (Gorga and Thornton, 1989). Insert earphones were

Table 3-3 Summary of selected stimulus factors that influence the ABR
*The definitions presented in this table have been compiled from Hall (2007)

Text has been removed due to copyright issues.

used where possible (unless indicated otherwise) for a number of reasons including increased interaural attenuation, increased patient comfort and an overall reduction in transducer ringing.

Overall, we were not concerned with using the ABR as a marker of hearing thresholds – these were measured using a number of other hearing tests previously, as described in sections 3.3 and 3.4. Therefore, stimulus intensity levels presented for air conduction were 80 and 90 dB nHL. These suprathreshold intensity levels were selected in order that the ABR would reflect maximal synchronous discharge (i.e. so that, where possible, all waveform components would be elicited, reflecting optimal auditory ability, see Table 3.3).

Stimulus intensities were calibrated relative to the averaged behavioural threshold of 20 normal hearing adults following the recommended procedure outlined by (Sininger, 1992). A broadband masking noise of 35-45 dB nHL was applied to the non-test ear to prevent a response from the non-test ear due to possible stimulus cross-over from the test ear. This was an important consideration in our study given the high stimulus intensities that were used to evoke the ABR (Burkard and Hecox, 1983, Burkard et al., 1990a, 1990b).

3.5.1.2 Recording (acquisition) factors

To measure ABR, a series of stimuli are presented to the ear at a constant rate by a transducer. The electrodes pick up the neural response, which is amplified, filtered and then averaged by a computer. Continuous recording information recorded at the scalp needs to

be converted into a non-continuous digital format. This is achieved by using an analogue-to-digital converter. The data can then be manipulated using digital filtering and/or artefact rejection (elimination of responses if too noisy). A typical ABR experimental set up is schematically represented in Figure 3.8.

Figure has been removed due to copyright restrictions

Figure 3-8 Typical ABR experimental set up.

The greatest challenge when recording the ABR is improving the signal to noise ratio. Typically the ABR signal is small – between 0.5 and 50 μV. In contrast, interference from noise (either physiological or non-physiological sources) is at least 50 μV. There are two possible sources of noise that can contaminate the ABR: physiological noise (bioelectrical activity including spontaneous EEG, muscle potentials, cardiac potentials, electro-ocular potentials, electrodermal potentials) and non-physiological noise (electromagnetic, electrostatic and instrumentation noise). It is not possible to eliminate all of these sources of noise from the signal of interest but we can overcome many of these

problems by manipulating different acquisition factors during the recording process (see Hall, 2007 for a review).

Detecting the ABR response is also influenced by electrode placement. An electrode is a device that makes contact with the body and conducts bioelectrical activity from a wire lead to the recording device. The electrodes used in the present study were disposable. These were chosen for ease of use and hygiene. Placement of electrodes is usually based on the 10-20 system (Hall, 2007).

For most of the experimental studies (see chapters 4-6), the ABR was recorded differentially from four electrodes: subject's forehead (Fpz) as ground; vertex (Cz) (non-inverting electrode); and both mastoid processes (Ml, M2) as the inverting electrode. A single recording channel (vertex and ipsilateral mastoid) was used in all ABR tests. Disposable electrodes were used, following skin exfoliation with Nu-Prep. Electrode impedances were maintained below 5000 Ω and inter-electrode impedances were balanced to within 2000 Ω in order to maximise rejection of common mode signals.

The EEG inputs were amplified by 100,000 and band-pass filtered from 150 to 3000 Hz (12 dB per octave roll-off) using a Nicolet Spirit system (Nicolet Biomedical Ltd). In order to maintain good resolution, an averaging window of 20ms and an artefact-rejection feature (a simple amplitude cut-off, or amplitude limiting, where the noise has a larger envelope than signal), set at 25 μV peak to peak, was selected.

3.5.1.3 Non-pathologic factors

In Chapter 2, we examined the influence of maturation and age on the ABR. However, a number of non-pathological subject characteristics can also significantly influence the outcome of the ABR recording. For example, distinct differences in ABR latency and amplitude have been reported for female versus male adults. Females have been shown to have shorter peak latency and larger waveform amplitudes (Jerger and Johnson, 1988, Dehan and Jerger, 1990). This is thought to be due to a combination of head size and hormonal influences. When interpreting the ABR, other 'human' factors that must be accounted for include age, hearing sensitivity, body temperature and medication. Table 3.4 presents a summary of the non-pathological factors that may impact on the ABR.

Non-pathologic factors	Effect on the ABR	Reference
Age	Older adults show an increase in ABR peak and interpeak latencies and a decrease in amplitudes despite considerable variability in hearing thresholds.	Jerger et al., (1988); Psatta et al., (1988)
Gender	Significant differences in latency reported for males and females. Males have been shown to have longer peak and inter-peak latencies and smaller amplitudes than females.	Dehan et al., (1990); Keith et al., (1987)
Hearing sensitivity	With increasing hearing loss, ABR components have been shown to increase in latency and decrease in amplitude. This finding is further complicated by an interaction between aging and gender.	Jerger et al., (1988);
Body temperature	Latencies of ABRs increase and amplitudes decrease with lower temperatures. The opposite is observed in cases of hyperthermia.	Hall et al., (1988); Koshi et al., (1990); Rodriguez et al., (1995); Rodriguez et al., (1999)
Drugs/medications	Few drugs have been reported to have any effect on the ABR. Some drugs used for anaesthesia or as a sedative have been reported to effect ABR latency. Ototoxic drugs (e.g) chemotherapy agents, alcohol etc can result in hearing loss, particularly a high frequency hearing loss and should always be checked prior to undertaking an ABR.	Hall (2007)

Table 3-4 Summary of selected non-pathologic factors that are known to influence the ABR

3.5.1.4 Interpreting the click-evoked ABR

To record the ABR, all participants reclined comfortably in an acoustically and electrically shielded booth. In order to help minimise the effects of any myogenic noise on the ABR, participants were instructed to relax fully, paying particular attention to their neck, shoulder and jaw muscles. They were advised that they could sleep if desired and to tell the tester should any of the test signals become uncomfortably loud. No sedatives were administered during any of the recordings. Two averages were made at each test signal and the presence of reproducible components was defined. A third replication was carried out if there was any uncertainty in the result.

Three measures are commonly used when interpreting the ABR – latency, amplitude and morphology. These measures are defined and quantified as follows:

- Latency refers to the length of time (usually specified in ms) between the introduction of the stimulus and the elicitation of the response. In this thesis, we report two measures of latency: a*bsolute latency and interpeak latency*.

- Amplitude refers to the magnitude (usually specified in μV) of each waveform component. In this these we report the a*bsolute peak amplitude*.

- Morphology refers to the overall appearance, shape and reproducibility of a waveform.

Each waveform response represents an average of 2048 stimulus presentations over a 20-ms analysis window. A normal ABR was determined by the examination of absolute latencies of waves I, III and V, and inter-wave latencies of I-III, III-V and I-V. The amplitude of waves I, III and IV/V (measured from the most positive peak of the fused IV/V complex to the vertex negative peak after wave V) were also measured.

Chapter 4
Characterising the 'audiological phenotype' in children diagnosed with Gaucher Disease

4.1 Introduction

Lysosomal storage diseases (LSD) are a group of rare disorders characterised by a deficiency in a single lysosomal enzyme or essential cofactor. This deficiency results in the abnormal accumulation of one (or more) natural compounds within the lysosome. Although individually rare, these 'orphan' diseases collectively represent a group of more than 40 inheritable disorders – affecting as many as 1 in 5000 births (Meikle et al., 1999). As a consequence, the LSD are the commonest cause of paediatric neurodegenerative disease (Aerts et al., 2003).

The most prevalent subgroup of LSD's are the glycosphingolipidoses (GSLs). These are inherited disorders that are characterised by excessive accumulation of one or multiple (glyco)sphingolipids. This group includes gangliosidosis, Niemann-Pick disease types A and B, Gaucher's disease, Farbers disease, Fabry's disease, metachromatic leukodystrophy, multiple sulphatase deficiency, Krabbe's disease, and sphingolipid activator protein deficiency. With one exception (Fabry disease, which is X-linked) all GSLs have an autosomal recessive inheritance pattern (Meikle et al., 2006).

These disorders pose a major challenge to the clinician – most become manifest during early infancy or childhood and present with a wide array of neurological signs and symptoms. These signs and symptoms can include seizures, respiratory distress, hypotonia, loss of motor skills, regression in developmental milestones, disturbed vision and impaired oculomotor function and deafness. Many of these signs occur as a direct result of neurological damage to brainstem circuits in the auditory and oculomotor centres, for example, the exaggerated startle response (beginning between ages 3-6 months) observed in Tay-Sachs disease (Kaback and Desnick, 1993) or the abnormal

vertical saccades, which is the hallmark of Niemann-Pick type C disease (Higgins et al., 1992, Patterson et al., 2007).

Diagnosis is particularly challenging in these disorders – because the same disease can have many different presentations (phenotypes) depending to some extent on the age of onset and the stage of development of the child's nervous system. The clinical picture is further complicated by our incomplete understanding of the pathophysiological processes which trigger many of these diseases.

While there have been significant advances in treatments for this group of diseases, our lack of suitable, quantitative tests to measure the neurological status in many of these patients remains a significant barrier to the early detection and diagnosis in many cases (Whitfield et al., 2005, Cachon-Gonzalez et al., 2006, Cox, 2010a). We will revisit this issue later in this chapter (see section 4.4) and again in Chapter 5.

In Chapter 1 of this thesis, we proposed that combined multimodal measures (eye movements and audiological tests) had a wide application in baseline diagnostic assessment and longitudinal monitoring of patients in clinical trials or on treatment for many neurodegenerative disorders. In this chapter, we begin to develop this argument by reviewing recent work on eye movement and audiological investigations in Gaucher disease (GD).

Gaucher disease (GD) is the prototypical GSL caused by a deficiency in the enzyme, glucocerebrosidase. This rare disease is characterised by the accumulation of the substrate glucocerebroside (glucosylceramide, ceramide β-glucoside) in macrophages (Gaucher cells) in the spleen, liver, bone, lungs and brain. Phenotypical features are markedly varied, but symptomatic GD patients typically manifest an array

of systemic signs including hepatosplenomegaly, anaemia, thrombocytopenia, growth retardation, lung and bone disease (Grabowski et al., 2004, Vellodi et al., 2009).

The majority of patients' exhibit only systemic signs (non-neuronopathic) and are usually classified as having Type 1 disease (GD1). However, approximately 5% of all GD cases will exhibit additional primary neurological signs (neuronopathic disease). These signs include eye movement abnormalities, auditory abnormalities, myoclonus, abnormal EEG/ seizures, cognitive impairment and progressive bulbar palsy (Harris et al., 1999, Goker-Alpan et al., 2003, Goker-Alpan et al., 2005, Goker-Alpan et al., 2008). The clinical features of GD are displayed in Table 4.1.

Table 4-1 Summary of the clinical features reported in GD.

Phenotype	**OMIM**	**Primary CNS Involvement**	**Bone Disease**	**Other**
Type 1 (Non-neuronopathic)	230800	Parkinsons Disease? Cognitive impairment?	+	Splenomegaly Hepatomegaly Cytopenia Pulmonary Disease
Type 2 (Acute or infantile)	230900	Bulbar signs Cognitive impairment Pyramidal signs	–	Splenomegaly Hepatomegaly Cytopenia Pulmonary Disease Dermatological changes
Type 3 (Neuronopathic; Subacute; Juvenile)	231000	SIF PME Seizures Cognitive impairment	+	Splenomegaly Hepatomegaly Cytopenia Pulmonary Disease

Abbreviations: OMIM = Online Mendelian Inheritance in Man; PME = progressive myoclonic epilepsy; SIF = saccade initiation failure; + signs refer to the degree of involvement; - lack of involvement

Why have we chosen to study GD? There are two reasons. Firstly, the abnormal eye movements seen in nGD are considered by many clinicians to be the universal indicator of the neurological (neuronopathic) subtypes (nGD). Audiological abnormalities have also been described in nGD (Dreborg et al., 1980, Bamiou et al., 2001, Grasso et al., 2006), although there is little agreement as to whether audiological

investigations could be considered reliable tests of neurological involvement in GD (Benko et al., 2011). Clearly further studies examining the potential role of auditory studies, particularly those that test the integrity of brainstem pathways are required. Secondly, GD has been hailed as a disease with *"totemic significance for the development of orphan drugs"* (Cox, 2010, p 299). Clearly, any novel insights in this rare neurodegenerative disorder may be directly applicable to the multitude of other 'orphan' diseases.

We begin this chapter by providing a detailed clinical description of GD, paying particular attention to the abnormal eye movements which are considered a hallmark of the neuronopathic subtypes (Harris et al., 1996, Harris et al., 1999). We then outline what is known experimentally about the use of eye movement and audiological techniques from the published literature relating to the diagnosis of GD, and draw attention to those results most relevant to the thesis, before introducing our empirical data.

We consider two issues in this chapter:

1. Firstly, we ask whether *audiological tests when coupled with eye movement studies, can be used to provide reliable subclinical and pre-symptomatic tests of neurological involvement in GD?* The inclusion of such studies may provide important clues in understanding the pathophysiology in GD.
2. Secondly, we ask whether *the use of these combined tests will enable us to correctly distinguish between phenotypes.* If the tests are sensitive enough, can they help reduce the phenotype-genotype discordancy that has been extensively described in this disease?

4.2 Gaucher Disease – clinical phenotypes

Historically, GD has been subdivided into one of three possible phenotypes – based upon the age of presentation and whether or not any primary neurological signs were observed (Table 4.1). This resulted in GD being classified as either: type-1 (GD1) without primary neurological involvement; type-2 (GD2) with severe neuronopathic disease leading to death by the 3rd year; or type-3 (GD3) with less severe and more variable neurological involvement (Table 4.1).

Since one of the objectives of this chapter is to determine whether the application of auditory testing can be used to improve phenotypic identification (section 4.1), we now expand upon each of these phenotypes in more detail to provide context for our later discussion.

4.2.1 Type 1 Gaucher disease (GD1)

Type 1 Gaucher disease (GD1)[21] is the most common type of GD with an estimated prevalence of 1:200,000 (Weinreb et al., 2008). It is relatively common across all ethnic groups but several studies have shown an increased incidence in the Ashkenazim Jewish population (Diaz et al., 2000, Koprivica et al., 2000). Studies in this population have estimated an increased frequency of 1 in 5000 with a carrier frequency as high as approximately 1 in 10 (Cox et al., 2008).

GD1 is highly variable in onset and severity. Some patients may show symptoms in infancy, while others may be asymptomatic and only come to medical

[21] This phenotype has been variously referred to in the literature as 'adult' GD, non-neuronopathic GD and type 1 GD. See Table 4.1 for a description of the clinical features of this phenotype. To avoid any confusion, we shall refer to this subtype as GD1.

attention because of affected relatives (Lachmann et al., 2004, Cox et al., 2008, Cherin et al., 2010). For this reason, the reported frequency of GD1 is probably underestimated (Cox et al., 2008).

Typically this phenotype will only have systemic disease (or rarely secondary neurological problems). The most common systemic signs include splenomegaly, thrombocytopenia, hepatomegaly and bone disease (Table 4.1). Atypical bone pain, pathological fractures, avascular necrosis and bone crisis are frequently reported (Grabowski et al., 2004). Secondary neurological signs including sciatica, paraesthesia, muscle weakness, cramps and tremor have also been widely reported in GD1 patients (Pastores et al., 2003).

The traditional view of this phenotype, as one *without primary neurological involvement*, has recently been challenged. CNS signs have been reported in GD1 patients, often later in adult life (Miller et al., 1973, Neudorfer et al., 1996, Tayebi et al., 2001, Guimaraes et al., 2003, Cherin et al., 2010, Giraldo et al., 2011). Neurological signs in this 'adult-onset form' (or late-onset neuronopathic subtype), have been reported to manifest anywhere from 17 – 55 years of age (Tayebi et al., 2001, Guimaraes et al., 2003).

Atypical Parkinson symptoms (PD) have also been widely described in the literature (Alfonso et al., 2007, Biegstraaten et al., 2008, Alonso-Canovas et al., 2010, Cherin et al., 2010, Giraldo et al., 2011). This 'akinetic-rigid syndrome' was reported as co-existing with other neurological signs including cognitive impairment in 19/60 cases; myoclonus in 2/60 cases, dementia and *abnormal horizontal eye movements in 3/60 cases*. A crucial assumption underlying each of these studies is that patients were correctly phenotyped (i.e. correctly identified as having non-neuronopathic disease). As

we will show later in this chapter (See section 4.4.4.2), it is possible that many of these studies, in the absence of formal eye movement studies, had failed to correctly distinguish the GD1 patient from the GD3 patient (Harris et al., 1999).

4.2.2 Type 2 Gaucher disease (GD2)

Type 2 Gaucher disease (GD2)[22] is a significantly less common but more severe form of the disease. Estimates of the frequency of classic GD2 disease in the general population have ranged widely from 1 in 100,000 to 1 in 500,000 births (Grabowski, 1993). The onset of the disease is usually in the first year of life and the affected infant typically presents with severe systemic disease and extensive neurological impairment, particularly in the brainstem. GD2 patients are usually seen clinically as an infant who is failing to thrive and recurrent infections. Hypertonicity, organomegaly, and bulbar palsy are common clinical findings (Table 4.1). The complications arising from the relentless brainstem deterioration usually results in early demise, typically by the age of two years.

More recently, a lethal perinatal phenotype associated with ichthyosis or collodion skin and hydrops fetalis has been found (Church et al., 2004). This group of infants have little, if any, detectable β-glucocerebrosidase activity and survival is not tenable (Finn et al., 2000, Stone et al., 2000). The mechanisms contributing to the devastating neurological manifestations seen in GD2 remain unknown. However, auditory electrophysiological abnormalities have been reported in an infant as young as 7 weeks old (Lacey and Terplan, 1984). It therefore seems plausible that neurological

[22] This subtype has also been called infantile' or 'acute' GD. The more severe neonatal form characterised by foetal hydrops and ichthyosis has been called the Collodion baby. In this thesis, we shall refer to this severe phenotype as GD2.

damage may be present prenatally in these cases. This is supported by the recognition of a prenatal lethal form of the disorder (Finn et al., 2000).

4.2.3 Type 3 Gaucher disease (GD3)

Type 3 Gaucher disease (GD3)[23] is neuronopathic disease that is less severe than GD2. Systemic disease is always present, but it is often more severe than systemic signs seen in GD1 and is usually evident in early infancy. Typically the neurological progression seen in GD3 is much slower than in GD2 but the degree of neurological involvement is highly variable (Table 4.1). For example, neurological signs may initially be absent or not recognised for years, and progression may be very slow and insidious, resulting in psychometric decline, oculomotor deficits, ataxia, myoclonus, epilepsy and dementia (Dreborg et al., 1980, Beutler and Grabowski, 1995).

4.2.4 Distinct phenotypes or part of a continuum?

"These observations raise an important question: Why is there such vast phenotypic variation in this presumably single gene disorder" (Sidransky, 2004, p7)

More recently, these phenotypic distinctions have been extended and blurred in the neuronopathic subtypes, (Abrahamov et al., 1995, Stone et al., 2000, Goker-Alpan et al., 2003). Figure 4.1 shows a highly simplified representation of the clinical subtypes and the spectrum of varying phenotypical signs and symptoms that have been reported.

[23] This neuronopathic subtype has also been referred to as 'juvenile' or sub-acute' GD. GD3 has been further subtyped into GD3a, GD3b and GD3c. For a detailed review of these different subdivisions see Abrahomov et al., (1995) or Patterson et al., (1993). Throughout this thesis, we shall simply refer to this phenotype as GD3.

Figure 4-1 Proposed model of phenotypical traits across GD

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Clearly, the question arises as to whether there is a similar continuum between neuronopathic and non-neuronopathic disease (Biegstraaten et al., 2008). Systemic disease tends to be more severe in the neuronopathic subtypes than in non-neuronopathic phenotype, implying that GD1 patients may have very mild and undetected neurology (Cherin et al., 2010, Giraldo et al., 2011). This is supported by recent audiological electrophysiological studies in GD1 (Perretti et al., 2005, Grasso et al., 2006). Unfortunately, these studies fail to mention how the initial phenotype diagnosis of type-1 disease was reached, other than on 'clinical grounds' (Perretti et al., 2005, Grasso et al., 2006).

One of our core aims in this chapter is to examine whether the use of combined eye movement and audiological investigations can improve the phenotype/genotype discordancy that has been previously described in this disease.

4.3 Genotypes

In the previous section, we described a wide variation in the different phenotypes that have been reported in GD. However, we have discussed little, if anything at all, about the corresponding genotype. We begin to address this matter in this section.

The glucocerebrosidase gene was mapped to a large gene on chromosome 1 q21-23 in 1985 (Ginns et al., 1985). Since then more than 200 different disease-causing mutations have been identified (Grabowski and Horowitz, 1997, Koprivica et al., 2000). However, it is still not possible to make the diagnosis of GD by genotyping alone.

Most alleles are rare. However four mutations – N370S, L444P, 84insG and IVS2 have been frequently identified and account for the majority of cases, but there are wide ethnic variations (Grabowski and Horowitz, 1997). For example, the N370S and 84GG alleles account for over 80% of GD in the Ashkenazi Jewish population, permitting screening for the disorder in this group (see Koprivica et al., 2000 for a review), but less than 45% in German, Spanish, Portuguese and British non-Jewish populations (le Coutre et al., 1997). In Japanese patients these alleles appear to be absent (Ida et al., 1995).

In contrast, the L444P mutation accounts for about 3% of mutant alleles in Ashkenazi Jewish populations, but about 25% in non-Jewish Caucasian populations and 44% in Japanese patients (Ida et al., 1999). In the genetically isolated region of Norrbottnian in Sweden all patients are homozygotic for the L444P allele. These ethnic distributions probably reflect founder effects.

The relationship between genotype and phenotype is surprisingly discordant for reasons that are unclear, but it is clear that the extensive heterogeneity observed between patients cannot be explained by a mutation in the glucocerebrosidase gene alone. Genetic background and other environmental factors must also play an important role. Figure 4.2 presents a summary of these factors.

Figure 4-2 Schematic showing the proposed relationship between phenotypic expression and other factors that may influence disease expression in GD

Figure has been removed due to copyright restrictions

4.3.1 Genotype-Phenotype correlation?

The remarkable variability seen in phenotype expression has severely impeded the diagnostic and prognostic value of genotyping. Nevertheless, some broad generalisations can be made based on the literature. For example, several studies have shown that the N370S allele is highly correlated with a mild form of the disease. Both heterozygotes and homozygotes appear to be free from neuronopathic signs and symptoms (Grabowski and Horowitz, 1997). As such, it is thought that even a single copy of this allele may confer some degree of 'neurological protection'. Other mutations such as R463C and R496H have also been associated with 'mild' disease.

In direct contrast, the L444P allele is strongly associated with neuronopathic disease. Studies have shown that 68% of GD3 and 48% of GD2 mutant alleles are L444P (L444P/N370S appears to be non-neuronopathic). Other mutations such as V394L and D409H have also been observed in cases of GD with more severe phenotypes.

However, the relationship between genotype and phenotype in the majority of GD patients is imperfect. For example, although the majority of L444P homozygotes demonstrate neuronopathic disease, some apparent type 1 phenotypes have been reported (Kawame et al., 1993, Sidransky et al., 1994, Ida et al., 1995, Beutler and Gelbart, 1996, Hatton et al., 1997). It is possible that some patients may have been

incorrectly identified as L444P/L444P due to failure to detect a recombination with the pseudogene. However, even with more elaborate genotyping techniques, some papers have reported some L444P homozygotes as having non-neuronopathic phenotype (Germain et al., 1998).

This picture is further complicated, not only by inaccurate genotyping, but by the persistent failure to correctly establish the phenotype in the first instance (Harris et al., 1999). This is supported by recent epidemiological work which estimates that only a small proportion of GD patients (10-20%) are correctly diagnosed and treated (Niederau et al., 2001). Clearly, it is imperative to develop sensitive quantitative measures that are able to detect subtle neurological abnormalities before irreversible overt signs appear. In this thesis, we propose that combined eye movement and auditory testing may offer a solution to this long-standing dilemma.

4.4 Diagnosis of GD

Throughout this chapter, we have made mention of the diagnostic challenges that face clinicians working with these rare orphan diseases. Until now, we have concentrated our discussion solely on the varying genotype and phenotypes that have been described in the literature. At this point in the thesis, we must consider in more detail *how* GD is diagnosed and, of more relevance to this thesis, we must address the question – how do we diagnose neuronopathic GD?

Until recently, diagnosis was based primarily on a clinical examination and an invasive bone marrow biopsy (Brady and Schiffmann, 2004). The presence of Gaucher cells in the biopsied sample, was considered confirmatory of the disease. Misdiagnosis

was frequent, as cells masquerading as Gaucher cells (pseudo-Gaucher cells), were also reported in a number of other conditions (Bain and Lee, 2010).[24]

The definitive diagnosis of GD is now made using relatively non-invasive blood tests measuring levels of glucocerebrosidase activity in leucocytes or alternatively from skin fibroblasts (Grabowski et al., 2004, Vellodi et al., 2009). Lowered glucocerebrosidase activity (between 0 and 30% of normal values) is considered diagnostic of GD. Other testing (e.g. DNA testing) is also routinely measured in asymptomatic homozygotes and carriers in close relatives of affected individuals (Vellodi et al., 2009).

4.4.1 Diagnosing neuronopathic disease

Although a simple blood test is now all that is required in order to diagnose GD, there is *no evidence* to show that this biomarker is able to differentiate between non-neuronopathic and neuronopathic phenotypes (Vellodi et al., 2009). Identifying the correct phenotype is based partially on genotype and partially on phenotype but as we have already remarked upon earlier in this chapter, there is limited value in this approach.

The clinical hallmark of neuronopathic disease is early brainstem disease (Table 4.1). Signs of brainstem involvement can include oculomotor abnormalities, dysphagia resulting in feeding problems, apnoeic episodes, laryngeal stridor due to bulbar palsy and abnormal auditory brainstem responses (Schiffmann et al., 1997, Goker-Alpan et al., 2005, Vellodi et al., 2009). The most widely accepted phenotypic indication of

[24] Pseudo-Gaucher cells have also been described in sickle cell anaemia, chronic granulocytic lymphoma, and Hodgkin's disease.

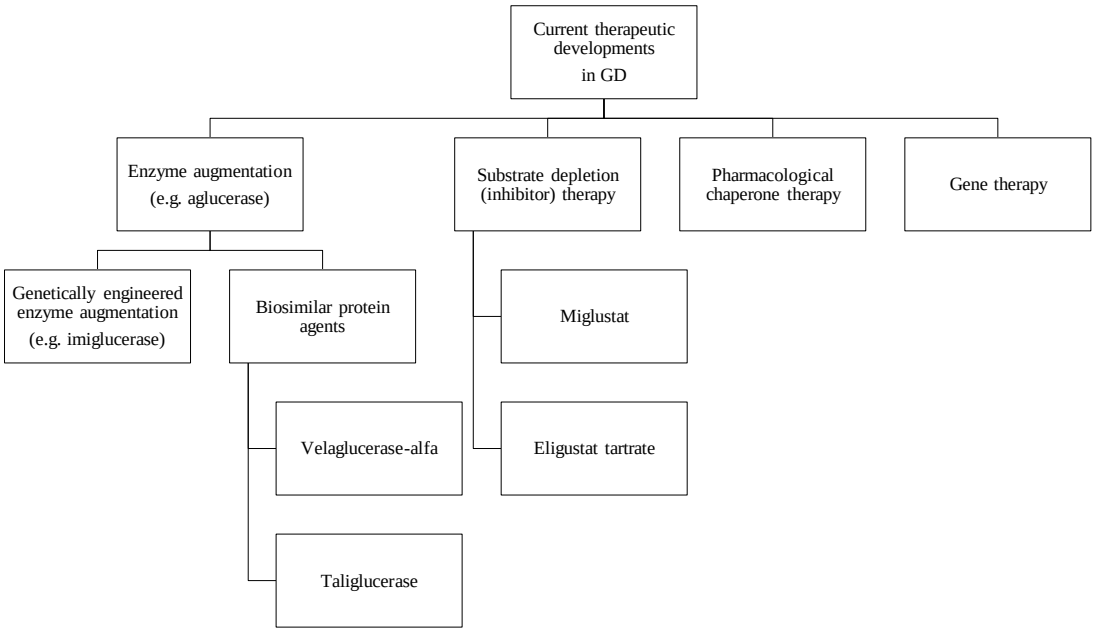
neurological involvement is the presence of specific eye movement abnormalities (Schiffmann et al., 1997). However, as Harris and colleagues (1999) have shown, it is all too common for eye movement abnormalities to remain undetected, resulting in patients with GD3 being erroneously labelled as having GD1. The long term consequences of these misdiagnoses are as yet unknown[25].

4.4.2 The importance of correct phenotyping in GD

Major advances have been made in developing treatments for GD. As a result of this remarkable progress, GD has become a prototype for treatments for a number of related orphan diseases (Elstein, 2011). Figure 4.3 presents a summary of the currently available therapies that have been developed or are currently undergoing clinical trials. The introduction of enzyme replacement therapy (ERT) (Cerezyme®) – a recombinant human glucocerebrosidase expressed in genetically engineered Chinese hamster ovary cells – was at the forefront of this revolution. Prior to the availability of ERT in the early 1990s, no specific or curative treatments were available for GD.

Figure 4-3 A summary of current treatments that have been developed or are in development for use in GD.

[25] There is a substantial literature that has recently been published following the contamination of one of the sites responsible for manufacturing ERT. This dramatic event saw world-wide rationing of ERT. It is possible that these studies may allow us a 'window' to examine this issue further (see e.g. Cox et al., 2010; Hollak et al., 2010 for a review).



Hailed as a '*pharmaceutical blockbuster'* by many investigators, ERT has been shown to clearly reverse many of the systemic manifestations of GD, but its therapeutic effect on the neurological component is much less certain (Cox and Schofield, 1997). In vitro studies have shown that the exogenous enzyme should work, but there is limited human data to support this (Pelled et al., 2000). A more recent therapeutic development is substrate depletion using N-butyldeoxynojirimycin (OGT 918; Zavesca®; (Platt et al., 1994, Platt and Jeyakumar, 2008). Clinical trials of Zavesca® have shown significant amelioration of hepatosplenomegaly and haematological manifestations in GD1 patients (Cox et al., 2000). It is still not known whether it can reverse the neurological deficits in nGD. We will address this issue in more depth in our next chapter (Chapter 5).

The development of these new treatments[26] has also placed an imperative on the early distinction between different phenotypes (non-neuronopathic and neuronopathic disease) to allow the appropriate treatment to commence as soon as possible. Patients with neuronopathic disease require a higher dose of ERT to slow down the neurological progression of the disease (Vellodi et al., 2001, Vellodi et al., 2009).

Treatments for GD are prohibitively expensive. As a result, it is estimated that only a minority of GD patients (10% or currently 5000 patients world-wide) have access to treatment (Cox, 2010b). The extreme cost also precludes high-dose treatment for all GD patients, making it absolutely essential to correctly identify the phenotype[27]. Scientific investment in the development of objective markers, such as saccadic and auditory measures, that allow the early accurate identification of the neuronopathic phenotype are urgently required. In the following sections, we consider these two objective assessments in more detail and critically evaluate their current utility in the diagnosis of GD.

4.4.3 Eye movement signs in neuronopathic GD

Patients diagnosed with nGD demonstrate specific saccadic eye movement abnormalities. These were first described by Reiss and Kato in 1932[28]. Since then a

[26] The development of new enzymatic preparations, velaglucerase alfa (VPRIV™) and taliglucerase alfa (UPLYSO™), as well as alternative treatments substrate reduction and pharmacological chaperones, are important additions to the management portfolio of this disease. A review of the therapies in GD is beyond the scope of this thesis but a number of excellent reviews are available (see Cox 2010).

[27] Annual average costs to treat an adult Gaucher patient with ERT is of the order of £100,000 and in the early debulking phases of the illness, about £200,000 per annum (Cox, 2010).

[28] Reiss and Kato described abnormal gaze (abduction defect or 6th N palsy) in two cases of GD.

variety of eye movement abnormalities in neuronopathic GD have been described in the literature (Herrlin and Hillborg, 1962, Kraoua et al., 2011). These include:

- Horizontal SIF (hSIF) (Schiffmann et al., 1997, Harris et al., 1999, Altarescu et al., 2001b)
- Slow horizontal saccades (Harris et al., 1999, Park et al., 2003, Goker-Alpan et al., 2005)
- Vertical SIF (especially downward) (Miller et al., 1973, Cogan et al., 1981, Garbutt and Harris, 2000)
- Slow vertical saccades (especially downward) (Miller et al., 1973, Cogan et al., 1981, Garbutt and Harris, 2000)
- Abnormal vestibulo-ocular reflex (Miller et al., 1973, Dreborg et al., 1980)
- Strabismus (Dreborg et al., 1980, Erikson and Wahlberg, 1985, Vivian et al., 1993, Bembi et al., 1994, Harris et al., 1999)
- 6^{th} N paresis (Miller et al., 1973, Winkelman et al., 1983, Erikson and Wahlberg, 1985, Vivian et al., 1993)

The combination of SIF and slow saccades are widely accepted to be a diagnostic sign of neuronopathic disease in a patient already diagnosed with GD. The onset of saccade abnormalities in unknown – in GD2, the onset is probably in utero (Stone et al., 1999, Tayebi et al., 1999, Finn et al., 2000, Stone et al., 2000, Gupta et al., 2010). The onset in GD3 is also unclear, although it appears to be very early (Cox-Brinkman et al., 2008). Clearly the earlier the onset of the disease, the more difficult it will be to accurately diagnose without the use of objective, non-invasive measures.

A number of studies have also reported that atypical eye movements are often 'precocious', preceding other neurological signs, often by many years (Harris et al., 1999, Garbutt and Harris, 2000, Accardo et al., 2010). Interestingly, ABR abnormalities have also been reported in very young nGD patients (as young as 7 weeks old in GD2; and 5/12 in GD3) (Lacey and Terplan, 1984, Cox-Brinkman et al., 2008). This makes oculomotor (and potentially auditory) testing a very useful diagnostic tool, but it also raises the important question as to why these irreplaceable brainstem circuits are so vulnerable to GD neurotoxicity.

The major abnormalities in nGD are difficulties in triggering horizontal and vertical (especially downward) saccades and a consistently severe degree of saccadic slowing. Harris et al., (1999) has argued that this combination of abnormalities almost certainly reflects brainstem dysfunction, consistent with post-mortem studies (Kaga et al., 1982, Kaga et al., 1998) and the other obvious brainstem signs seen in advanced cases of nGD (Akdag et al., Conradi et al., 1991, Patterson et al., 1993, Cox and Schofield, 1997, Michelakakis et al., 2002, Goker-Alpan et al., 2003, Gupta et al., 2010, Kraoua et al., 2011).

At this juncture in the thesis, we will limit our discussion and focus only on the most common eye movement abnormalities in GD – saccade initiation failure (SIF) and slowing of saccadic eye movements which we have discussed extensively, earlier in Chapter 2.

4.4.3.1 Saccade Initiation Failure ("ocular motor apraxia") in GD

Saccade initiation failure (SIF) is a difficulty in triggering or generating a saccade (Vivian et al., 1993, Harris et al., 1996, Harris et al., 1999, Cassidy et al.,

2000a). This rare defect has been referred to in the literature as 'ocular motor apraxia', 'supranuclear gaze palsy', 'looping', or 'saccade initiation failure' (SIF) (Harris et al., 1996, Harris et al., 1999).

In neuronopathic subtypes, the triggering mechanism responsible for initiating horizontal eye movements (h-SIF) appear to be preferentially affected, making it extremely difficult for the child to shift their gaze along the horizontal plane (i.e.) from left and right. Although the horizontal and vertical saccade centres are anatomically remote from each other, a number of studies have also shown that vertical eye movements (particularly downward SIF) can also be impaired in nGD (Miller et al., 1973, Garbutt and Harris, 2000). This usually occurs in the more advanced stages of the disease (Benko et al., 2011), but there have been no systematic studies examining this issue.

Two adaptive strategies have evolved to compensate for this defect in gaze: a hypermetric head movement (head-thrust)[29] and synkinetic blinking[30] (Harris et al., 1999). These overt signs are strongly suggestive of neuronopathic disease in a child already diagnosed with GD. These adaptive strategies are not pathognomonic of h-SIF but a number of clinicians incorrectly attributed these signs as evidence of neuronopathic disease (Harris et al., 1999). One study that clearly underscores the need for objective measurement in nGD is outlined in Harris et al., (1999). They recorded eye movements in a group of 8 enzymatically diagnosed GD children. They found SIF in 6

[29] In a head-thrust the patient rotates the head rapidly in the direction of the peripheral visual target. These very large head movements drive the eyes to the mechanical limit of gaze (lock-up) and allow them to drag gaze over to the target. Once the target is foveated the head is then returned to the straight-ahead position while the VOR keeps the eyes on target. For an excellent review of this topic see Cassidy et al., (2000) or Harris et al., (1999).

[30] Synkinetic blinking (SB) is a compensatory mechanism in which the child is able to initiate a saccade by a blink. SB is difficult to detect and the underlying mechanism is unclear. See Rottach et al., 1998 for a review on the effects of blinking on the saccadic system.

cases – leading to the diagnosis of GD3 in 2 young children who were too ill for reliable clinical examination, and a revision of diagnosis from GD1 to GD3 in 3 cases.

The pathophysiology of SIF is still unknown although there is a substantial literature implicating a number of CNS structures including the brainstem (Shawkat et al., 1995). This topic is reviewed extensively in Harris et al., (1999).

4.4.3.2 Saccade Slowing

Although SIF has considerable diagnostic value when properly assessed, it is difficult to use as a longitudinal quantitative measure. An alternative measure that is more suited for monitoring purposes is saccade slowing. When saccades can be generated, most neuronopathic patients will also exhibit slow horizontal saccades (Harris et al., 1999, Park et al., 2003, Goker-Alpan et al., 2005). In advanced cases of GD2, no saccades are made, so it is impossible to measure their speeds (Vivian et al., 1993).

Few studies have successively managed to record saccades in uncooperative patients or in children younger than 6 to 7 years of age. As we will show later in this chapter, there is a paucity of saccadic data, particularly in children (Stowens et al., 1982, Grafe et al., 1988, Gross-Tsur et al., 1989, Harris et al., 1996, Garbutt and Harris, 2000) and even fewer quantitative examinations (Harris et al., 1999, Garbutt and Harris, 2000, Cohen CS, 2001, Garbutt et al., 2001). One study has shown that the saccades are grossly slow in four children diagnosed with GD3. Interestingly, horizontal saccades were of a normal speed in the single GD1 case (Harris et al., 1999). We have summarised the available quantitative studies of GD in Table 4.2.

We highlighted earlier in Chapter 2 of this thesis that measurement of saccade speed is very difficult to detect by simple clinical observation alone. This view is endorsed by a retrospective case study review of 32 L444P homozygote patients outlined in Goker-Aplan et al., (2005). They reported that slowed horizontal saccades were a common finding in these patients but were not always documented at the time of diagnosis. For example, in two cases, slow horizontal saccades were detected several years (11 years in one case and 27 years in another) after the initial diagnosis.

There are two possible explanations for this finding. Either abnormal eye movements are not always adversely affected in neuronopathic disease or more likely – as we show later in this chapter – that in the early stages of the disease, it is not easy to clinically differentiate between GD1 and GD3. This is because neurological signs in GD3, particularly abnormal eye movements are subtle and easy to miss without precise measurement.

Table 4-2 Summary of previous studies that have formally assessed eye movements in GD.

Author	Year	N=	Age (yr)	Sex		Study design	Genotype	Phenotype	Treatment?	EM	Summary of key findings
				M	F						
Cogan et al.	1981	4	5/12 – 1	3	1	CR	-	GD3	SPL	AB	¾ cases had eye movements that 'simulated' congenital OMA. Similar age of onset. Headthrusts routinely observed. Vertical movements relatively spared. In one case eye movements were the presenting sign of GD (at 5 months).
Vivian et al.	1993	1	40884	1	-	CR	-	GD2	-	AB	HSIF and VSIF reported. The presence of a functional sensory visual pathway was indicated by the presence of flash ERGs and pattern reversal EPs.
Harris et al.	1996	1	NR	-		CS	NR	GD2	NR	AB	Investigated a wide group of children with OMA using objective eye movement studies. The infant diagnosed with infantile GD never exhibited any saccades or quick phases.
Harris et al.	1999	8	-	-		CS	L444P.	GD1 and GD3	ERT	AB	Measured EOG and video – revised GD diagnosis for patients with GD1.
Garbutt and Harris	2000	7	-	-	7	CS	NR	1 GD1, 1 GD2, 5 GD3	NR	AB	Vertical saccade initiation failure (in either direction, up/down, or both).
Garbutt et al.	2001	1	NR	NR	NR	CR	NR	GD3	NR	AB	OKN quick phases had a longer duration and lower peak velocity compared with the age matched control OKN quick phases may be a simple means for approximating the main sequence and thus a useful clinical tool for identifying brainstem pathology.
Accardo et al.	2005a	1	3.5	-	1	CR	L444P/F213I	Initially diagnosed as GD1 – revised to GD3	ERT	AB	Record and quantify the saccadic eye movements in the case of a 3.5-year old cooperative child who was affected by GD.
Accardo et al.	2005b	15	19694	NR	NR	CS (?)	NR	GD1	ERT	N*	Main sequence and saccade latency were WNL in all GD1 cases. *But authors report anomalies in number of

Author	Year	N=	Age (yr)	Sex		Study design	Genotype	Phenotype	Treatment?	EM	Summary of key findings
											oscillations in the velocity profile in 8/5 cases. 2/8 cases later developed seizure activity but no clinical details given.
Capablo et al.	2007	1	5	1	-	CR	L444P/E326K+, N188S	Initially diagnosed as GD1 – revised to GD3	1^{o} ERT but followed with comb ERT/SRT	N?	Neurological signs first seen at age 23 years – seizures. No 'oculomotor paralysis' observed clinically Combined therapy resulted in progressive improvement of his neurologic picture was observed. Walking a short distance (20 m) with aid became possible again.
Cox-Brinkman et al.	2008	3 sib	Birth-1.5	1	2	CR	L444P homozyg.	GD3	1^{o} ERT but followed with comb ERT/SRT; One case has only had comb therapy	AB in all cases#	#Diagnosed with 'partial OMA' initially in one case (this later deteriorated and h-SIF and v-SIF recorded); one case had no observable OMA until age 15 mo which also deteriorated. The final case also had slow saccades and 'partial OMA'. Authors report neurological signs stabilised in all 3 cases following combined therapy.
Accardo et al.	2010	2	-	-	2	CR	R353G homozyg.	GD3?	Miglustat	AB	Quantitative measure of miglustat in GD by estimating the characteristic parameters of saccadic main sequence. Miglustat treatment effective and resolved SEM.

4.4.4 Are abnormal eye movements *truly* diagnostic of neuronopathic disease?

Several investigators have also recently questioned whether there is value in distinguishing between the phenotypes – arguing that the division between the three phenotypes is no longer valid, particularly with emerging evidence that patients with GD1 *may have* neurological disease – albeit with a later onset in many cases (Cherin et al., 2010, Giraldo et al., 2011).

More importantly with regards to this thesis, a number of studies have also asserted that *eye movements are also abnormal in some patients with non-neuronopathic disease* (Miller et al., 1973, King, 1975, Harris et al., 1999, Ida et al., 1999, Garbutt and Harris, 2000, Tayebi et al., 2001, Tayebi et al., 2003, Varkonyi et al., 2003, Goker-Alpan et al., 2004, Wong et al., 2004, Accardo et al., 2005a, Accardo et al., 2005b, Alfonso et al., 2007, Capablo et al., 2008, Goker-Alpan et al., 2008, Accardo et al., 2010, Alonso-Canovas et al., 2010).

These studies clearly contradict those investigators who have made the claim[31], that SIF (and by association, slow saccades) are an 'obligatory feature' or a 'universal finding' in patients diagnosed with neuronopathic disease (Schiffmann et al., 1997, Altarescu et al., 2001b, Frei and Schiffmann, 2002, Goker-Alpan et al., 2003, Vellodi et al., 2009, Benko et al., 2011).

This discrepancy is highly disconcerting – particularly as we have already clearly emphasised in Section 4.4.2, that the nosology of nGD is determined by the presence or absence of abnormal eye movements. Clarification of this issue is particularly important as classification of patients with GD as one type or another is

[31] It could be argued that this claim is based on opinion and/or clinical experience alone and is therefore unsupported. At the time of writing, there is no published study which has systematically evaluated the evidence for this statement.

used to predict the outcome or response to treatment and more importantly, the correct level of dose.

In the following sections, we examine the available scientific literature to see whether there is any evidence to support the theory that GD1 and GD3 are *not* distinct phenotypes on the basis of eye movement studies.

4.4.4.1 Do non-neuronopathic GD patients have abnormal eye movements?

We undertook a systematic review of the available literature, using the electronic database PUBMED. The search strategy, inclusion and exclusion criteria are shown separately in Appendix 2. Sixty-five papers published across a 40-year period (from 1970-2010) reported eye movement abnormalities in GD (Appendix 2). Of these, a total of 17 studies (26%) reported atypical eye movements in patients diagnosed with GD1. We present the key findings from each of these studies in Table 4.3.

The data provided four possible interpretations – each of which may explain why abnormal eye movements are reported in GD1. These include: a) application of outdated phenotype classifications b) incorrect phenotyping and lack of qualitative measurements resulting in misdiagnosis; c) co-morbidity and d) late-onset disease associated with PD. We now provide a discussion on each of these below.

4.4.4.1.1 Application of outdated phenotype classification

Historically, GD3 was considered to be restricted to children and adolescents, i.e. GD was categorised on the basis on age of disease onset, hence the terms ‘infantile’

and ‘juvenile’. However, over time it has been recognized that neurological involvement in GD3 sometimes emerges in adulthood. Before this recognition, several case reports have described patients with GD1 disease and adult-onset neurological manifestations (Miller et al., 1973, King, 1975, Tayebi et al., 2001) who could better be classified as late-onset GD3.

4.4.4.1.2 Incorrect phenotyping and lack of qualitative measurements resulting in misdiagnosis

A number of studies shown in Table 4.4 have published case reports in which patients originally thought to be GD1 were misdiagnosed and which required revision to GD3 (Harris et al., 1999, Ida et al., 1999, Tayebi et al., 2001, Accardo et al., 2005a, Accardo et al., 2005b, Capablo et al., 2008, Accardo et al., 2010). Furthermore, several studies have regrettably published little (if any) detail regarding how they obtained their eye movement results. For example, Alfonso et al., (2007) reported eye movement disorders in 3 cases but failed to provide any specific details about the type of abnormalities or how these were measured. Furthermore it is not clear whether these patients had a revised diagnosis (from GD1 to GD3) or what their neurological course was.

4.4.4.1.3 Co-morbidity known to be associated with nervous system diseases

A third explanation for the abnormal eye movements seen in Table 4.4 is the existence of co-morbid neurological disease (Garbutt and Harris, 2000, Accardo et al., 2010). For example, Accardo et al., (2010) describe atypical eye movements in two

patients with epilepsy. The seizure activity was initially considered 'casual' in both cases but led to a revised diagnosis, from GD1 to GD3, in one case[32]. Garbutt and Harris (2000) reported vSIF in a child diagnosed with GD1 who had normal horizontal OKN responses. An MRI scan in this child revealed a lesion in the rostral midbrain with the appearance of an old haemorrhage. This presumably was the cause of the vSIF. Both of these examples lend strong support for our argument in this thesis for the development and application of additional tests in phenotype identification.

4.4.4.1.4 Late-onset disease associated with PD.

A relatively new obstacle that may impede correct phenotypic classification is the case of the GD1 patient who develops Parkinsonism and abnormal horizontal saccadic eye movements (Tayebi et al., 2001, Tayebi et al., 2003, Varkonyi et al., 2003, Alonso-Canovas et al., 2010); see our earlier discussion in section 4.2.1. Eye movement abnormalities especially in the saccadic eye movements, are also associated with Parkinsonism, although they usually develop when the disease progresses (Zee, 1986, Leigh and Zee, 1999a). Unfortunately, it is unclear whether the abnormal eye movement signs seen in this small group of GD1 patients are present before the onset of Parkinsonism or whether they are developed in the course of Parkinsonism. This most likely reflects the lack of precise quantitative eye measurements. This is clearly illustrated in a study described by Tayebi et al. (2001).

[32] It is of interest that the treatment for seizures (barbituates) used in one of these cases may have accounted for the slow saccades in one case but it is not clear whether the authors of this study accounted for the potential side-effects of this treatment.

Here the authors report what they describe are the initial signs of neurological involvement (Parkinson-like neurological signs) in a 42 year old woman with GD1. They say that

"…w*e were not able to establish whether the oculomotor abnormality preceded the parkinsonian symptoms, although the patient always had to move a book in order to read" (p319).*

It is not clear whether these sporadic cases should still be classified as GD1 in the presence of neurological symptoms such as Parkinsons. Biegstraaten et al. (2008) has argued that while

"the term non-neuronopathic Gaucher disease does not seem to be an appropriate characterization of type I Gaucher disease. However, the neurological signs and symptoms in type I Gaucher disease are of a totally different kind from and, in the majority of cases, of much less severity than the signs and symptoms associated with types II and III disease" (p337).

Clearly, clarification of this issue and a world-wide consensus on correct phenotyping, using precise quantitative procedures, is urgently required to prevent further confusion in this area.

Author	Year	N=	Genotype	Phenotype	Treatment?	Eye movements	Recording method	Summary of key findings
Miller et al.	1973	2	-	GD1	-	AB	C	First report of oculomotor disturbances in GD in adult siblings. First neurological signs observed were seizures and deterioration in IQ.
King	1975	1	-	GD1	-	AB	NR	PME – first sign of neurological involvement. Saccadic eye movements 'slow' in all directions. Authors concluded that this case was evidence for neurological involvement in the 'adult' phenotype of GD.
Harris et al. ,	1999	8	L444P	MIX	ERT	AB	O	Revised diagnosis in 2 cases
Ida et al.	1999	15	MIX	MIX	NR	AB in 4/15	C	No significant details available other than evidence of abn eye movments
Garbutt and Harris	2000	7	NR	1 GD1, 1 GD2, 5 GD3	NR	AB	O	Vertical saccade initiation failure (in either direction, up/down, or both).
Tayebi et al.	2001	1	L444P/ D409H	GD1	SPL ERT	AB	C	First neurological signs were PD at age 42 yrs. Her eyes could fix and follow, but she had difficulty initiating movements and had almost no horizontal saccadic eye movements. Pursuit was only possible with a great deal of blinking. 'We were not able to establish whether the oculomotor abnormality preceded the parkinsonian symptoms, although the patient always had to move a book in order to read. This distinction is relevant because the longevity of children and adolescents with type 3 Gaucher disease and oculomotor abnormalities is now increased due to enzyme replacement therapy and it will be important to closely monitor these patients for movement disorders.' (p319)
Accardo et al.	2005 a	1	L444P/ F213I	Initially diagnosed as GD1 – revised to GD3	ERT	AB	O	Record and quantify the saccadic eye movements in the case of a 3.5-year old cooperative child who was affected by GD.
Accardo et al.	2005 b	15	NR	GD1	ERT	N*	O	Main sequence and saccade latency were WNL in all GD1 cases. *But authors report anomalies in number of oscillations in the velocity profile in 8/15 cases. 2/8 cases later developed seizure activity but no

								clinical details given.
Alfonso et al.	2007	193		178 GD1 7/193 GD2 8/193 GD3	NR	AB in 3/178 GD1	NR	characterise the GBA mutations and analyze genotype/phenotype relationships in 193 unrelated patients from the Spanish GD Registry
Capablo et al.	2007	1	L444P/ E326K+ N188S	Initially diagnosed as GD1 – revised to GD3	1^{o} ERT but followed with comb ERT/SRT	Norm	O	Neurological signs first seen at age 23 years – seizures.No 'oculomotor paralysis' observed clinically but they note bilateral blepharospasm. During the following 12 months of combination therapy,a progressive improvement of his neurologic picture was observed; visceral and laboratory parameters of the disease remained stable or improved , myoclonic epilepsy improved, and tonic–clonic seizures decreased. Sleep, speech, and swallowing, as well as social interaction, also improved markedly. Walking a short distance (20 m), with aid, became possible again.
Accardo et al.	2010	2	R353G homozyg.	GD1 initially but this was revised in 1 case to GD3 following onset of EM abnorm.	ERT initially but followed with SRT alone	AB	O	Serial eye movement recordings made using limbus eye tracker. Normal control data also presented. Both cases had seizures in adolescence but this was considered 'casual' . One case showed no further neurological signs later in life and EM studies were always normal. The second case however showed a deterioration in EM on the main sequence but this improved after 2 years of SRT.
Alonso-Canovas et al.	2010	1	N370S/ L444P	GD1	ERT	AB	Check video [C]	Authors report a case of atypical parkinsonism with apraxia and supranuclear gaze abnormalities in GD1. There was difficulty in initiating eye movements and a supranuclear gaze abnormality with restriction and hypometric saccades, especially in the vertical plane.

Table 4-3 Selected eye movement studies that have reported that GD1 patients with abnormal oculomotor signs.

4.4.4.2 Do neuronopathic GD patients have normal eye movements?

Only four studies (6%) that we included in this review actually reported that patients diagnosed with GD3 had normal eye movements (Chabas et al., 1995, Erikson et al., 1995, Ida et al., 1999, Suwannarat et al., 2007). We have presented a summary of these studies in Table 4.4.

The data from this small group of studies was plagued by inconsistent reporting and a lack of any formal eye movement measurement. For example, the patient described by Suwarannet et al., (2007) with 'normal' eye movements was the youngest in a series of four GD3 patients. The same patient (who presented at 0.65 years) and was described as '*asymptomatic'* and *'appeared normal'* on the physical exam was later described in the same publication as showing signs of *'devastating neurological regression'* (p349). Regrettably, no further details are made available[33] (Suwannarat et al., 2007). In another study, undertaken in a series of 8 patients diagnosed with GD3 L444P homozgygotes, the authors describe gaze abnormalities in 7/8 cases. They note that the single case that had no eye movement abnormalities had in their opinion *'type 1 disease'* (p 204) as she did not display any other neurological signs except a general slowing on her EEG (Erikson et al., 1995).

None of these studies have objectively measured the eye movements in their patients despite incontrovertible evidence to show that it is easy to miss abnormal eye movements based on purely clinical observation. There is insufficient evidence from these studies to conclude that neuronopathic GD patients (at this time) have normal eye movements.

[33] The information regarding this patient is limited and the authors report that the patient developed seizures at 11 months. No further mention is made regarding eye movements or how they are measured.

Table 4-4 Previous eye movement studies that have reported normal eye movements in GD3 cases.

Author	Year	Genotype	Phenotype	Treatment?	Summary of key findings
Chabas et al.	1995	D409H	GD3		Three sisters suffering from an unusual form of Gaucher's disease are described. These patients had cardiovascular abnormalities consisting of calcification of the ascending aorta and of the aortic and mitral valves. Neurological findings included ophthalmoplegia and saccadic eye movements in two patients, and tonic-clonic seizures in the third. The three patients died, two of them after having undergone aortic valve replacement. Tissue was obtained from one of the sibs and fibroblast and liver beta-glucocerebrosidase activity was reduced to 4% and 11% of mean normal values. Genotype analysis indicated that the patient was homozygous for the D409H mutation.
Erikson et al.	1995	L444P	GD3	NR	Reported 1 case as having normal eye movements although some speculation regarding neurology
Ida et al.	1999	MIX	Varied	NR	Normal eye movements in some cases, although no clear discussion of how SEM measured
Suwarannet et al.	2007	F2131 in 3 cases and various mutations	AB in 3 cases of GD3	NR	Report SIF in 3 cases and normal eye movements in one case classed as GD3 who was asymptomatic at initial diagnosis (later developed seizures).

4.4.5 Auditory signs in GD

There are comparatively fewer studies that have investigated the auditory system in GD. The majority of these studies have used the ABR to investigate brainstem involvement in GD. We have constructed a series of tables that summarise these studies across the three different GD phenotypes (see Table 4.5 for ABR studies in GD1; Table 4.6 for ABR studies in GD2 patients and Table 4.7 for ABRs performed in GD3 patients).

As we discussed previously in Chapter 2, the ABR is a well-validated measure for testing the integrity of auditory brainstem pathways which has been available since the early 1970s as a clinical tool. However, we could find only five studies that have measured the ABR in GD1 patients (Ida et al., 1999, Accardo et al., 2005a, Pensiero et al., 2005, Grasso et al., 2006, Accardo et al., 2010); seven studies in GD2 patients (Kaga et al., 1982, Lacey and Terplan, 1984, Vivian et al., 1993, Kaga et al., 1998, Ida et al., 1999, Grasso et al., 2006, Miyata et al., 2006) and eight studies in GD3 patients (Abrahamov et al., 1995, Schiffmann et al., 1997, Altarescu et al., 2001a, Aoki et al., 2001, Bamiou et al., 2001, Goker-Alpan et al., 2005, Grasso et al., 2006, Cox-Brinkman et al., 2008).

All of the ABRs recorded in GD2 have been reported as abnormal (Kaga et al., 1982, Lacey and Terplan, 1984, Kaga et al., 1990, Kaga et al., 1998), corresponding to histopathological abnormalities of loss of neuronal cells and gliosis in the CN and SOC (Lacey and Terplan, 1984, Kaga et al., 1998).

Table 4-5 A comparison of previous studies investigating the ABR in GD1 patients

Author	Year	N=	Age (onset) Yrs	Sex		Genotype	Treatment	SIF?	Hearing	Recording method				Conclusions
				M	F					*Stim*	*Rate*	*Intensity*	*Waveforms shown?*	
Ida et al.	1999	4	1 - 5 yrs and 3/12	3	1	L444P homozyg.	ERT (11/12) BMT (1/12)	N (4/12)	Hearing loss in 2/12 cases ? normal in other cases	NR	NR	NR	N	12 cases originally diagnosed as GD1 – of these, 9/12 cases later developed neurological signs. ABRs recorded only in 4/12 cases and was abnormal in the 2 cases which later developed additional neurological signs. No detail given regarding abnormalities seen.
Accardo et al.	2005a	1	3.5	-	1	L444P/ F213I	ERT	Y	NR	NR	NR	NR	N	Initially diagnosed as GD1 – revised to GD3. ABR showed abnormal morphology with poor repeatability and increased latencies. No details about abnormalities.
Perretti et al. (2005)	2005	16	3yr -48 yr	6	10	various	NR	N	NR	Click	11/s	65 dB SL	Y	ABR recorded in 16 cases (11 adults and 5 children) 5/16 ABRs abnormal Peak latencies and amplitudes not reported.
Grasso et al.	2006	54	NR	26	28	NR	NR	NR	HL reported in 13/54 cases of GD1	Click	11.1/s	≥90 dB nHL	Y	ABR abnormal in 1/54 and elongated in 8/54 GD1 cases Peak latencies and amplitudes not reported.
Accardo et al.	2010	2	16-22 yrs	-	2	R353G/ R353G	ERT & comb SRT	Y	NR	NR	NR	NR	N	Normal

Author	Year	N=	Age (onset) Yrs	Sex		Genotype	Treatment	SIF?	Hearing	Recording method				Conclusions
				M	F					*Stimuli*	*Rate*	*Intensity*	*Waveforms shown?*	
Kaga et al.	1982	1	6/12	1	-	NR	None	Y	NR	Click	10/s	NR	Y	Delayed waveforms and loss of waves III-V.
Lacey and Terplan	1984	1	Birth	1	-	NR	None	Y	NR	Click	10/s	65, 80, 88 dB	N	Wave I-II present; wave III only present at intensity of 88 dB.
Vivian et al.	1993	1	7/12	1	-	NR	None	Y	NR	NR	NR	NR	N	Report a "major disturbance of function in aud. pathways through the upper part of the brainstem" No other details reported
Kaga et al.	1998	1	Birth	-	1	NR	None	Y	'poor response'	NR	NR	NR	Y	Wave I-II present at normal latencies and loss of waves III-V.
Ida et al.	1999	3	3/12 – 5/12	1	2	L444P/ L444P	NR	Y	NR	NR	NR	N	N	Abnormal ABR in 1 case; ABR not examined in the other 2 cases
Miyata et al.	2006	1	18/12	-	1	?	NR	Y	NR	NR	NR	NR	N	Abnormal ABR Lesions in CN

Grasso et al.	2006	5	NR	4	1	NR	NR	NR	NT	Click	11.1 /s	≥90 dB nHL	Y	ABR abnormal in all cases with absent IV-V bilaterally. As the disease progressed the ABR showed a gradual loss of waves II and III. Peak latencies and amplitudes not reported.

Table 4-6 A comparison of previous studies investigating the ABR in GD2 patients

The picture is less clear in GD1 and GD3 patient groups. For example, several investigators have compared the ABR findings in GD3 and while some degree of similarity has been identified, several inconsistencies have also been reported. Patients with GD3 may have normal ABR waveforms or delayed/absent waves II-V and ERT may stabilise the ABR findings in some cases (Schiffmann et al., 1997), although they have not been thoroughly investigated. We explore the utility of the ABR as a marker of treatment efficacy in our next chapter.

The discrepancies seen in the GD1 and GD3 literature may reflect methodological differences (different stimuli, intensity, stimulation rates, polarity) or different recording techniques (montages) may have lead to different conclusions. Generally the recording method is never reported (Table 4.5 and 4.7) and a description of waveform analysis is typically missing. Furthermore other essential auditory investigations are often not performed. For example, many of these studies have never established that the peripheral hearing mechanism required to generate an ABR is normal (Schiffmann et al., 1997, Altarescu et al., 2001b, Perretti et al., 2005).

More recently, it has been claimed that patients with GD1 have sub-clinical neurological signs as indicated by audiological measurements (Perretti et al., 2005, Grasso et al., 2006). Clearly, as we mentioned earlier in Section 4.4.4, the finding of neurological abnormality in non-neuronopathic disease (similar to those seen in GD3 disease) is highly significant. However, before we can accept these data, two important issues need to be carefully clarified: a) the questionable diagnosis of neuronopathic disease in this study and b) the dubious interpretation of their ABR and other electrophysiological data. We begin to address these questions later in this chapter.

Author	Year	N=	Age (onset) Yrs	Sex		Genotype	Treatment	SIF?	Normal Hearing	Recording method				Conclusions
				M	F					*Stimuli*	*Rate*	*Intensity*	*Waveforms shown?*	
Abrahamov et al.	1995	12	2-20	7	5	D409H/ D409H	NR	Y	NR	NR	NR	NR	N	ABRs all normal but no data presented
Schiffman et al.	1997	5	3 – 8	4	1	L444P/ L444P in 3/5, D409V/ RecNciI in 1/5 and U in 1/5	3/5 partial SPL ERT	Y	NR	NR	NR	NR	N	3/5 children had normal ABRs; 1/5 had wave I only; 1/5 had delayed latencies and developed hyperacusis.
Altarescu et al.	2001	21	8/12 – 35 yrs	12	9	various	ERT	Y	NR	NR	NR	NR	N	ABRs recorded in all 21 cases: 11/21 patients were reported with normal ABRs, of which only 2 showed deterioration. Of the remaining 10 with abnormal ABRs, there was no deterioration, and 2 even showed improvement with ERT. No details about abnormalities
Aoki et al.	2001	1	8/12	1	-	L444P homozyg.	ERT	Y	NR	NR	NR	NR	N	Wave I only present at baseline After 12 months waves, I-II, IV-V on one side and wave V on other ear were observed.
Bamiou et al.	2001	9	3.3 – 11.7	1	8	L444P homozyg. in 8 cases, Pending in 1/9	ERT BMT (2)	Y	Normal in all 9 cases	Click	11.1/s	90 - 100 dB nHL	N	ABR abnormal in 6/9 cases Abnormalities incl delayed waveforms and absent waves from II-V.

Goker-Alpan et al. (2005)	2005	32	2/12-2.5	15	17	L444P homozyg.	9/32 SPL 2/32 BMT 23/32 ERT	Y	NR	NR	NR	NR	N	ABR abnormal in 9/32 cases; normal in 10/32 cases and NA in 13/32 cases
Grasso et al.	2006	8	NR	4	4	NR	NR	NR	HL reported in 1/8 GD3 case	Click	11.1/s	Min intensity of 90 dB nHL	Y	ABR abnormal in 3/8 cases of GD3 cases Peak latencies and amplitudes not reported.
Cox-Brinkmann et al.	2008	3 sib	Birth-1.5	1	2	L444P homozyg.	1^{o} ERT but followed with comb ERT/SRT One case has only had comb therapy	Y	NR	NR	NR	NR	N	Serial ABRs undertaken. In case 1 - initially a normal ABR reported which became abn (absent wave IV/V and prolonged I-III); Case 2 – report a 'grossly abnormal ABR' – waves I and V only Case 3 – mildly prolonged waveforms which were later reported as normal following comb therapy.

Table 4-7 A comparison of previous studies investigating the ABR in GD3 patients

What is clear from the data presented across Tables 4.5 and 4.7 is that there is still no agreement as to whether audiological investigations could be a sensitive marker of disease burden with some studies reporting a wide range of latency abnormalities (Schiffmann et al., 1997) and other reporting normal ABRs in GD3 patients (Altarescu et al., 2001b). Despite these differences, there are common trends in the data that appear. It would seem from the limited data that the ABR is *always* abnormal in severe neuronopathic disease (GD2). Furthermore, it would seem that the ABR *may be able to detect* even subtle neurological changes in the less severe neurological subtypes. More importantly, the potential role of the ABR as an outcome measure for monitoring neurological disease is also unclear. We will examine this issue later in Chapter 5 of this thesis.

A number of other auditory abnormalities have been reported in the scientific literature. These include:

- Sporadic reports of hearing loss (conductive and sensorineural) in some cases (Dreborg et al., 1980, Grasso et al., 2006, Alfonso et al., 2007)
- Middle ear reflexes are absent or elevated (Bamiou et al., 2001, Grasso et al., 2006)
- Suppressed or absent medial olivocochlear suppression (Bamiou et al., 2001)
- Hyperacusis has also been reported (Schiffmann et al., 1997)

In summary, much of the published literature which has investigated auditory function in GD is characterised by a poor description of experimental paradigms, often providing limited data regarding data processing methods and criteria. Clearly, there is a need to undertake a more systematic approach. In this first experimental chapter, we

begin to address this issue by undertaking a systematic assessment of auditory function by carrying out investigations on a wide range of GD patients, with known eye movement status[34]. Our primary aim in this study is to construct a fuller, more complete 'audiological profile' of the possible effects of GD on auditory pathways.

4.5 Methodology

4.5.1 Subjects

Twenty five cases diagnosed with GD were identified from referrals to the Metabolic unit at Great Ormond Street Hospital. Diagnosis of GD had been previously made by demonstration of deficient β-glucosidase activity either in peripheral leucocytes or fibroblasts (See section 4.4 for a description of this process). Table 4.8 summarises the clinical features of each of the patients.

Subjects were classified into 3 phenotypic groups based on enzymatic or DNA analyses in conjunction with a thorough history and physical examination and a complete oculomotor assessment by an experienced clinician. Group 1 (cases 1-7; n=7) consisted of seven non-neuronopathic (GD1) patients (age range 6-17 years, 5 females). Group 2 consisted of neuronopathic patients (cases 8-21, n = 18) and included two GD2 patients (cases 8-9, 2 females) and twelve GD3 patients (cases 10-21; 8 females). The data presented was collected over a four year interval from 2002 to 2006.

An equal number of age (within 6 months) and gender-matched children (n=25, 15 females, 18month – 17 years; mean age – 8 years) with normal hearing threshold levels (normal pure tone audiogram and tympanometry) with no known predisposing

[34] Some of the discussion presented in this chapter has appeared in two papers (Campbell et al., 2003; 2004).

factors for hearing loss were used as controls (Appendix 2). Control subjects were initially recruited using convenience sampling, (e.g. posters and emails with information about the project were sent to co-workers and colleagues known to the investigators). In the latter stages of the study, controls were recruited using snowballing techniques. Ethical Committee approval was obtained for the study and informed consent was obtained from the parents of the younger children and from the older subjects themselves.

A history of ear disease was reported in 7/25 cases of GD. All of the 7 cases who identified a hearing loss in the case history were categorised as neuronopathic GD (n=2/7 GD2; n=5/7 GD3). Both cases with the GD2 phenotype were reported as having fluctuating problems with their hearing, although it was unclear whether the hearing problems were the result of intermittent middle ear infections or difficulty accurately recording hearing thresholds as a result of tracheostomy in both cases. In the remaining 5 cases, only case 11 had been fitted with binaural hearing aids. The other 4 cases had a history of middle ear disease which would resolve spontaneously or required treatment with antibiotics. None of these cases had ever required surgical intervention (i.e. grommets).

Interestingly, case 17 reported having been diagnosed as profoundly deaf and was fitted with hearing aids. The hearing aids were later withdrawn, when it was noted that the hearing aids were causing discomfort and hearing was later discovered to be within the normal range.

The modified listening profile is a 36-item checklist, typically administered by the parents and/or teachers and is designed to identify possible listening difficulties in

children. It does this by asking the observer (parent or educator) to compare the child to a reference population of other children of similar age and background. The profile concentrates on six different listening conditions (noisy, quiet, ideal, multiple inputs, auditory memory/sequencing, and auditory attention span) described in Appendix 3.

Parents (or educators working directly with the child) were asked to judge whether or not the child had 'more' difficulty than other children in each listening condition using a five point scale (i.e.) did they feel that their child had 1) less difficulty, 2) same amount of difficulty, 3) more difficulty, 4) considerably more difficulty or 5) doesn't function at all when compared to children of similar age and background. The overall responses for each child are summarised in Table 4.8.

The questionnaire was completed and returned in 17/21 cases. The questionnaire was not administered to four families (cases 8, 9, 15 and 24) simply because the child involved was too young.[35] The questionnaire was not returned in four other cases (cases 4, 10, 11 and 21).

All children diagnosed with non-neuronopathic GD children reported either having 'less difficulty' or 'the same amount of difficulty' in each of the six listening conditions (n=6) (Table 4.8). Interestingly, several concerns were identified using the

[35] The questionnaire requires that the parent or teacher compare the listening behaviour with their peers. Four of the cases enrolled in the study were too young and were not attending nursery or school, therefore any comparison of their listening behaviour would not have been possible.

Table 4-8 Summary of patient clinical characteristics

If a parent or educator indicated that the child had 'less difficulty' or 'same amount of difficulty' then listening in that condition was not considered to be concern. However, if parents or educators ticked the boxes showing either 'more difficulty', 'considerably more difficulty' or 'doesn't function at all', then that was considered a concern. Abbreviations: BMT – Bone marrow transplant, ERT – enzyme replacement therapy, F- female, HL – hearing loss, M- male, N – No, Norm – normal, NR – no response, SIF – saccade initiation failure, Y – Yes.

Case	Age (yrs)	Sex	Neurological Signs	Genotype	Phenotype	Treatment	Ear problems?	Problems/Concerns reported in the listening profile?					
								Noise	*Quiet*	*Ideal*	*Multiple inputs*	*Auditory memory/sequencing*	*Auditory attention span*
1	6	M	None	N370S/U	1	ERT	N	N	N	N	N	N	N
2	8	F	None	N370S/U	1	ERT	N	N	N	N	N	N	N
3	14	M	None	L444P/R463C	1	ERT	N	N	N	N	N	N	N
4	15	F	None	U/U	1	ERT	N	NR	NR	NR	NR	NR	NR
5	12	F	None	U/U	1	ERT	N	N	N	N	N	N	N
6	7	F	None	R463T/53bp del	1	ERT	N	N	N	N	N	N	N
7	17	F	None	N370s/84gg	1	ERT	N	N	N	N	N	N	N
8	2	F	SIF	L444P/c1263del55	2	ERT	Y	NR	NR	NR	NR	NR	NR
9	1.5	F	SIF	L444P/U	2	ERT	Y	NR	NR	NR	NR	NR	NR
10	4	M	SIF, epilepsy	L444P/L444P	3	ERT	N	NR	NR	NR	NR	NR	NR
11	10	M	SIF	V15L/S339L	3	ERT	HL	NR	NR	NR	NR	NR	NR
12	10	F	SIF, ataxia	L444P/L444P	3	BMT	N	Y	N	N	Y	Y	Y
13	5	F	SIF	L444P/L444P	3	ERT	Y - Ear infections	Y	N	N	Y	Y	Y
14	4	F	SIF	L444P/L444P	3	ERT	N	N	N	N	N	N	N
15	2	M	SIF	U/U	3	ERT	Ear infections	NR	NR	NR	NR	NR	NR
16	5	F	SIF	L444P/L444P	3	ERT	Y	Y	N	N	N	N	N
17	8	M	SIF, Tourettes	L444P/L444P	3	ERT	N	Y	Y	Y	Y	Y	Y
18	6	F	SIF	L444P/L444P	3	ERT	N	N	N	N	N	N	N
19	12	F	SIF	L444P/L444P	3	ERT	Y	Y	Y	Y	Y	Y	Y
20	15	F	SIF	L444P/L444P	3	BMT	N	Y	Y	Y	Y	Y	Y
21	12	F	SIF	L444P/L444P	3	ERT	N	NR	NR	NR	NR	NR	NR
22	4	F	SIF, ataxia	L444P/L444P	3	ERT	N	N	N	N	N	Y	Y
23	9	F	SIF	L444P/L444P	3	ERT	N	Y	N	N	N	N	Y
24	2	M	SIF	L444P/L444P	3	ERT	N	NR	NR	NR	NR	NR	NR
25	6	F	SIF	L444P/L444P	3	ERT	N	N	N	N	N	N	N

listening profile within the neuronopathic GD group (Table 4.8). Three children diagnosed with neuronopathic disease reported no problems in any of the listening conditions (cases 14, 18 and 25). Unsurprisingly, there were fewer auditory difficulties reported when the listening conditions were described as either 'quiet' or 'ideal' (n=8/11), although 3 cases consistent reported experiencing 'more difficulty' than their peer group even in an optimal listening environment. The listening conditions identified by the questionnaire as the more challenging in the neuronopathic GD group were 'noisy' environments (n=7/11 children) and at times when the children need to attend to multiple auditory inputs (n=6/11) or when recalling auditory information (n=7/11).

4.5.2 Procedure

All subjects had standard baseline audiological assessment as previously described in Chapter 3. The presence of hearing loss in GD subjects as assessed by abnormal audiometric and impedance audiometry tests was a criterion of exclusion from the study. This criterion was essential in view of the known influence of the transmission properties of the middle ear on otoacoustic emissions, acoustic reflexes and the ABR.

Audiometric results were obtained from all subjects in the same session in which electrophysiological tests were performed. The ABRs were recorded to clicks (100 μs in duration) of alternating polarity presented at a rate of 11.1/s at an intensity of 90 dB nHL using TDH-49 headphones.

Clinical examinations were carried out by a neurologist and/or ophthalmologist using techniques previously outlined in Table 2.7. Once the initial clinical assessment

had been completed, a more detailed assessment was undertaken by an experienced eye movement specialist using DC-coupled electro-oculography (EOG) and simultaneous video (Harris et al., 1992, Jacobs et al., 1992).

EOG recording was bitemporal, with disposable electrodes placed on the outer canthi with reference electrode in the mid-forehead. Infants sat on their parent's lap. GD subjects were also examined for locking up during optokinetic nystagmus elicited by horizontal moving stripes using a full field system high-contrast coloured curtain rotated about the infant at 25 and 50 degrees per second. For this test, eye movements were recorded with EOG only. Subsequent assessments were by bedside video only. Saccades were recorded and analysed off-line. Duration and peak velocity of saccades were measured using a standard computer algorithm previously described in Harris et al., (1999). The findings are summarised in Table 4.14

4.5.3 Data analysis

The following summary statistics were presented for continuous variables: number of values, mean, standard deviation (SD), median, minimum, and maximum. For categorical data, frequencies and percentages were to be presented. Distribution normality and equality of variance between groups were assessed by one-sample Kolmogorov--Smirnov test (with Lilliefors correction) and Levene's tests, respectively.

Because of the small sample size, performance on audiological measures in the GD patients was compared with that of control participants using the nonparametric Mann-Whitney *U* test or the Wilcoxon Rank Sum Test. Two-tailed statistics were used

throughout, and p< 0.05 was considered significant. All statistics were computed with SPSS (v14) statistical software (SPSS, Chicago, IL).

4.6 Results

4.6.1 Baseline Audiometric Tests

4.6.1.1 Pure tone audiometry

Pure tone audiometry was measured in 24/25 (96%) of all cases. We were unable to measure behavioural thresholds in one subject (case 15) because of poor co-operation. Results of air conduction audiometry across, confirmed normal hearing in 23/25 (92%) of GD subjects (95CI=73.97-99.02). A bilateral moderate mixed hearing loss was recorded in one subject (case 11) and was excluded from the remainder of the study. Overall mean thresholds are plotted in dB HL for the three different phenotypes and the age matched control group in Figure 4.4.

Hearing thresholds were comparable across the groups and the control subjects for both ears. Differences between the groups were tested for significance using the Wilcoxon signed-rank test which showed that pure tone thresholds did not differ significantly between the control group and the Gaucher group, except at 2 kHz on the left ear (z = -2.974, p< .05, r = - .44).

However, the hearing thresholds were elevated at 0.5, 1 and 2 kHz for the two infants in the GD2 group when compared with the other GD phenotypes. This is not an unexpected finding. It has been well documented in the literature that younger children typically require a higher level of auditory stimulus than older children when

performing audiometry – although the reason for the variability is still unclear (Trehub et al., 1989). But it is commonly attributed to either the maturation of auditory sensitivity with increasing age or may simply reflect the maturation of the child in terms of improved attention or listening skills. Mean threshold levels across frequencies 0.25 to 8 kHz are shown below in Table 4.9.

Figure 4-4 Mean (± SEM) hearing threshold levels for the right and left ears.
Data is shown for the right and left ears in the conventional for the control group and in three GD phenotypes (n=23) in dB HL; error bars show the 95% confidence interval of the mean at each frequency.

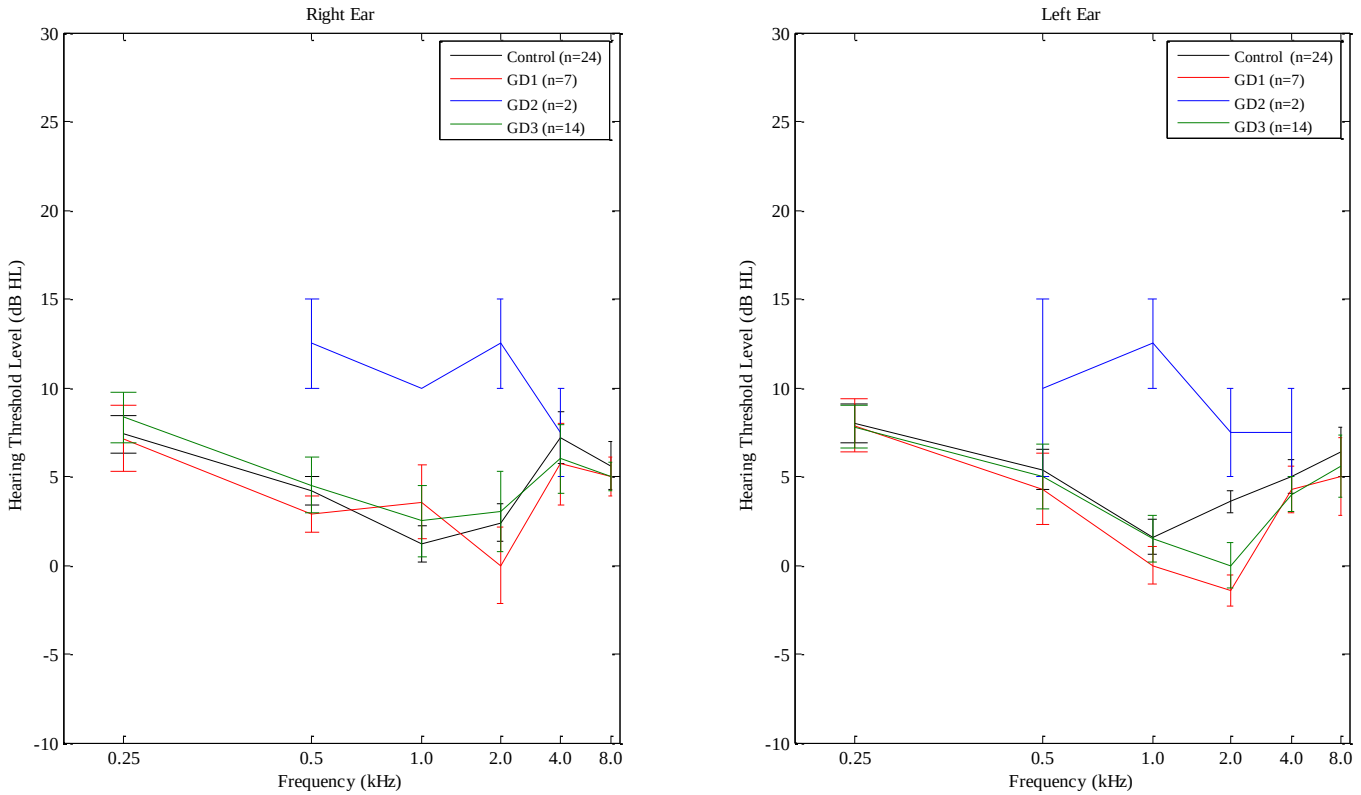


Table 4-9 Mean air conduction thresholds.
Data is shown across both ears, for GD1 (neuronopathic subgroup); NGD (neuronopathic subgroup which includes the GD2 and GD3 subtypes) and for age-and-gender matched normal control subjects.

Group		*Rt 0.25 kHz*	*Rt 0.5 kHz*	*Rt 1 kHz*	*Rt 2 kHz*	*Rt 4 kHz*	*Rt 8 kHz*	*Lt 0.25 kHz*	*Lt 0.5 kHz*	*Lt 1 kHz*	*Lt 2 kHz*	*Lt 4 kHz*	*Lt 8 kHz*
GD1	Mean	7.14	2.86	3.57	0.00	5.71	5.00	7.86	4.29	.00	-1.43	4.29	5.00
	N	7	7	7	7	7	7	7	7	7	7	7	7
	Std. Dev.	4.88	2.67	5.56	5.77	6.07	2.89	3.93	5.35	2.89	2.44	3.45	5.77
	SEM	1.84	1.01	2.10	2.18	2.30	1.09	1.49	2.02	1.09	0.92	1.30	2.18
	Median	5.00	5.00	5.00	0.00	5.00	5.00	5.00	5.00	0.00	0.00	5.00	5.00
NGD	Mean	9.23	5.00	3.44	4.69	6.56	5.00	7.70	5.63	3.12	0.94	4.38	6.92
	N	13	16	16	16	16	13	13	16	16	16	16	13
	Std. Dev	4.00	5.16	5.69	6.44	6.25	2.04	3.30	5.44	5.12	4.17	3.10	5.22
	SEM	1.11	1.29	1.42	1.61	1.56	0.57	0.92	1.36	1.28	1.04	0.77	1.45
	Median	10.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	0.00	0.00	5.00	5.00
Control	Mean	7.40	4.20	1.20	2.40	7.20	5.60	8.00	5.40	1.60	3.60	5.00	6.40
	N	25	25	25	25	25	25	25	25	25	25	25	25
	Std. Dev	5.23	4.00	4.85	5.42	7.23	6.66	5.40	5.58	4.94	3.07	4.79	7.00
	SEM	1.05	.80	.97	1.08	1.45	1.33	1.08	1.12	0.99	0.61	0.96	1.40
	Median	5.00	5.00	0.00	0.00	5.00	5.00	10.00	5.00	0.00	5.00	5.00	5.00

4.6.1.2 Tympanometry

All 25 GD subjects had normal middle ear function as determined by tympanometry on the day of testing and the middle ear pressure was comparable between the groups. Table 4.10 shows the mean and standard deviation of the middle ear analysis for the right and left ear across the Gaucher phenotypes and the age-and-gender matched controls. The ear canal volume was noticeably smaller in the GD2 group but this most likely reflects the age of the participants.

Table 4-10 Mean tympanometric values in normal controls and in patients with GD.
SD is shown in brackets. Abbreviations: ECV – ear canal volume

	RIGHT EAR			**LEFT EAR**		
	ECV (ml)	**Compliance (ml)**	**Pressure (daPa)**	**ECV (ml)**	**Compliance (ml)**	**Pressure (daPa)**
Control (n=25)	0.90 (0.19)	0.70 (0.60)	7.00 (30.83)	0.89 (0.21)	0.72 (0.58)	-4.38 (32.41)
GD1 (n=7)	1.03 (0.43)	0.59 (0.19)	-9.29 (39.63)	1.09 (0.42)	0.66 (0.47)	0.71 (25.73)
GD2 (n=2)	0.48 (0.21)	0.60 (0.57)	-17.5 (45.96)	0.55 (0.21)	0.45 (0.21)	-20.1 (44.18)
GD3 (n=16)	0.87 (0.09)	0.60 (0.44)	-36.3 (31.67)	0.93 (0.14)	0.58 (0.38)	-28 (35.71)

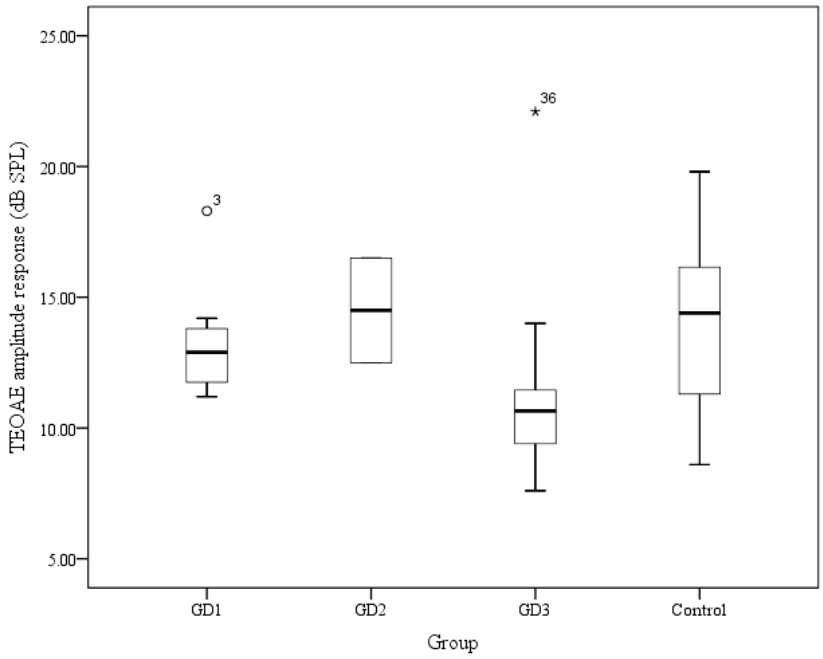
4.6.1.3 Transient evoked otoacoustic emissions

Transient evoked otoacoustic emissions were recorded in all 24 GD subjects. The mean TEOAE amplitudes in the non-neuronopathic group was 13.4 ± 2.4 dB SPL for the right ear and 12.5 ± 1.35 dB SPL on the left ear compared with 14.6 ± 3.6 dB SPL for the right ear and 12.5 ± 4.9 dB SPL for the control group. In contrast, a smaller mean amplitude was observed in both ears for the neuronopathic group (10.9 ± 2.2 dB SPL for the right ear and 10.2 ± 1.8 dB SPL for the left ear).

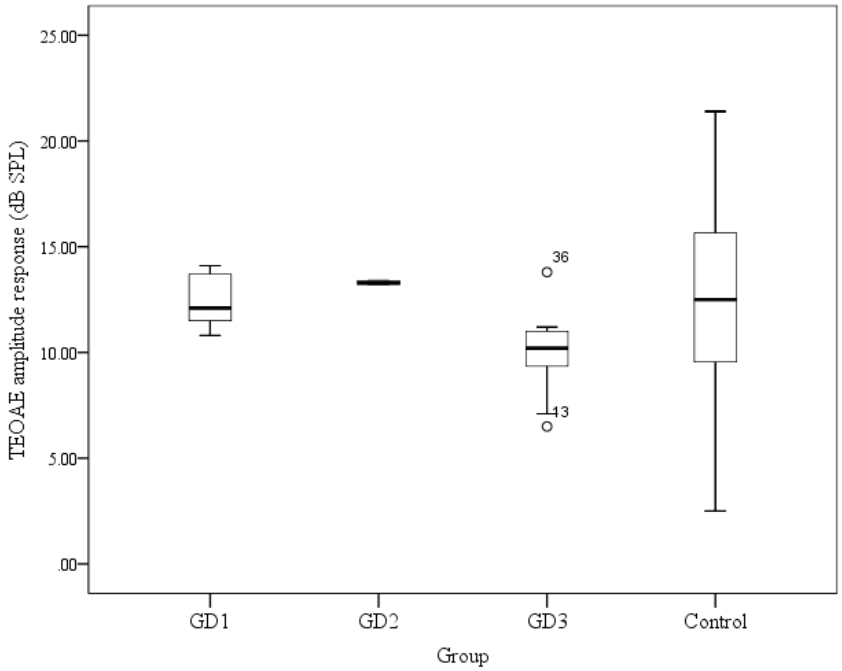
Figure 4.5 shows the 95% confidence intervals (95% CIs) for the means of each of the GD subgroups. The TEOAE amplitude responses are comparable across right and

Figure 4-5 Box-plots illustrating transient evoked otoacoustic emissions (TEOAE) amplitudes across the different GD phenotypes and in the age-and-gender matched controls. Amplitude response (dB SPL) for the right ear is shown in (A) and amplitude response data for the left ear is shown in (B).

A.



B.



left ears. The GD2 subgroup was too small (n=2) to perform any meaningful statistical analysis. However, when compared to the other two GD groups, the magnitude of the OAE amplitude response is greater in the two GD cases. TEOAEs are widely reported

as being larger in younger age groups compared with older age groups. For example, clinically normal adult ears have been reported to have a weaker response of < 3 dB SPL with little or no response at higher frequency bands. This decrease in cochlear response associated with the ageing process is thought to reflect the sensitivity of the OAE to damage (e.g. ototoxic damage, noise exposure) (Kemp et al., 1990, Hurley and Musiek, 1994, Oghalai, 2004, Robinette and Glattke, 2007).

Interestingly, there is a marked difference in variability between the left and right ears for GD2 cases. (i.e.) there is little variability on the left ear (13.4 dB SPL in case 1 and 13.2 dB SPL in case 2) compared with the OAE measurement on the right side (12.5 dB SPL in case 1 and 16.5 dB SPL in case 2). TEOAE measurement is highly sensitive to physiologic and ambient noise levels (Kemp et al., 1990, Norton and Widen, 1990, Hurley and Musiek, 1994, Robinette and Glattke, 2007) and the greater response on the right side of case 2 is due to an improved signal-noise ratio during recording (i.e. the infant was asleep and required no suctioning of the tracheostomy tube as a result).

Because there were no observable differences between ears, we combined the data from both ears to perform statistical analysis. The mean TEOAE amplitude was significantly different across the groups ($H(2) = 17.80$, $p<.05$). Mann-Whitney tests were used to follow up this finding. A Bonferroni correction was applied and so all effects are reported at a 0.0167 level of significance. It appeared that the TEOAE amplitude was not significantly different between the GD1 phenotype and the control group ($U = 297$, $r = - 0.08$). However, TEOAE amplitude was significantly lower in the GD3 phenotype when compared to normal age and gender matched controls ($U = 434$, $r = -0.40$) and when compared with the GD1 phenotype ($U = 69$, $r = -0.53$).

4.6.2 Brainstem tests

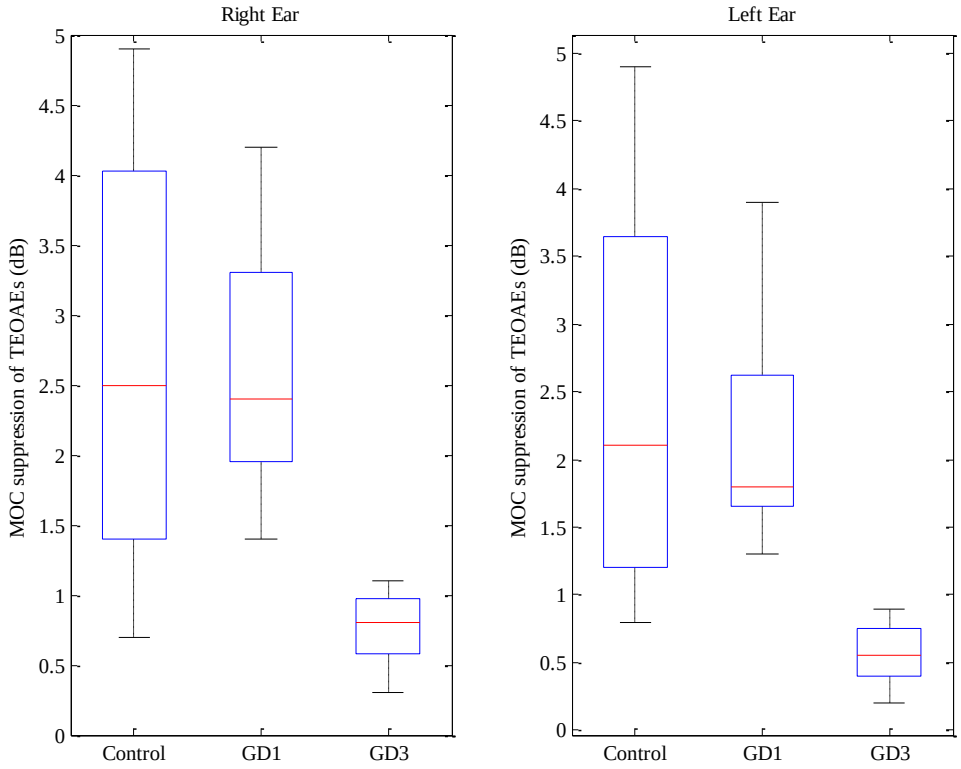
4.6.2.1 Acoustic reflex thresholds

We were unable to record the acoustic reflex threshold in 8/24 (33%) cases, because of poor co-operation (cases 8 – 10, 12, 22 – 25). In one case (case 17) acoustic reflex thresholds could not be obtained due to the patient's oversensitivity to the acoustic stimuli. ARTs were normal with ipsi-and-contralateral stimulation in 7 cases (cases 1-7) (range 80 – 100 dB) but abnormal (elevated or absent) in the remaining cases (cases, 13-16, 18-21).

4.6.2.2 Medial olivocochlear suppression

The suppression effect of the medial olivocochlear system was obtained by subtraction of the TEOAE responses under contralateral stimulation from those without contralateral stimulation. Medial olivocochlear suppression with contralateral stimulation was measured in 15/24 GD patients. We were unable to record MOCS in the remaining nine cases because the subjects were unable to accurately report 40 dB SL required to complete the test in 6/9 cases and because of poor co-operation in the remaining 3 cases.

Figure 4-6 Box-plots showing the medial olivocochlear suppression in patients diagnosed as GD1 and GD3 compared with control group.



The mean MOC suppression in the non-neuronopathic group was 2.8 ± 0.8 dB for the right ear (range: 1.9 – 4.2 dB, n=7) and 2.3 ± 0.8 dB on the left ear (range: 1.6 – 3.9 dB, n=15) compared with 2.9 ± 1.2 dB for the right ear (range: 1.4 – 4.6, n=15) and 2.4 ± 1.1 dB for the left ear (range: 0.8 – 4.6, n=15) for the control group. In contrast, in the neuronopathic group, suppression was found to be reduced or absent in both ears (0.7 ± 0.3 dB, range: 0.3 – 1.1 dB, n=8, right ear and 0.6 ± 0.2 dB, range: 0.4 – 0.9, n=8, left ear). Figure 4.6 shows a box and whisker plot of the GD phenotypes and the control group. Only 2/16 ears (12.5%) of GD3 subjects (95CI = 1.55-38.35) had normal suppression values greater than 1 dB.

Differences between the groups were tested for significance using the Kruskall-Wallis test. MOC suppression was significantly different across the groups ($H(2) = 33.68$, $p<.05$). Mann-Whitney tests were used to follow up this finding. A Bonferroni correction was applied and so all effects are reported at a 0.0167 level of significance. MOC suppression was not significantly different between the GD1 phenotype and the control group ($U = 199$, $r = - 0.04$). However, suppression was significantly lower in the GD3 phenotype when compared to normal age and gender matched controls ($U = 5.5$, $r = -0.79$) and when compared with the GD1 phenotype ($U = 0$, $r = -0.81$).

4.6.2.3 Auditory Brainstem Responses

The ABR was measured in all subjects across both ears. ABR waveforms, latencies and interpeak latencies were normal in 7/20 (cases 1-7) and abnormal in 13/20 cases (cases 8-10, 12-25). ABR waveforms, morphology and latencies were normal in all of the non-neuronopathic subjects (cases 1-7) but the ABRs in the neuronopathic group had a wide range of abnormalities including absent waveforms, delayed peak and interpeak waveform latencies in all subjects. The absolute latencies of waves I, III, and V and the interpeak intervals I-III, III-V, and I-V for each individual case are shown in Table 4.11

In the neuronopathic group, only wave I was identifiable all subjects although it was delayed in 1/17 (case 15). Wave III was normal only in 3 of the 17 cases studied (absent in 12/17; delayed in 2/17) on the right ear and normal in 6 of the 17 (absent in 9/17 cases and delayed in 2/17) for both ears. Wave V was abnormal on the right ear for all of the neuronopathic subjects (absent in 12/17; delayed in 5/17) and normal only in 1

Table 4-11 Individual ABR data for peak latencies.

Latencies are grand mean averages of two or more traces. Each ABR was recorded at an intensity level of 90 dB nHL. Abbreviations: AB – absent; NT – not tested

Case	Right Ear						Left Ear					
	Wave I	Wave III	Wave V	Interpeak I-III	Interpeak III-V	Interpeak I-V	Wave I	Wave III	Wave V	Interpeak I-III	Interpeak III-V	Interpeak I-V
1	1.56	3.74	5.66	2.18	1.92	4.10	1.56	3.67	5.45	2.11	1.78	3.89
2	1.49	3.60	5.33	2.11	1.73	3.84	1.49	3.50	5.47	2.01	1.97	3.98
3	1.51	3.50	5.40	1.99	1.90	3.89	1.51	3.54	5.44	2.03	1.90	3.93
4	1.63	3.65	5.35	2.02	1.70	3.72	1.49	3.60	5.46	2.11	1.86	3.97
5	1.68	3.60	5.28	1.92	1.68	3.60	1.59	3.66	5.37	2.07	1.71	3.78
6	1.54	3.49	5.45	1.95	1.96	3.91	1.50	3.53	5.50	2.03	1.97	4.00
7	1.49	3.52	5.43	2.03	1.91	3.94	1.52	3.49	5.37	1.97	1.88	3.85
8	1.75	4.2?	AB	2.45?	-	-	1.69	AB	AB	-	-	-
9	1.78	AB	AB	-	-	-	1.83	AB	AB	-	-	-
10	1.61	AB	AB	-	-	-	1.59	AB	AB	-	-	-
11	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
12	1.43	AB	AB	-	-	-	1.51	3.93	AB	2.42	-	-
13	1.64	4.40	6.32	2.76	1.92	4.68	1.58	3.80	6.18	2.22	2.38	4.60
14	1.50	AB	5.90	2.07	2.33	4.50	1.47	3.54	5.79	2.07	2.25	4.32
15	2.2	AB	AB	-	-	-	2.14	AB	AB	-	-	-
16	1.56	4.18	6.40	2.62	2.22	4.84	1.51	3.97	6.25	2.46	2.28	4.74
17	1.79	AB	AB	-	-	-	1.73	AB	AB	-	-	-
18	1.46	3.52	5.94	2.06	2.42	4.48	1.51	3.43	5.91	1.92	2.48	4.40
19	1.45	AB	AB	-	-	-	1.48	AB	AB	-	-	-
20	1.64	AB	AB	-	-	-	1.52	AB	AB	-	-	-
21	1.59	AB	AB	-	-	-	1.52	AB	AB	-	-	-
22	1.67	AB	AB	-	-	-	1.73	3.67	AB	1.94	-	-
23	1.78	AB	AB	-	-	-	1.70	AB	AB	-	-	-
24	1.73	3.5	AB	1.77	-	-	1.80	3.67	6.72	1.87	3.05	4.92
25	1.51	3.55	5.83	2.04	2.28	4.32	1.58	3.50	5.66	1.92	2.16	4.08

of the 17 cases (case 25) on the left ear (absent in 11/17; delayed in 5/17). Table 4.12 displays the peak latencies for each phenotype.

Amplitudes were measured for all peak waveforms. A nominal value of 0 μV was allocated when no waveform was evident. For illustrative purposes we also constructed a series of whisker and box plots of the amplitudes for the control group and across the different GD subgroups (Figure 4.8). Typically, the wave V amplitude is larger than other peak waveforms in the GD1 and control group. In contrast, in the GD3 group, wave I is the largest (range: 0.09-0.56 μV, right ear; 0.12-0.76 μV, left ear).

Table 4-12 Mean peak latency data presented for each GD phenotype.
Abbreviations: R – right ear; L – left ear

PHENOTYPE	**LATENCY (MS)**			
	Ear	**Wave I**	**Wave III**	**Wave V**
Non-neuronopathic (GD1)	R	1.56 (0.07)	3.59 (0.09)	5.41 (0.12)
	L	1.52 (0.04)	3.57 (0.07)	5.44 (0.50)
Neuronopathic (GD2)	R	1.77 (0.02)	-	-
	L	1.76 (0.10)	-	-
Neuronopathic (GD3)	R	1.64 (0.19)	3.83 (0.43)	5.69 (0.20)
	L	1.62 (0.18)	3.69 (0.20)	6.09 (0.38)

There were no observable differences between any of the groups for wave I amplitude, however the greatest decrease in amplitude for waves III and V was seen in the neuronopathic subtypes (GD2 and GD3) when compared to the control group and the non-neuronopathic GD subtype.

To compare the amplitude of each peak waveform for the GD groups and control subjects statistically we performed the non-parametric Kruskall-Wallis test. This showed highly significant differences between the groups and peak amplitude for wave III and wave V on the right ear and for all of the peak waveforms on the left ear ($p<0.05$). Post-hoc analysis showed that the peak amplitude was not significantly different between the GD1 phenotype and the control group ($U = 297$, $r = -0.08$).

However, amplitude was significantly lower in the GD3 phenotype when compared to normal age and gender matched controls (U = 434, r = -0.40) and when compared with GD1 patients (U = 69, r = -0.53).

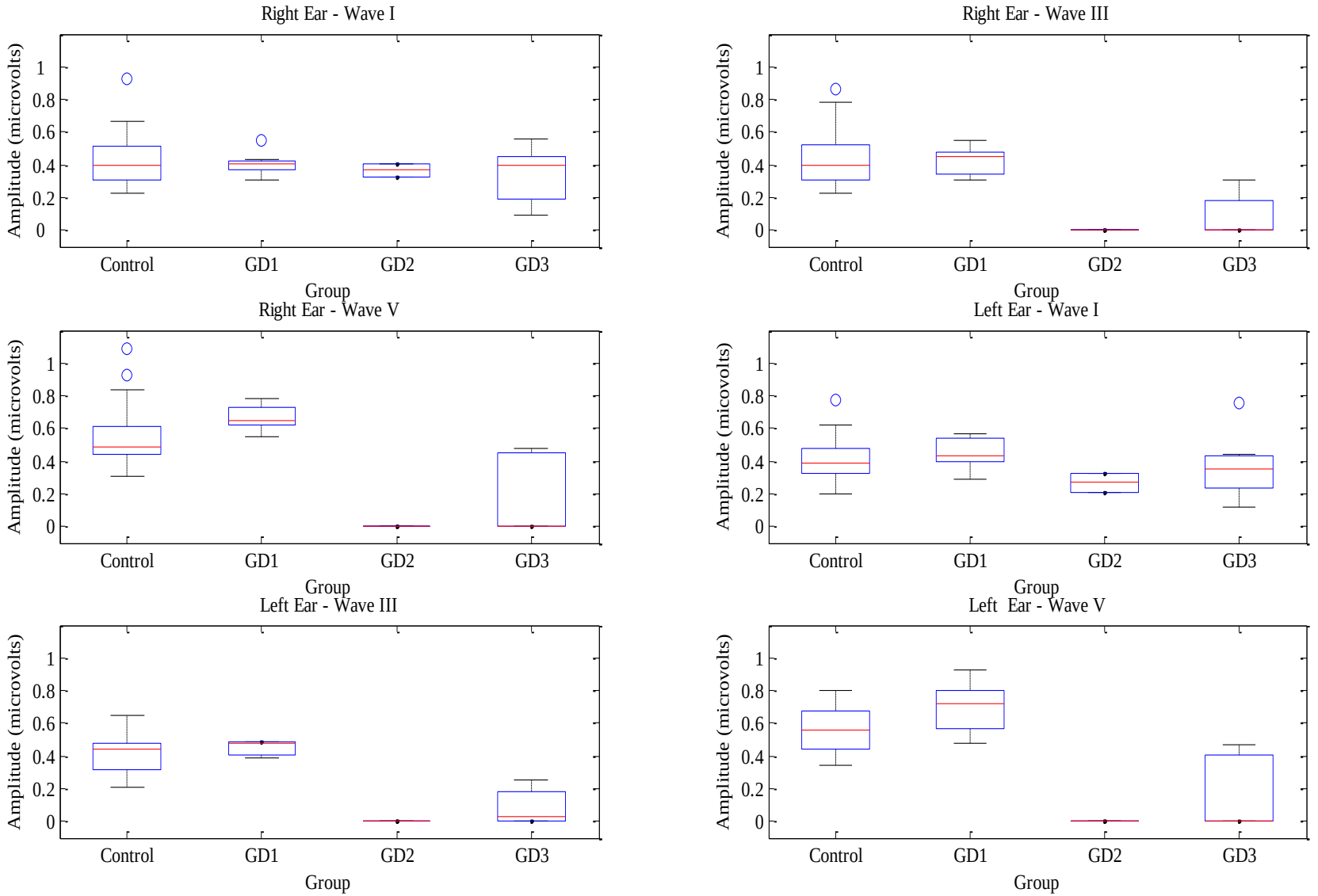


Figure 4-7 ABR amplitudes for peak waveforms (I, III and V) in patients diagnosed as GD1 and GD3 compared with an age-and-gender matched control group

4.6.3 Comparison of audiological findings and oculomotor signs

A summary table of oculomotor signs and auditory assessments for all of the subjects is shown in Table 4.14. Subjects clustered into two distinct groups. One group (n=7) had normal eye movements and normal auditory investigations (cases 1-7). The other group (n=18) exhibited SIF and or slow saccades, absent waveforms and poor morphology on the ABR, absent or elevated ARTs, abnormal MOCs and normal peripheral hearing (cases 8-9, 11-21). Table 4.13 shows a contingency table of eye movement and audiology test results. There was a 100% concordance between oculomotor and auditory findings and there is no gradation between the two groups.

Table 4-13 A 2x2 Contingency table of auditory and oculomotor tests in GD.

		EYE MOVEMENTS	
		Abnormal	Normal
AUDITORY	Normal	0	7
	Abnormal	18	0

Table 4-14 Summary of oculomotor and auditory tests (n=25) during the present study.
Abbreviations: ARTs = Acoustic reflex thresholds (dB), HL = Hearing loss (dB HL), MOCS = medial olivocochlear suppression test (dB), N/A = test not applicable due to the presence of a conductive hearing loss, N/T = not tested due to poor co-operation, OAEs = Otoacoustic emissions, PTA = Pure tone audiometry, TEOAE = Transient evoked otoacoustic emissions, TYMP = Tympanometry.

Case	Age	Sex	Phenotype	Eye Movements		Audiology					
				OKN	Saccades	PTA	TYMP	ARTs	OAEs		ABR
									TEOAEs	MOCS	
1	5	M	1	N	NT	N	N	N	N	N	N
2	8	F	1	N	N	N	N	N	N	N	N
3	14	M	1	N	N	N	N	N	N	N	N
4	15	F	1	N	N	N	N	N	N	N	N
5	12	F	1	N	N	N	N	N	N	N	N
6	7	F	1	N	NT	N	N	N	N	N	N
7	17	F	1	N	N	N	N	N	N	N	N
8	2	F	2	SIF	NT	N	N	NT	N	NT	AB
9	1.5	F	2	SIF	NT	N	N	NT	N	NT	AB
10	4	M	3	SIF	NT	N	N	NT	N	AB	AB
11	10	M	3	SIF	Slow L, R	AB	AB	NT	NT	NT	NT
12	10	F	3	SIF	Slow L, R, D	N	N	NT	N	AB	AB
13	4	F	3	SIF	NT	N	N	AB	N	AB	AB
14	3	F	3	SIF	NT	N	N	AB	N	AB	AB
15	1	M	3	SIF	NT	N	N	AB	N	NT	AB
16	4	F	3	SIF	NT	N	N	AB	N	AB	AB
17	7	M	3	SIF	NT	N	N	NT	N	NT	AB
18	5	F	3	SIF	Slow L, R, D	N	N	AB	N	AB	AB
19	11	F	3	SIF	Slow L, R, D	N	N	AB	N	AB	AB
20	14	F	3	SIF	Slow L, R	N	N	AB	N	AB	AB
21	11	F	3	SIF	Slow L, R	N	N	AB	N	AB	AB
22	4	F	3	SIF	NT	N	N	NT	N	NT	AB
23	9	F	3	SIF	NT	N	N	NT	N	AB	AB
24	2	M	3	SIF	NT	N	N	NT	N	NT	AB
25	6	F	3	SIF	NT	N	N	NT	N	NT	AB

4.7 Discussion

4.7.1 Baseline audiometry measures

Pure tone audiometry revealed normal hearing in 23/24 GD participants. Only one child (case 11) was found to have a bilateral moderate mixed hearing loss. We found no statistically significant difference in the hearing thresholds across the different phenotypes.

To the best of our knowledge this is the first study to have formally assessed the peripheral hearing in the acute neuronopathic subtype (GD2) despite previous studies providing vague behavioural descriptions (e.g. "unresponsiveness" (Lacey and Terplan, 1984) or a "poor response" (Kaga et al., 1998). It is surprising that this is the first time that any formal assessment of peripheral hearing has been undertaken in GD2; particularly as precise interpretation of *all auditory measures*, particularly the ABR depends upon establishing accurate levels of hearing.

There are sporadic reports of hearing loss scattered throughout the GD literature, although details provided in these publications are often ambiguous or lacking in detail (Dreborg et al., 1980, Schiffmann et al., 1997, Alfonso et al., 2007). The incidence of hearing loss reported in these papers varies widely from 4% report by Alfonso et al., (2007) to as high as 21% in Grasso et al., (2006). Moreover, the type of hearing loss also differs across studies. For example, one study describes 'neurogenic' loss in one case and a 'slight conduction impairment' in three GD patients (Dreborg et al., 1980).

The largest audiological study in GD showed that hearing was normal in 48/62 cases (41 GD1, 7GD3); abnormal in 13 GD1 patients and in 1 GD3 case (Grasso et al., 2006). This high incidence of hearing loss is alarming and potentially has wide implications for patients diagnosed with GD.

The authors highlight this concern in their study, arguing that *"...the prevalence of hearing loss among the general population is 10%. In our study, hearing loss is present in the 24% of subjects with GD1"* (Grasso et al., 2006, p70).

Our findings are very different. We found only one case of hearing loss in GD3 and no discernible hearing loss in any patient diagnosed with GD1. Indeed our TEOAE data were within normal limits for all GD1. Interestingly, the mean TEOAE amplitude data for the GD3 group was significantly below the GD1 and age matched control groups. This finding may indicate that there is subtle subclinical damage in the cochlea of these patients which has not been detected on the audiogram. This finding may reflect subclinical microvascular damage within the cochlea which may be due to abnormal blood flow, altered blood vessel properties or abnormal blood composition.

The reason for the discrepancy between our results and previously published studies is not clear. One possibility is that there are significant differences between patient groups; age differences, stage of the disease and treatment parameters. For instance, our sample are much younger (age range 1-17 years) than those described in the Grasso et al., (2006) study (age range of 5 months – 50 years; median 32 years). Furthermore, many of the children in our sample were on high-dose ERT which may have slowed or halted any potential degeneration (Vellodi et al., 2009).

Unfortunately, Grasso et al., (2006) have only published the degree of hearing loss (mild loss in 12 GD1 and profound in 1 GD1). In view of their normal tympanometry results – a finding consistent with our own study – it is unlikely that the hearing loss reported in their series of GD1 patients is conductive. Regrettably, they also failed to publish any individual frequency or ear specific data. Without more detailed information regarding their age range and onset of hearing loss we can draw no further conclusions.

Whether hearing loss is seen in patients with GD1 and/or GD3 at a later onset has still to be determined but because of the discrepancy between our audiometric findings and those reported by Grasso et al., (2006), we would recommend that all children diagnosed with GD undergo serial auditory evaluation regardless of phenotype. We will revisit the issue of the value of serial audiometric measures again in Chapter 5.

4.7.2 Brainstem auditory tests

4.7.2.1 Acoustic reflex thresholds

In our study, we found that acoustic reflexes thresholds were normal in all GD1 patients and abnormal in 8 cases of GD3. We were unable to reliably record the ART in 10/25 cases (40%) because of poor co-operation and hypersensitivity to loud sound in one case. In spite of this limitation, our data still lead us to conservatively conclude that acoustic reflexes are elevated or absent in *at least* 50% (8/16) of GD3 children.

This data is consistent with those previously reported by Bamiou et al., (2001) but differs significantly from those results reported in Grasso et al., (2006). They reported normal ARTs in 94% (51/54) of GD1 patients and normal ARTs in 87% (7/8) GD3 patients. It is conceivable that the differences between our studies may simply be a result of different testing techniques and interpretation. For example, Grasso et al., (2006) applied a stricter criterion (compliance change > 0.03ml), while we adopted the UK recommendations of >0.02ml (BSA, 1992).

The presence of both ipsilateral and contralateral abnormalities in the 8 cases of neuronopathic disease usually indicates central disease, localised to the cochlear nuclei and the olivary nuclei. These findings could reflect involvement of the facial nucleus or

its fascicle. In GD2 disease, 6th N palsies are common. The lengthy 7th fascicle proceeds dorsomedially and bends around the 6th nucleus at its genu and returns ventrolaterally before exiting the brainstem. Thus, it is conceivable that a midline lesion near the genu could affect both fascicles to give a bilateral lesion and also affect the horizontal saccade centres in the PPRF.

The functional role of the ART is unclear. It is thought to be important in protecting the inner ear from damage by continuous loud sound, but it is also thought to play an important role in improving speech perception particularly in noisy background environments. It is thought to achieve this by attenuating low frequency noise that can cause the upward spread of masking of the high frequency sounds. Our findings that the ART is impaired in GD3 patients results may indicate increased listening difficulty in an acoustically challenging background or an increased sensitivity to sound as described by a number of individual GD3 cases (Table 4.8).

Several post-mortem studies have previously implicated the central auditory nervous system in nGD (Winkelman et al., 1983, Erikson, 1986). However, because of a lack of studies in this area we cannot judge what the functional implications might be. Clearly future studies should consider including well-defined psychoacoustic studies and other behavioural measures of central auditory pathways as a logical and necessary development in this new area of research.

4.7.2.2 Medial olivocochlear suppression

In our study, we have clearly shown that patients diagnosed with GD3 demonstrated a significantly reduced suppression of TEOAEs when compared to both

the control group and GD1 patients. These findings are consistent with data previously reported in Bamiou et al., (2001). We could find no evidence to show that the MoCB was impaired in patients with GD1. As far as we are aware, this is the first time that MOCs has been reported in patients diagnosed as GD1.

It is difficult to interpret these findings because of the complexity of the anatomical pathways that are responsible for generating the response. The lack of suppression in the GD3 patients could reflect either (a) abnormal MOCS feedback or (b) may be due to inability to access efferent system via a dysfunctional afferent auditory pathway. Evidence from cases of unilateral auditory neuropathy would seem to indicate that impaired efferent suppression is due to poor synchrony in the afferent pathways.

The pathway involves the projection from olivocochlear neurons mostly in the medial region of the superior olivary complex (SOC) to the contralateral cochlea. Crossing olivocochlear axons pass the midline in the dorsal brainstem near the floor of the 4th ventricle passing under the genu of the facial nerve. Thus, it is plausible that lesions affecting the horizontal saccades could also compromise contralateral suppression.

The functional role of the efferent auditory system remains largely unknown. Moreover, the clinical significance of these findings is equally unclear. Evidence from animal and human studies has suggested that the efferent pathway enhances the frequency resolving capacity of the auditory system, particularly in the presence of competing acoustic signals or degraded acoustic signals (Kumar and Vanaja, 2004, Guinan, 2006, 2010).

Abnormal MOC suppression has been reported in a number of clinical populations including acoustic neuroma (Prasher et al., 1994), vestibular neurectomy (Williams et al., 1994), auditory neuropathy (Starr et al., 1996), patients with tinnitus (Ceranic et al., 1998), and in children with auditory processing disorder (Kumar and Vanaja, 2004).

Although our sample size is small, these results taken together with our ARTs data suggest extensive abnormalities within the auditory brainstem in GD3. These abnormalities could result in significant encoding and processing deficits. Further study is needed to clarify the extent of these abnormalities and their clinical relevance.

4.7.2.3 Auditory brainstem response

We found normal ABRs in the non-neuronopathic group and abnormal ABRs in all of the neuronopathic GD subjects. The severity of abnormalities in the nGD group were diverse and ranged from delayed waveforms, poor or absent wave III-V and in many cases with only one peak waveform (typically wave I) being identifiable in many cases. Our findings are unequivocal: we have shown no evidence for any auditory abnormalities in children with non-neuronopathic GD. We have also clearly shown that children with neuronopathic GD have a number of widespread auditory brainstem abnormalities.

We have previously shown in this chapter that the existing literature that has investigated the ABR in GD is baffling and difficult to interpret, because of a clear lack of control data and other methodological issues (See section 4.4.5). We do not wish to

add to this confusion, so in the following sections, we will compare our findings directly with the previously published studies for each phenotype.

4.7.2.3.1 Comparison of our findings with previous ABR studies in GD1

In our study, we could find no evidence of any ABR abnormality or on any of our other audiological measures in children with the GD1 subtype. Our finding is consistent with those reported in Accardo et al., (2005). Only five studies (n=77; 35 male, 42 females) that have used the ABR as a core index test have been reported in the literature (Ida et al., 1999, Accardo et al., 2005a, Perretti et al., 2005, Grasso et al., 2006, Accardo et al., 2010). We have summarised these studies and presented their key findings in Table 4.5.

The ABR findings across these studies were inconclusive – the ABR was reported as normal in 60/77 cases and abnormal in 17/77 cases; however two of the studies that had initially reported their patients here as 'type 1' later revised this diagnosis to 'type 3' (3 cases) (Ida et al., 1999, Accardo et al., 2005a). Eye movement data across these studies – which we have established is essential in establishing the correct phenotype – was limited, (Ida et al., 1999, Perretti et al., 2005) and in some cases, absent (Grasso et al., 2006). Without objective phenotyping with eye movement recording, the data presented by Grasso et al., (2006), Perretti et al., (2005) or Ida et al (1999) cannot be taken as evidence for significant abnormalities in type 1 disease.

Furthermore, it is not clear whether the abnormalities seen in some of the ABRs were due to peripheral hearing loss. Peripheral hearing was measured only in two studies (Ida et al., 1999, Grasso et al., 2006) and hearing loss was identified in 15

patients. However, it is not entirely clear whether hearing was actually measured in all of the patients described in the Ida et al., (1999) study. It is not possible to determine whether the prolonged latencies reported occurred as a result of the hearing loss (typically high frequency) or whether the ABR reflected an impairment in auditory pathways as a result of the disease process. An example of this is outlined in Grasso et al., (2006). They report a high incidence of hearing loss in the GD1 population (in 13/54 or 24%) which was a mild high frequency hearing loss in 12 cases and profound hearing loss in 1 case. Obviously we would not expect to see any ABR trace in a patient who for whom the stimulus was not audible. Unfortunately, audiometric data and ABR data for individual patients are not presented so we are unable to draw any further conclusions about whether hearing loss may explain the elongation in I-V. Further audiometric studies including pure tone audiometry, stapedial reflexes and tympanometry would be needed to clarify the origins of these abnormalities

Furthermore, none of the studies reported here presented any quantitative latency or amplitude data. Three studies failed to report any technical details regarding the ABR recording and did not publish their waveforms so we are unable to draw any conclusions (Schiffmann et al., 1997). The ABR waveform presented in the Perretti et al., (2005) study is from a L444P homozygote and typically expected in GD3 disease showing prolonged I-III interval and poor morphology. In the absence of definitive eye movement recording, we cannot accept these data as evidence of neurological involvement in GD1.

Control data was also limited across these studies. The question of abnormal electrophysiological findings is usually based on the standard deviation of in-laboratory control data. In the absence of control data, it would seem that several studies have

made independent decisions on each individual test in each individual. This is highly questionable as it will almost certainly lead to false positive claims.

4.7.2.3.2 Comparison of our findings with previous ABR studies in GD2

In contrast with the data presented for the GD1 patients, our findings in GD2 are extremely consistent with previously reported studies in this phenotype. Seven studies have previously reported ABR data in 11 GD2 (6M, 5F) (Kaga et al., 1982, Lacey and Terplan, 1984, Vivian et al., 1993, Kaga et al., 1998, Ida et al., 1999, Grasso et al., 2006, Miyata et al., 2006). The age of onset of these cases was variable, but most of the patients were diagnosed at birth or early in infancy; only one study measured the ABR in the latter stages (at 18/12 months; (Miyata et al. 2006)). SIF was reported in all of the cases with the exception of the Grasso et al., (2006) study which failed to report any other clinical details other than the audiological data.

In all of these cases, the ABR was abnormal, although none of the studies examined the peripheral mechanism to ensure normal hearing. All of the studies concluded that the ABR was abnormal in the 11 cases, with many of the studies identifying a progressive loss of components waves (Table 4.6). In each case, the patient died before their 2nd birthday. Abnormalities were very similar and all of the studies reported a loss of wave V and in some papers a loss of wave III was also observed. Generally the earlier ABR components (waves I and II) were preserved.

The technical data presented in each of the studies was limited. For example, click stimuli was used to elicit the ABR in 3 studies but was unreported in four studies; intensity levels used to elicit a response was reported in two studies (Lacey and Terplan,

1984, Grasso et al., 2006). Representative ABR waveforms were shown in only in 3 studies (Kaga et al., 1982, Kaga et al., 1998, Grasso et al., 2006).

4.7.2.3.3 Comparison of our findings with previous ABR studies in GD3

Our results in this study clearly showed that the ABR was markedly abnormal in all GD3 patients, without exception. Over the last 3 decades, only 8 studies have been undertaken in GD3 patients using the ABR (Abrahamov et al., 1995, Schiffmann et al., 1997, Altarescu et al., 2001b, Aoki et al., 2001, Bamiou et al., 2001, Goker-Alpan et al., 2005, Grasso et al., 2006, Cox-Brinkman et al., 2008) (Table 4.7). These studies represent the results from a total of 78 individual GD3 subjects. Collectively, the ABR was reported as being 'normal' in 56% of reported cases with GD3 and abnormal in 44% of the reported cases. We found that the ABR was abnormal in 100% of our patients. Eye movement abnormalities were a common finding across the other 7 studies, with one exception – one patient reported in Altarescu et al., (2001) showed no clinical signs of hSIF at the time enrolment into a clinical trial investigating ERT in GD3. Interestingly, they developed 'mild' hSIF during the initial treatment phase.

Why is there such a discrepancy between our data and previously reported studies? One possibility is the technical differences in experimental paradigms, standards, data processing methods and criteria used across studies, e.g. the implementation of different experimental conditions, type of stimulus used, etc. We have summarised these in Table 4.8. Another concern is the lack of multivariate statistical comparison to a control group. In the majority of the studies presented, limited descriptive analysis (e.g. ABR is normal or abnormal) is presented. In a number

of studies, no control data is shown. Furthermore, although the ABR is a quantitative measure of brainstem function - none of the studies included here have published any quantifiable latency and/or amplitude measures. This is the first study to present quantitative data not just descriptive analysis.

Alternatively, it is likely that there are group differences in treatment, particularly dose; genotype and age of the patients investigated which may explain the variation. Most of the patients enrolled in these studies were on ERT. Only two studies fail to mention whether their patients are receiving treatment (Abrahamov et al., 1995, Grasso et al., 2006).

Clearly the greatest barrier to using the ABR as a measure of brainstem dysfunction in GD is the lack of standardised testing and interpretation. If the ABR is to be used as a routine application in this area, then investigators in this area urgently need to reach some consensus.

4.7.3 Pathophysiology

At the beginning of this thesis (Section 4.1) we asked whether audiological tests when coupled with eye movement studies, can be used to provide reliable subclinical and pre-symptomatic tests of neurological involvement in GD? We believe that we have clearly demonstrated the sensitivity of the ABR as a marker of subclinical deterioration in nGD patients. In this section, we ask what the results of the ABR (and other audiological tests) tell us about the pathophysiology disease mechanisms in nGD.

The ABR test assesses whether auditory information reaches the brainstem in a synchronised manner or not (Hall, 2007) and does not provide any information on

clinical auditory correlates. The precise anatomical correlates of abnormal ABRs are uncertain and the ABR test is frequently considered as a whole rather than with separate analysis of its anatomically distinct generators. Waves I and II are thought to denote activation of its distal and proximal extra-axial portion of the 8th nerve, and waves III–V activation of more central relays in the brain stem, from the region of the dorsal cochlear nucleus to that of the inferior colliculus, although waves III–V will be affected secondarily when waves I and II are impaired or absent. If the cell body was the primary site of pathology in nGD then the inevitable outcome would be prompt death of its afferent dendrites and efferent axon and myelin sheath (Wallerian degeneration). This would have resulted in a loss of waves I-II on the ABR even in the presence of functional OHCs. However we have clearly shown that wave I was always evident in nGD.

However, it may be misleading to focus on the absence of specific waves because in all the nGD patients, the ABR morphology, with the exception of wave I, was extremely poor. The morphological abnormalities seen could be explained by problems in timing disparity. The ABR occurs within a few milliseconds of stimulation therefore volleys must be precisely timed to be recordable with surface averaging techniques. Poor synchronization results in abnormal waveform morphology and plummeting of the ABR amplitude (Starr et al., 2001). Minor jitter in timing within the auditory nerve can also interfere with precise phonemic decoding and, consequently, with the perception of speech sounds and other auditory tasks particularly in background noise (Starr et al., 1991, Berlin et al., 1994, Starr et al., 1996, Starr et al., 2001). It is tempting to postulate that part of the progressive intellectual decline as

reported in various studies in GD could be accounted for by specific central auditory deficits.

To our knowledge no systematic studies of auditory processing in GD have been undertaken although neuropathological studies have implicated several central auditory structures in GD including cerebral and cerebellar Purkinje cells, neuronophagia in the cerebral cortex, brainstem and other areas (Winkelman et al., 1983, Erikson, 1986). Further research is urgently needed to clarify these issues.

The combined findings of normal evoked otoacoustic emissions (TEOAE) and absent or markedly disturbed auditory evoked potentials from the brainstem are reminiscent of 'auditory neuropathy' (Starr et al., 1996). A set of salient features defining auditory neuropathy include elevated auditory thresholds to pure tone stimuli, absent to severely abnormal ABRs to high level stimuli, the presence of otoacoustic emissions (that do not suppress with contralateral noise stimulation), absent acoustic reflexes to both ipsilateral and contralateral tones and word recognition ability poorer than expected from the audiogram.

The pathology underlying auditory neuropathy is unknown, but can be caused by disruption in neural synchrony or by patchy damage to inner hair cells in the cochlea. Harrison (1998) developed an animal model of selective IHC loss resulting in many of the characteristics of auditory neuropathy (Harrison, 1998). We propose that the 'paradoxical' pattern of auditory abnormalities that we have seen in neuronopathic GD could reflect preferential damage to the IHC pathways with preservation of normal or near-normal OHC pathways. Following IHC degeneration there is associated loss of type I afferent fibres (Takeno et al., 1998) (which constitute 90-95% of the auditory

nerve) and that more central neurons (at least to the mid-brain) will also show some degeneration. Thus, central involvement is not precluded by this hypothesis (Salvi et al., 1999). Further audiometric studies including middle latency and cortical evoked responses are urgently needed to clarify the origins of these abnormalities.

4.7.3.1 Is there a link between auditory signs and eye movement signs?

It is not clear why either the auditory and saccadic systems appear to be preferentially affected in neuronopathic disease. Our data suggests that there may be a link between the eye movement and auditory abnormalities in neuronopathic GD. One possibility is that these pathways are more vulnerable as a result of some direct (or indirect) neurotoxic event. The unusual pattern of audiological and oculomotor abnormalities is consistent with an excitotoxic mechanism predisposing nerve cells to glucocerebroside toxicity, which is further exacerbated by high average neuronal firing rates. Recent animal studies have identified a possible candidate as glutamate, an excitatory amino acid that may be responsible (Korkotian et al., 1999, Pelled et al., 2000).

Recent studies have shown that neuronal cell cultures treated with glucocerebrosidase inhibitor have been found show an increased sensitivity toward glutamate and other metabolic inhibitors, suggesting compromised neuronal function. A release of intracellular Ca^{2+} appears to be related to this increased sensitivity, resulting in neuronal cell death (at least in neurons with elevated glucocerebroside levels) (Korkotian et al., 1999, Pelled et al., 2000). Therefore, GD neurotoxicity might be directly attributed to accumulation of the substrate itself. The addition of

glucocerebrosidase to the cultured cells prevents neuronal cell death. However, contributions to raised intracellular Ca^{2+} from other toxic agents, such as glucosylsphingosine, cannot be excluded (Nilsson and Svennerholm, 1982, Conradi et al., 1984, Orvisky et al., 2000).

Glutamate is known to have neurotoxic properties when released in large amounts or when incompletely recycled. In the cochlea, this 'excitotoxicity' leads to neuronal death in the spiral ganglion (Pujol and Puel, 1999).

Glutamate is also an important neurotransmitter in the supranuclear control of saccades. It is the dominant neurotransmitter for excitatory burst units (EBNs) (Leigh and Zee, 1999a), which provide the intense phasic pulse that drives the eyes at high speed during saccades. Dysfunction of these very active cells would lead to slow saccades. In addition, excitatory afferents of omnipause neurons (OPN) have been shown to be glutamanergic, possibly reflecting signals from the superior colliculus. OPNs are cells that fire continuously at high rates except during saccades. Dysfunction of these cells can also cause saccade slowing but could also interfere with saccade triggering.

Further elucidation of these mechanisms in GD is clearly needed, but we raise the hope that pharmacological agents directed at the neurotoxic process itself may halt or even reverse neurological progression.

4.7.4 Implications for correct phenotyping in GD

One of the aims of this chapter was to determine whether the combined use of ocular motor and auditory tests, could help improve phenotypic identification in GD. In

this study, we have clearly shown, for the first time, two distinct phenotypes with one group exhibiting normal eye movements and no audiological abnormalities that we could detect. A second group had uniformly abnormal saccadic eye movements and ABRs. Remarkably there were no exceptions in spite of a range of severity of disease. Our findings lead us to conclude that GD1 and neuronopathic GD (GD2 and GD3) phenotypes are distinct based on these tests.

These results are exciting and the application of audiological testing may offer an inexpensive solution to the dilemma that we are now seeing in patients diagnosed with GD1 but who are presenting with additional PD signs.

4.8 Conclusions

In this chapter, we systematically investigated the audiological function in 25 children with enzymatically diagnosed GD using a series of tests that measured the integrity of overlapping but not identical efferent and afferent pathways and brainstem structures in the auditory system. All of the children enrolled in this study had undergone formal eye movement studies. The data from our study has clearly shown a) that combined auditory and oculomotor studies can be used to better delineate the underlying neurological deficits in nGD and b) GD1 and GD3 are really distinct phenotypes based on auditory and eye movement testing.

We could find no indication of gradations between the two groups, consistent with the notion of two distinct phenotypes –neuronopathic and non-neuronopathic. Although our sample size is small, the data also suggests that audiological abnormalities are more commonplace than previous electrophysiological studies have shown. This

novel finding has important implications for the habilitation of children diagnosed with nGD.

We believe that these findings provide sufficient evidence to warrant the inclusion of audiological testing as part of the standard assessment of newly diagnosed GD patients and recommend that they undergo these tests prior to commencing treatment. Furthermore, these tests may have a wider application as longitudinal outcome measures for use in clinical trials or as markers of neurological burden in GD. We examine this in more detail in the next chapter.

Chapter 5
The utility of the ABR as a longitudinal measure in neuronopathic Gaucher disease

5.1 Introduction

"The development of valid clinical instruments for appraising each LSD and its effects on key life outcomes is but a science in the earliest period of embryonic development" (Cox, 2009, p2)

The general outlook for patients with GD (and related orphan diseases), for which new treatments have been introduced, has infinitely improved (Goker-Alpan et al., 2008, Benko et al., 2011). However, in the race to develop these 'extremely profitable' new treatments, some researchers have criticised the lack of scientific investment in developing outcome measures for monitoring brainstem dysfunction in these patients and for use in clinical trials. They have forcefully argued that resources have been focussed solely on developing (or modifying) drug therapies, to the exclusion of developing tools to monitor the progression (or remission) of neurological involvement in GD (Cox, 2010b, Elstein, 2011).

These philosophical arguments – concerning the ethics of orphan drug treatments – are highly contentious and scientifically laudable, but they have also inadvertently unveiled a parallel concern, highlighted in Miekle and Hopwood (2003):

"the effectiveness of these therapies, particularly for those LSD involving CNS and bone pathologies, will rely heavily upon the early diagnosis and treatment of the disorder, before the onset of irreversible pathology" (S36).

Our ability to test the neurological efficacy of new therapies is very limited, if not absent, for the young or cognitively impaired child. What tools or techniques can we use to determine the success of these different therapies that are tasked with halting (and ideally reversing) the neurological component and meet an unprecedented demand for *early diagnosis* and longitudinal monitoring of disease status in these conditions.

In our previous chapter, we showed that combined auditory and oculomotor studies could be used reliably to detect the presence of neuronopathic disease before overt neurological signs and symptoms become manifest. As such these tests have the potential to provide a means for preclinical diagnosis of brainstem disease in nGD in the very young child. In this chapter, we build on these results and ask whether the auditory tests could be used to measure the disease burden for those patients on treatment.

Two key questions arise:

(3) *Can the ABR be used for long term monitoring of any neurological progression in GD?* Little is known about the fluctuations in the ABR over time and if we hope to exploit the full potential of the ABR for longitudinal monitoring, then it is imperative that we address this issue.

(4) If the ABR is shown to be a sensitive and reliable longitudinal measure of subclinical neurological disease burden, then *can the ABR be used to capture any drug efficacy over the limited time frame of clinical trials?*

In this Chapter, we present two empirical studies. Our first study is concerned with our question of whether the ABR can be used to reliably measure the disease burden in the neuronopathic subtypes of GD. We present data for the first time of serial ABR recordings in type 2 and type 3 GD. In our second study, we take an in-depth look at an emerging and promising treatment, i.e., substrate reduction therapy (SRT), an alternative approach to the current enzyme replacement therapy. We then present our data on use of the ABR as a secondary outcome measure in the substrate restriction therapy GD3 trial.

5.2 Experiment 1: The utility of the ABR in monitoring the disease burden in nGD

5.2.1 Introduction

Biomarkers are objective, biological measurements that are used to aid in the diagnosis of disease and to monitor disease progression. However it is clear that not all biomarkers that have great value for diagnosis are suitable when it comes to monitoring disease progression. Indeed, some offer little value in this regard. This is particularly true in the study of GD where the application of systemic biomarkers *do not* necessarily reflect the neurological component of the metabolic disease.

Let's consider one example relating to the diagnosis of GD. At present, the definitive diagnosis of GD is based on the activity level of the enzyme glucocerebrosidase. In individuals with GD, the enzyme activity is significantly lower than in individuals without GD. However, as we have previously argued in Chapter 4, there is no evidence to show that this biomarker is able to differentiate between non-neuronopathic and neuronopathic phenotypes (Pentchev et al., 1983). Furthermore, it has no value in predicting the rate of neurological progression or severity (Beutler and Gelbart, 1996, Cox, 2010b, Elstein, 2011).

In order to monitor neurological progression in GD, we need to look for a different set of markers that specifically serve this purpose – suitable neurological markers that can detect change in neurological function. Several neurological biomarkers have been proposed as having a potential utility as a biological marker of disease burden in nGD. These putative biomarkers include the clinical evaluation of a subset of neurological symptoms, (e.g. assessing mental state, cranial nerves, motor skills, and other neurological symptoms; (Schiffmann et al., 1997, Altarescu et al.,

2001b)), somatosensory evoked potentials (Garvey et al., 2001) and a series of neuropsychological assessments (such as Purdue Peg Board test, Wechsler Scale, Benton visual retention test, Rey auditory verbal learning test, d2 test of attention, continuous performance test, and Trail Making Test (Benko et al., 2011).

However, many of these routine clinical markers have no quantitative data and their use in monitoring response to therapy is poorly defined. Furthermore, many of these assessments are critically dependent upon a considerable degree of co-operation and communication.

An alternative approach, which has been used to document neurological involvement in GD, has involved the use of a questionnaire-based severity scoring measurement. Two scales are routinely used to assess the severity of involvement for patients with GD (Zimran et al., 1992, Di Rocco et al., 2008). Of note, these scales calculate all neurological involvement in one domain only, and are not sensitive enough to track neurological changes seen over time. More recently, Davies et al. (2007) developed a list of clinical signs and symptoms, along with a severity-scoring system, for patients with nGD. Unfortunately during the development of this tool, authors excluded reports of type-2 patients, and focused only on the severity and progression of symptoms in patients with GD3 (Davies et al., 2007).

One attempt to address this gap in our clinical knowledge resulted in the establishment of a specialist (European-wide) Task Force on nGD. They were tasked with developing guidelines for the assessment and monitoring the neurological aspects of GD (Vellodi et al., 2001). These guidelines recommended a comprehensive clinical examination at the initial assessment to include formal eye movement studies (where

possible), audiometry, neuro-ophthalmology assessment, brain imaging (MRI or CT), electrophysiological measures (EEG and ABR) and psychometric testing to establish the distribution and types of neurological abnormalities that may be present. Table 5.1 shows a summary of the serial measures and schedule that are recommended, following the initial clinical assessment.

How well these guidelines were received or more importantly, how well they were implemented remains unclear. In the following sections, we consider the evidence to support the application of the ABR as a longitudinal measure in normal hearing subjects and in patients diagnosed with GD.

Table 5-1 Minimum clinical protocols for longitudinal monitoring neurological involvement in GD.

Follow up assessment		**Time Frame (mo)**					**Other information**
		3	*6*	*9*	*12*	*O*	
Clinical examination	Neurological exam	X	X	X	X		In year 1, a neurological exam should be undertaken every 3 months and then every 6 months after.
	Eye movement examination (*preferably formal*)		X				
	Neuro-ophthalmological investigation				X		
	Measurement of peripheral hearing				X		
Brain imaging	MRI (or CT if MRI not available)					X	Only if clinically indicated
Neurophysiology	EEG					X	Only if clinically indicated (eg) seizures.
	ABR				X		
Neuropyschometry	IQ tests				X		Recommend widely available tests (eg) WISC-III

5.2.1.1 The longitudinal study of the ABR in normal human subjects and in neurological cases

Previously in Chapter 1, we argued that the ABR test had the potential to objectively track changes in the status of the auditory brainstem pathways. The literature offers a number of examples of this attribute, including documentation of the natural fluctuations in ABR activities that occur in normal human subjects (Chiappa et al., 1979, Edwards et al., 1982, Tusa et al., 1994). Longitudinal studies of the ABR have also been used to assess changes in brainstem function in patients diagnosed with MS and head trauma (Robinson and Rudge, 1978, Aminoff et al., 1984, Nuwer et al., 1987, Schoenhuber et al., 1987).

One the earliest studies to assess the reliability of the ABR, as a longitudinal measure, was reported by Chiappa et al., (1979). They measured the ABR in a small group of normal hearing participants (n=8), over an interval spanning 2 – 9 months. Their study showed no statistically significant changes in ABR latency or amplitude, suggesting that the ABR is a robust technique for serial application (Chiappa et al., 1979). Similar findings were also reported in Edwards et al., (1982) and Robinson and Rudge (1978).

Repeated ABR measurements were also examined in a larger sample (n= 87 normal hearing subjects) over a longer time frame (2 years). Although the ABR was shown to have an increased variability in latency and amplitude compared to previous studies, it was not considered clinically significant. The authors concluded that the ABR was a 'stable' electrophysiological measure, that could be used longitudinally to capture changes (if any) in auditory brainstem disease (Tusa et al., 1994).

Serial ABRs have also been used to assess the integrity of brainstem pathways in a number of neurological disorders, particularly in MS. In contrast to the studies in normal hearing participants, studies that have used the ABR as a longitudinal measure in neurological disease have reported wide variation in the latency and amplitude of the ABR component (Robinson and Rudge, 1978). One study reported that the ABR was an extremely reliable measure in control subjects and in clinically stable MS patients over 2.5 years. However, the ABR in *some* patients who had clinical relapses during the study showed significant fluctuations in both the latency and amplitude parameters (Robinson and Rudge, 1978). This finding was supported by another study in 12 patients diagnosed with MS, that also showed excessive variability between test sessions even when the clinical deficit was stable (Aminoff et al., 1984). In contrast, Nuwer et al., (1987) described the ABR as a 'sensitive and objective measurement' based on recordings that took place over a 3 year double-blind, placebo-controlled drug trial in MS (Nuwer et al., 1987).

The reason for the discrepancy between these studies is not clear. One possibility may reflect different technical applications of the ABR, or a heterogeneous MS population with varying expression of symptoms and ages. Clearly, the role of the ABR in monitoring disease progression requires further study.

5.2.1.2 The longitudinal study of the ABR in GD

Six studies have recorded serial ABR measures, since the introduction of the European guidelines more than a decade ago (Altarescu et al., 2001a, Aoki et al., 2001,

Cox-Brinkman et al., 2008, Benko et al., 2011). These longitudinal studies are summarised in Table 5.2.

Two studies have monitored the ABR in GD2 infants. Both documented a clear deterioration in the ABR over a very short time interval (1-2 months). Moreover, they identified that ABR abnormalities were present at a very young age (age 7 weeks) (Lacey and Terplan, 1984). Because of the relentless neurological progression observed in GD2, treatment is usually palliative. There have been no studies investigating whether ERT (or any other treatment options) can modify the ABR in GD2.

The picture in GD3 is more confusing and the potential for the ABR to be used as an outcome measure for monitoring neurological disease remains unclear. One study has recently claimed that it is:

"possible to study the longitudinal course of a complex neurometabolic disorder such as GD3 with objective neurological, neurophysiological and neuropsychological endpoints" (Benko, 2011, p.8).

However, from the limited data presented in this study, it is not entirely clear how well the ABR fulfils that role. From the small number of studies conducted, it is clear that although a few investigators have recorded ABRs in GD, there is still no agreement as to whether audiological investigations could be a sensitive longitudinal marker of disease burden. Our goal in this experiment was to determine whether the ABR could be used to provide objective evidence of the time course of neurological involvement (progression or remission) in patients diagnosed with nGD who were undergoing high dose ERT.

Table 5-2 Summary of longitudinal ABR studies previously reported.

Author	Year	n	Phenotype	Treatment	ABR Baseline	Follow up (yrs)	ABR Final
Kaga et al.	1982	1	GD2	None	Abnormal – waves I,II and III present but prolonged	2/12	Abnormal – waves I and II present but delayed. Wave III present but prolonged on left ear only
Lacey and Terplan	1984	1	GD2	None	Abnormal – waves I, II and III present in both ears	1.5/12	Abnormal – only waves I and II present
Altarescu	2001	21	GD3	ERT	Normal ABR in 11 cases; Abnormal ABR in 10 cases	2 – 8	11 patients were reported with normal ABRs, of which only 2 showed deterioration. Of the remaining 10 with abnormal ABRs, there was no deterioration, and 2 even showed improvement
Aoki	2001	1	GD3	ERT	Only wave I was present in both ears	1	ABR improved following treatment with ERT - waves, I-II, IV-V on one side and wave V on other ear were observed.
Cox-Brinkmann	2008	3	GD3	Cases 1 and 2 were on ERT only but followed with 12 mo. Of comb ERT/SRT Case 3 has only had comb therapy	Case 1 - normal ABR reported Case 2- report a 'grossly abnormal ABR' – waves I and V only Case3 - mildly prolonged waveforms	3 – 7	Case 1 - ABR deteriorated (absent wave IV/V and prolonged I-III) Case 2 – no change Case 3 – ABR normalised following comb therapy.
Benko et al.	2011	15	GD3	All patients except one were on enzyme replacement therapy (ERT) years, while patient 14 was successfully treated with hematopoietic stem cell transplantation. 10 patients 'temporarily' received investigative SRT as part of a clinical trial	4/15 had normal ABRs, varying degrees of ABR abnormalities were reported in the other 11 patients.	2 – 4	Of the 15 cases presented, only 12 had serial measures. 9/12 cases had an abnormal ABR on the right ear and 8/12 had an abnormal ABR on the left.

Here we present our serial ABR findings in a group of GD3 children who were receiving high-dose ERT and in two infants diagnosed with GD2 (pre and post ERT treatment). A preliminary account of this work has appeared in two papers (Campbell et al., 2003, Campbell et al., 2004).

5.2.2 Methodology

5.2.2.1 Subjects

Out of a pool of 14 potential patients with nGD, 10 patients underwent serial ABR testing. The remaining 4 cases were not included in this study: two children had received bone marrow transplants (cases 12, 20) and were not receiving ERT. One case had a mixed moderate hearing loss and has since died (case 11). We were unable to complete ABR testing in one child (case 10) as they were no longer resident in the UK.

All of the remaining 10 patients (2 males) had been enzymatically diagnosed with GD and subsequently genotyped – 17/20 alleles were the L444P mutation, 1/20 alleles were D409H, 1/20 c1263del55 and 1/20 unknown. A summary of individual clinical characteristics and treatments is presented in Table 5.3. All of the GD3 patients had been receiving high-dose ERT (120 IU/kg/2weeks) for 1- 3 years. Each of the patients had a significant improvement in their overall systemic response with no overt neurological changes observed during this study.

5.2.2.2 Procedures

All subjects had standard baseline audiological assessment as described in detail in Chapter 3. All of the patients described in this chapter had established SIF (Table 5.3) as confirmed by an experienced clinician. Other oculomotor abnormalities were

identified in cases 8 and 9 following clinical examination. These are described in detail in the Section 5.2.3.1 below.

The ABRs were recorded to clicks (100 μs in duration) of alternating polarity presented at a rate of 11.1/s at an intensity of 90 dB nHL using TDH-49 headphones as previously described in Chapter 3 (section 3.5).

Table 5-3 Summary of clinical characteristics and treatments

Case	Sex	Neurological signs	Genotype	Phenotype	Treatment
8	F	*	L444P/c1263del55	2	ERT
9	F	*	L444P/U	2	ERT
13	F	SIF	L444P/D409H	3	ERT
14	F	SIF	L444P/L444P	3	ERT
15	M	SIF	L444P/L444P	3	ERT
16	F	SIF	L444P/L444P	3	ERT
17	M	SIF, Tourette-like Symptoms	L444P/L444P	3	ERT
18	F	SIF	L444P/L444P	3	ERT
19	F	SIF	L444P/L444P	3	ERT
21	F	SIF	L444P/L444P	3	ERT

Abbreviations: U- unknown, ERT – enzyme replacement therapy, SIF = saccade initiation failure.
* Neurological features for cases 8 and 9 are described in further detail in section 3.2.3.1.

For cases 8 & 9, the initial ABR test and audiometry were carried out *before commencement of ERT*; thus the follow-up studies, in these two infants, described ABR, post-ERT treatment. We discuss cases 8 & 9 individually in section 5.2.3.1, below and present a comparison of the two cases who were not receiving any treatment at baseline.

The ABR data presented in Section 5.2.3.2, describes the remaining 8 cases of GD3 (cases 13-19, 21; 2 males), which was undertaken in GD3 patients who were already receiving ERT; as such no pre-and-post ERT data was available for comparison. The follow-up ABR test and audiometry were carried out 1 to 3 years later. Ethical Committee approval was obtained for the study and informed consent was obtained from the parents of the younger children and from the older subjects themselves.

5.2.3 Results

5.2.3.1 Clinical course and audiological findings in GD2

5.2.3.1.1 Case 8

This female child was born at 37 weeks to healthy unrelated parents following an uncomplicated pregnancy. She was admitted to the special care baby unit because of meconium aspiration. At this time she was also noted to have conjugated hyperbilirubinaemia and thrombocytopenia, which resolved spontaneously, and she was discharged at one month of age. Hepatosplenomegaly was noted prior to discharge. She was re-admitted at 6 months for poor feeding and failure-to-thrive. At 7 months she was cachectic, with weight and head circumference below the 3rd centile. Head control was poor and there was generalised hypotonia with mildly increased reflexes. She had marked hepatosplenomegaly. At 7 months, oculomotor testing revealed full ocular motor range, and Doll's head manoeuvre was normal. No strabismus was seen.

There was a complete absence of horizontal saccades, and horizontal headthrusts were evident although head control was generally poor. Optokinetic stimulation showed an absence of downward saccades, but some upward saccades could be elicited. White cell enzymes showed a low level of beta-glucosidase and elevated plasma chitotriosidase. Mutational analysis demonstrated compound heterozygocity for the L444P and c1263 del 55 mutations, confirming the diagnosis of Gaucher disease. Brain MRI and EEG were normal. EMG showed clear evidence of neurogenic change in the genioglossal and minor changes in the two facial muscles with masseter possibly showing some neurogenic change, consistent with a bulbar palsy. Video fluoroscopy demonstrated moderate oral phase dysphagia.

Initial audiological investigations were carried out at 7½ months. Visual reinforcement audiometry showed normal responses to stimuli between 0.25 and 4.0 kHz consistent with normal peripheral hearing in at least one ear. Tympanometry was normal indicating normal middle ear function. Transient evoked otoacoustic emissions (TEOAEs) were normal with amplitudes of 12.5 dB on the right ear and 13.4 dB for the left ear. The ABR was abnormal with waves I – III being recorded at normal peak latencies and wave V absent. An emergency tracheostomy was performed following frequent laryngospasms and upper airway obstruction. She was commenced at 8 months on high dose infusions of macrophage-targeted placental glucocerebrosidase (Ceredase®) at 120 IU/kg administrated every two weeks.

She responded systemically to ERT over the next 1-2 months however, her neurological signs deteriorated. Table 5.4 shows the biomarkers commonly used to assess the efficacy of systemic responses to treatment. At 8½ months a gastrostomy was performed due to aspiration.

Table 5-4 Systemic response to ERT at the commencement of treatment and at follow-up.

Parameter	**Normal Range**	**Case 8**		**Case 9**	
		Baseline	*Final*	*Baseline*	*Final*
Dose		120	120	120	120
Haemoglobin (g/dl)	10.5 - 13.5	10	13.8	9	13.8
White cell count (l)	6 - 18 (x 10^9)	**12.7**	**14.56**	**5.43**	**12.15**
Neutrophils (l)	2 - 8.5 (x 10^9)	4.44	6.11	2.33	**8.91**
Platelets (l)	150 - 450 (x 10^9)	**99**	**617**	**94**	251
Chitotriosidase (nmol/hr/ml)	0-150	**21008**	**2992**	**10164**	**3903**

Subsequent ABRs carried out at 9 months showed increased latencies of waves I, II and III. At 11 ½ months myoclonic seizures occurred. Repeat ABR performed at this stage showed only wave I. Pre-and-post ERT ABR recordings are shown for both

infants in Figure 5.1. At 16 months she succumbed to an overwhelming chest infection. Post mortem was not carried out.

5.2.3.1.2 Case 9

This female infant was born by normal delivery to healthy non-consanguineous parents at 41 weeks following an uneventful pregnancy. There were no neonatal concerns, but feeding difficulties, recurrent vomiting and failure-to-thrive emerged. By 4 months there was uncoordinated swallowing and abdominal distension. Hypotonia and a bilateral convergent strabismus had developed by 6 months, and she was referred to our tertiary referral centre at 7 months. Examination revealed loss of motor skills, strabismus, respiratory difficulties, abnormal oesophageal motility and hepatosplenomegaly. Oculomotor examination revealed some limitation of up and down gaze, full adduction and limited abduction of each eye. A vestibulo-ocular reflex was present to Doll's head manoeuvre.

Optokinetic testing revealed a tonic deviation in the direction curtain rotation, with a complete absence of horizontal and vertical saccades, and headthrusts and synkinetic blinks were occasionally observed. Supranuclear brainstem lesions and probable bilateral abducent nerve palsy were indicated, and GD2 was suspected. Other ophthalmic investigations were unremarkable. EEG, motor and sensory nerve conduction studies were normal. White cell enzymes showed a low level of beta-glucosidase and elevated plasma chitotriosidase. Molecular genetic analysis demonstrated that she was a compound heterozygote for the L444P and an unknown mutation, confirming the diagnosis of GD.

Initial audiological investigations were carried out at 8 months. Air conduction thresholds measured between 0.25 and 4.0 kHz were normal, consistent with normal

peripheral hearing in at least one ear. Tympanometry was within normal limits indicating normal middle ear function. Transient evoked otoacoustic emissions (TEOAEs) were normal with the response amplitude signal-to-noise ratio of at least 4dB and waveform reproducibility in at least three octave bands of >75%. TEOAE amplitudes were 16.5 dB right ear and 13.2 dB left ear. The initial ABR measured before the commencement of high dose ERT. All recordings were abnormal with waves I – IV being recorded at normal peak latencies and wave V absent.

At 9 months, she was commenced on high-dose infusions of macrophage-targeted placental glucocerebrosidase (Cerezyme®) at 120 IU/kg administrated every two weeks. Over the next 3-4 months, there was a systemic response, with decreased chitotriosidase levels and haematological improvement (Table 5.4). However, neurological signs did not improve. Serial ABRs (at 9, 10 months) showed a gradual increase in latencies of waves I, II, and III, and by 11 months only wave I and II could be detected (Figure 5.1).

At 10 months a tracheostomy was performed following life threatening laryngospasm. At 13 months, oculomotor responses deteriorated with reduce vertical motility, and facial nerve palsy emerged. A gastrostomy was performed at 15 months for swallowing difficulties. By 18 months there were myoclonic seizures and dystonic posturing. She was unable to sit unsupported, and head control was poor. ERT was discontinued at 19 months At 20 months there were uncontrolled seizures and EEG revealed an excess of slow background frequencies and frequent inter-ictal epileptiform discharges. Respiratory distress with probable central apnoeic episodes emerged, and she died at 25 months from hypostatic pneumonia. A postmortem was not performed.

Figure 5-1 A: ABR in case 1 showing traces at baseline (before commencement of ERT) and final recording (after commencement of ERT). B: shows the ABR recorded from case 2.

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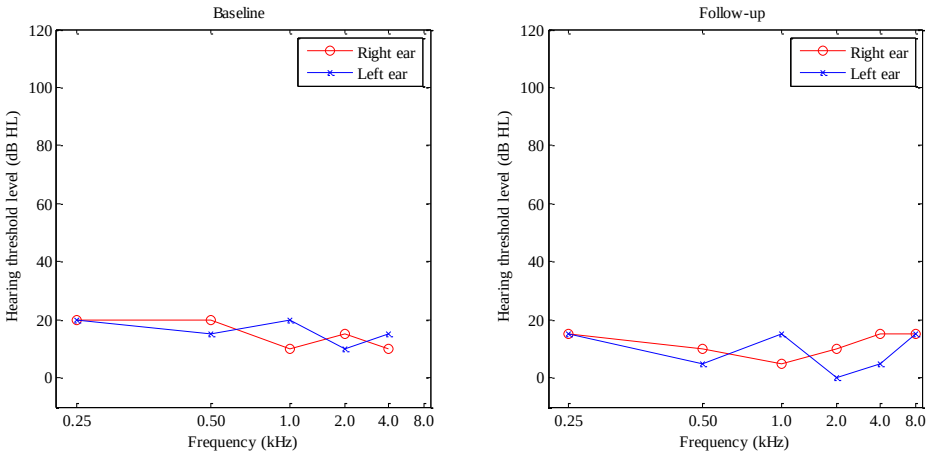
5.2.3.2 Audiological monitoring in GD3 children

Pure tone audiometry, tympanometry, otoacoustic emissions were normal in all subjects. A representative audiogram of the most severely affected case (case 18) is shown in Figure 5.2. The audiogram shown in Figure 5.2 shows no change in hearing levels' (i.e.) hearing is normal on both sides when measured at baseline and at the final measurement (Table 3.1), despite deterioration in the ABR, over the same 18 month interval (Appendix 2, section 9.2.6). Clearly, in the case, the change in ABR cannot be attributed to deterioration in the peripheral auditory system.

ABR findings revealed diverse results including prolonged peak and inter-peak latencies for waves I-V and generally dysmorphic wave formation. ABRs had normal latencies for waves I-V in 3 patients (cases 15, 19, 21) and prolonged or absent waves in 5 cases (cases 13-14, 16-18).

At a stimulus intensity of 90 dB nHL, wave I was identifiable in 8/8 cases. Wave III was recorded in all of the cases but was delayed in 1/8 cases (case 16). Wave V showed the greatest abnormalities and was absent in 2/8 of all the cases (cases 13, 18) and delayed in 3/8 cases (cases 14, 16, 17). The morphology was abnormal in all patients, with markedly reduced peak-to-peak amplitudes and wave V/I amplitude ratio reversal (Figure 5.3).

Figure 5-2 The pure tone audiogram of the most severe ABR abnormality (case 18) is shown at the commencement of the study and at follow-up (18 months later).



ABRs recorded after 1-3 years showed a clear deterioration in all cases, with worsening morphology leading to extinction of waves, and where discernible, waves III and V became delayed. Wave I was still present in all 8 cases, wave III was absent in 5/8 cases (cases 13, 16-18, 21) and wave V absent in 4/8 cases (cases 13, 16-18). We have summarised the ABR findings over the 1-3 years interval in Table 5.5 below and presented the individual waveforms for each subject in Figure 5.3.

Figure 5-3 The initial (baseline) and final ABR measurements are shown for each GD3 case.
Initial and final ABRs for cases 1-8 are shown. Each trace is the average of with two or three separate trials (1024 clicks, rate 11.1/s). The active electrode was positioned at Cz with the reference electrode positioned at the mastoid contralateral to ear stimulated and a ground electrode placed at Fz. Data was collected with a bandwidth of 150-3000 Hz, 12 dB per octave roll-off, and analysed employing an epoch of 0-20ms. In all cases two runs were carried out to check for replicability. Latencies were measured for waves I, III, and V and for the inter-peak intervals I-III, III-V and I-V. A normal ABR recorded from a child (age 12 years) with characteristically normal peak and interpeak latencies is shown for comparison.

Figure has been removed due to copyright restrictions

Table 5-5 Summary of the patient's clinical characteristics and ABR findings over a 3 year period.

Case	Age (yrs)	Sex	Neurology	ABR Baseline	Follow-up (years)	ABR Final
13	1	M	SIF	Absent wave V	1	All waves absent except I and II
14	4	F	SIF	Delayed V	3	Delayed III/V
15	4	F	SIF	Normal	3	Delayed wave V
16	9	M	SIF, Tourette-like Symptoms	Delayed III/V	1	All waves absent except I
17	13	F	SIF	Delayed V	1.5	All waves absent except I
18	12	F	SIF	Absent wave V	1.5	All waves absent except I
19	5	F	SIF	Normal	2	Delayed wave V
21	3	F	SIF	Normal	3	Delayed wave V, absent wave III

5.2.4 Discussion

5.2.4.1 Monitoring neurological progression in GD2

To our knowledge, this is the first report of the use of serial objective audiological testing to monitor the neurological progression in acute neuronopathic Gaucher disease (GD2). For cases 8 & 9, both patients responded to the ERT systemically, as measured haematologically and by chitotriosidase activity although complete correction was not achieved as has been reported previously (Erikson et al., 1993). Despite this improvement, both infants continued to deteriorate neurologically. Both infants exhibited audiological progression, as indicated by abnormal ABRs with absence of waves IV and V, and a gradual loss of waves II and III during the course of their illness, with preservation of wave I.

Our findings of rapid deterioration in later ABR waveform components are consistent with the previously reported studies in GD2 (Kaga et al., 1982, Lacey and Terplan, 1984, Kaga et al., 1998). Interestingly, the remarkable changes in the ABR reported by Kaga et al., (1982) preceded any pathological changes. Kaga and colleagues

(1982) reported a “relative preservation of the nuclei and tracts of the auditory pathways in the brainstem” (p210).

Only one other study has reported serial ABR measures and post-mortem data in an infant with GD2 (Lacey and Terplan, 1984). ABR measurements were abnormal at the initial baseline recording (age 7 weeks), with only the earlier components being spared (waves IV, V were absent). By age 12 weeks, only waves I and II were evident. The infant died at age 13 weeks. In contrast to the post-mortem findings reported by Kaga et al., (1982), there was a complete neuronal absence in the cochlear nuclei (bilaterally), hypoplastic superior olivary complex, a complete neuronal loss in the vestibular nuclei and scattered neuronal loss and gliosis throughout the inferior olives.

Although the ABR ‘signature’ is comparable in both cases – clearly the histopathological findings are very different. This is not surprising and may simply reflect the different rates of neurological progression in each case reported here. Neither of our cases underwent post-mortem examination so we cannot draw any further conclusions regarding this aspect.

5.2.4.2 ERT does not halt neurological progression in GD2

The GD2 patients previously reported in these studies were not undergoing ERT (Table 5.2). Thus, as far as we are aware, this is the first objective evidence for progressive neurological involvement, despite ERT, which has included measurements pre-and post enzyme treatment**.** Our conclusion is inescapable – ERT does not halt neurological progression in this severe form of the disease.

The reason for this failure is not clear. In vitro studies of rat hippocampal cells have shown that delivery of imiglucerase (glucocerebrosidase) can reverse the effects of glucosylthioceramide (an inhibitor of glucocerebrosidase) (Pelled et al., 2000). Thus, the failure to halt neurological progression may reflect the known poor permeability of the blood-brain barrier to imiglucerase or rapid CNS turnover of this enzyme or other contributory toxic substances, such as glucosylsphingosine (Orvisky et al., 2000).

One attempt to overcome the difficulties posed by the blood-brain barrier has been to infuse 'therapeutic' levels of glucocerebrosidase directly into the brain. Human studies in GD2 have been undertaken (Lonser et al., 2007). Infusions to the right paramedian frontal lobe, and brainstem regions (region of the right facial and abducens nuclei) were given. Despite this more aggressive approach, there was no evidence to support a change in the neurological status of the patient. In the absence of any therapy to halt, slow down or stabilise the rate of neurological deterioration in these patients, recent studies argue that treating the GD2 patient with ERT may merely result in the prolongation of pain and suffering (Vellodi et al., 2009). Our data emphasize the urgent need to understand the neurotoxic mechanisms in neuronopathic GD in order to develop effective treatments for application in this devastating phenotype.

5.2.4.3 Monitoring neurological progression in GD3

In this study, we have shown that the ABR deteriorated in all 8 children diagnosed with GD3 who were receiving high-dose ERT. Our data lead us to conclude that high-dose ERT does not prevent the deterioration of ABRs in children with GD3.

Although our sample is small, the data presented here is significantly different from data reported previously, as shown in Table 5.2. For example, Altarescu and colleagues (2001) showed that of 21 GD3 patients (8 months - 35 years) undergoing ERT, 11 patients were reported with normal ABRs, of which only 2 showed deterioration. Of the remaining 10 with abnormal ABRs, there was no deterioration, and 2 even showed improvement, leading to the conclusion that most patients did not deteriorate neurologically under ERT. This study was supported by a claim of improvement, including ABR, in a 20-year-old GD3 male following 1 year of ERT therapy (Aoki et al., 2001).

The reason for the discrepancy between our finding and Altarescu et al. (2001) study is not clear. One possibility may reflect different testing techniques and interpretation. Unfortunately, previous studies (Schiffmann et al., 1997, Altarescu et al., 2001a, Aoki et al., 2001) have not published ABR waveforms or recording techniques, so we cannot draw further conclusions.

Another possibility is that there are significant differences between patient groups. In our patients 15/16 alleles were the L444P mutation, which is the most common type-3 mutant allele. In the Altarescu study, 13 out of 21 patients were of the L444P homozygote, 1/21 were the L444P heterozygote and 7/21 were unknown.

The waveforms in the ABR reflect the synchronous neural activity in the brainstem auditory pathways, including the 8^{th} cranial nerve, cochlear nucleus, superior olivary complex, lateral lemniscus and/or the inferior colliculus (Chapter 2, section 2.4). However, because the exact generator sites of the ABR are not known, the pathophysiology of abnormalities is speculative, but we suggest that multiple factors

could be involved including axonal and neuronal loss and demyelination. Neuronopathic GD is known to involve brainstem (Lacey and Terplan, 1984, Harris et al., 1999, Bamiou et al., 2001) thus it seems most likely that the progressive deterioration in ABRs reflects underlying sub-clinical brainstem degeneration in our patients.

We cannot exclude the possibility that some GD3 patients may have a more positive response to ERT, but caution is needed as lack of ABR deterioration may simply reflect slower neurological progression in some patients. The insidious nature of the neurological disease may mean that outcome measures of the efficacy of ERT or any other treatment for neuronopathic disease may require years of follow-up.

5.3 Experiment 2 - The ABR as a 'Neuromarker' for Neuronopathic Gaucher Disease

5.3.1 Introduction

There is no doubt that ERT is very successful in the treatment of systemic GD (Kauli et al., 2000), but its therapeutic effect on the neurological component is much less certain (Kraoua et al., 2011). We have shown that in our earlier experiment that ERT is not beneficial in GD2. It is possible that the overwhelming severity of the infantile condition and/or irreversible foetal damage may be insurmountable with post-natal treatment. However, even in GD3, our results from auditory studies are not encouraging.

In vitro studies have shown that exogenous enzyme should work, however the difficulty in delivering sufficient enzyme to nerve cells remains a significant challenge for researchers in this field (Pelled et al., 2000).

An alternative strategy that has been proposed in a number of centres is to combine ERT with concomitant substrate depletion therapy, Miglustat® (N-butyldeoxynojirimycin) (Cox et al., 2000). Clinical trials in GD1 patients have shown that Miglustat® has a number of beneficial effects on the systemic component of the disease (Cox et al., 2000, Zimran and Elstein, 2003, Elstein et al., 2004). Preclinical studies in animal models have also indirectly shown that it crosses the blood-brain barrier raising hopes that this treatment could potentially enter the brain and reduce the neuronal burden of this lipid, thus improving the function of the CNS (Platt et al., 1994, Platt et al., 1997a, Platt et al., 1997b, Jeyakumar et al., 1999, Platt et al., 2001, Platt and Jeyakumar, 2008) .

Preliminary ABR data has been optimistic and has indicated that combined therapy with Miglustat® may improve neurological manifestations in GD3 (Table 5.2) (Cox-Brinkman et al., 2008). One study describes a marked improvement in the ABR in one case. Initially the ABR in the youngest sibling was reported abnormal with prolonged latencies. However, by the age of 3 years, the ABR had normalised.

Our aim in this study was to evaluate the potential for clinical improvement of the neurological aspects of 13 children diagnosed with GD3 who were undergoing a clinical trial to evaluate the efficacy and safety of Miglustat®. Here we report the longitudinal ABR data from a 24 month clinical trial that used the ABR as a secondary outcome measure.

5.3.2 Methods

5.3.2.1 Study design including the choice of control groups

This was a randomized, controlled study in patients with GD3. This study aimed to evaluate up to 30 patients diagnosed clinically with neuronopathic Gaucher disease who, if receiving ERT, had been stable on ERT for at least 6 months, or had successfully undergone a bone marrow transplant (BMT) at least 1 year prior to study entry. The original study took place across two centres: the UK and the US. The data we present in this chapter is from the UK centre only (n=13).

Patients were required to meet all of the following inclusion criteria shown in Table 5.6, and none of the exclusion criteria shown in Table 5.7, in order to be recruited into the original 12-month treatment period.

Table 5-6. Inclusion criterion

Inclusion criteria	Patients must meet the clinical diagnoses for nGD Be stable on ERT for at least 6 months or had a successful BMT *at least* 1 year prior to study entry Able to swallow a capsule

Once it had been determined that a patient was eligible for the study, he or she was to be randomly allocated to either the OGT 918 group or the No Treatment group. The randomization schedule was to yield an overall ratio of 2:1. To randomize a patient, sequentially numbered scratch cards were used by the Clinical Project Manager to reveal the individual patient assignment. The Clinical Project Manager (central randomization) confirmed recruitment and the treatment group to which the patient had been randomized, and allocated the next patient number by faxed reply. The

randomization sequence used a block size of six within each stratum (splenectomy status), and was generated by Quintiles UK, Biometrics department.

Randomization was stratified based on whether the patient had undergone a splenectomy. The rationale behind this decision was based on data from previous studies which have shown that splenectomised GD3 patients have a greater accumulation of glucosylceramide in liver and brain compared to non-splenectomized patients[36].

Table 5-7. Exclusion criterion

Exclusion criteria	Patients < 18 yrs who were unable to give informed assent or whose legal guardian was unable to provide informed consent. Patients > 18+ yrs who could not provide informed consent or whose legal guardian was unable to provide witnessed informed consent. Fertile patients who, at the time of the study, were sexually active and did not agree to use adequate contraception throughout the study and for 3 months after cessation of OGT-918 treatment. Patients who could not tolerate the study procedures or were unable to travel to the study center as required by this protocol. Concurrent therapy which may interfere with gastrointestinal absorption or motility. Clinically significant diarrhoea without definable cause within 3 months of screening visit, or who had a history of significant gastrointestinal disorders. Intercurrent medical conditions that rendered them unsuitable for the study e.g., human immunodeficiency virus (HIV), hepatitis infection. Creatinine clearance of less than 70 ml/min/1.73m^2 (crcl < 70).

On completion of the 12-month treatment period, patients randomized to no treatment were to have the option of receiving Migustat as part of the Extension study, a further option to enter a 12-month Extended Use study, and on completion of Month 36 (study end) an option of continued therapy with Miglustat was to be provided.

[36] Partially splenectomized patients were grouped with patients with an intact spleen.

A placebo control group was not chosen for this study because of known side effects of the Miglustat treatment[37] and the well documented swallowing problems that have been seen in patients with GD3. [38]

5.3.2.2 Subjects

Thirteen subjects (2 male; age range 2-19 years, mean age 8.4 years) diagnosed as having GD3 were recruited into the clinical trial, after informed consent had been obtained. The subjects were recruited from a larger multicentre study evaluating the efficacy and safety of Miglustat® in GD3 in adult, juvenile and paediatric patients, with particular emphasis on changes in neurological and systemic parameters.

Table 5-8. Demographic and baseline characteristics

Group	*Age*	*Gender*	*Current treatment*
Treatment (TX)			
TX 1	7	F	ERT
TX 2	3	F	ERT
TX 3	2	M	ERT
TX 4	16	F	ERT
TX 5	19	F	BMT
TX 6	7	F	ERT
No-treatment (NTX)			
NTX 1	8	F	ERT
NTX 2	5	F	ERT
NTX 3	6	F	ERT
NTX 4	8	F	ERT
NTX 5	9	F	ERT
NTX 6	11	M	ERT
NTX 7	8	F	ERT

[37] Diarrhoea is strongly associated with OGT 918 treatment (with onset occurring shortly after the treatment commences), no benefit would be obtained by using placebo to blind the treatment groups.
[38] Patients with NGD have swallowing difficulties, it was considered unethical to administer placebo for a continuous 12-month period with no potential benefit, in view of the likely discomfort patients would experience swallowing placebo.

At baseline, the 13 patients were to be randomized in to either a Treatment group (n=7) or a No Treatment group (n=6). The No Treatment group (n=6) received their normal clinical care.[39] Table 5.8 shows the individual subject characteristics for the two groups. Subjects were all allocated a unique patient identifier number and the local assessors were blinded to the treatment. The CONSORT diagram in Figure 5.5 shows the flow of participants through each stage of the clinical trial.

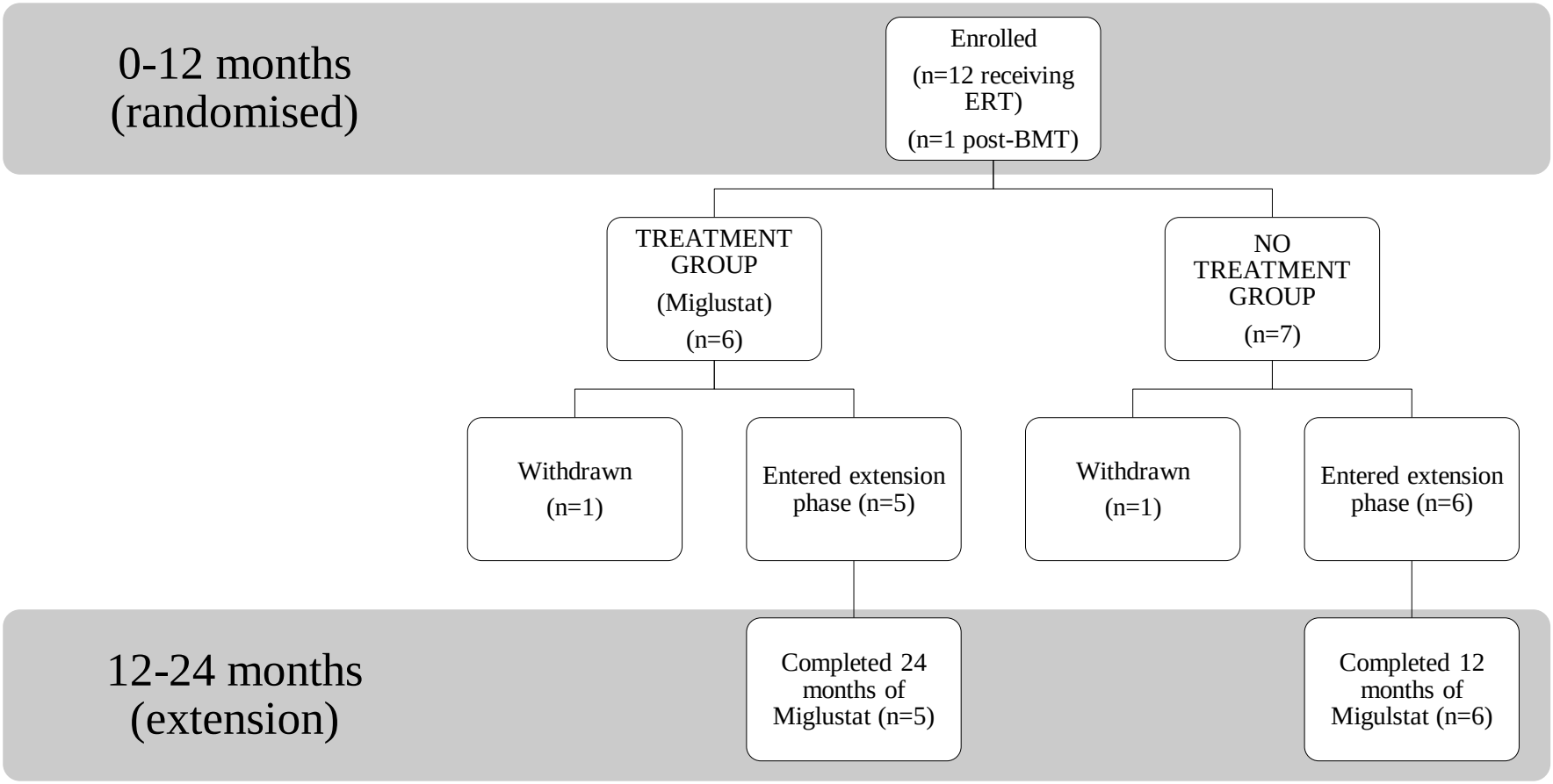
5.3.2.3 Procedure

All patients were to complete a follow-up visit 4 weeks after either completion of the 12-month study or withdrawal from the 12-month study (Figure 5.4). An analysis of the 12-month data was planned to be carried out to compare treatment with Miglustat (TX) to no treatment (NTX). All patients in the study were given the option to continue in the Extension study for an additional 12 months (total of 24 months) as long as no safety reasons prohibited this. All patients were to receive Miglustat in the Extension study, and the Extension study was performed to collect safety and efficacy data.

To avoid any confusion however, we will simply to refer to the groups as 'treatment' (TX) or 'no treatment' (NTX) and highlight the length of time each group has participated within the trial as either baseline (t=0); month 12 (t=12) and month 24 (t=24).

[39] A No Treatment control group was chosen as an alternative to placebo. Patients randomized to the No Treatment group received the standard clinical care that would normally have been available. These patients continued to receive concomitant ERT throughout the study, and provided data from which a natural history for this disease could be built.

Figure 5-4 CONSORT diagram



Serial audiological assessments were performed at baseline (t=0); month 12 (t=12) and month 24 (t=24). These included pure tone audiometry and auditory brainstem responses which we have previously described in detail in Chapter 3. Where participants were unable to perform audiometry, we have used additional screening tests (e.g.) otoacoustic emissions or free-field audiometry as previously described in Chapter 3. The equipment that was used to measure each of these assessments was the same for each subject and did not require significant recalibration at any of the annual maintenance checks.

5.3.2.4 Statistical Analysis

The following summary statistics were presented for continuous variables: number of values, mean, standard deviation (SD), median, minimum, and maximum. Distribution normality and equality of variance between groups were assessed by one-sample Kolmogorov--Smirnov test (with Lilliefors correction) and Levene's tests, respectively.

The data is examined as group data and on a case-by-case basis to determine the trajectory of change. Unfortunately we were only able to perform limited inferential statistical testing on the ABR amplitude trial data. This is due in part to the heterogeneity of the groups, small sample sizes and number of missing data values[40] . We were also unable to combine the audiometric data in any meaningful statistical analysis because of the small number of participants; particularly following the withdrawal of some patients and failure to complete the test by others which resulted in

[40] There are several missing data sets from the trial data, particularly latency data. This is due to the effects of the neurological component of the disease and ABR and not due to the ABR itself.

heterogeneous samples within the two groups over the 24 month interval, so a descriptive analysis of PTA measures was undertaken instead.

Performance on continuous ABR amplitude data measures (waves I, III and V) in the treatment group was compared with that of the non-treatment group at 24 months using the nonparametric Mann-Whitney *U* test or the Wilcoxon Rank Sum Test. A descry All statistics were computed with SPSS (v16) statistical software (SPSS, Chicago, IL).

5.3.3 Results

Thirteen patients (12 on ERT, one post-BMT) were randomized to receive either Miglustat (n = 6) or 'no treatment' (n = 7) for 12 months. All patients completed the first 12 months. Eleven patients (85%) entered the 12-month extension. Two patients withdrew: one from the TX group (1 female) and one from the NTX group (1 male) so that 5 patients completed 12 months of extension treatment with Miglustat (i.e. 5 received combined therapy for a total of 24 months) (Figure 5.4).

5.3.3.1 Pure tone audiometry

Pure tone audiometry was completed by 10 GD3 (76.9%) subjects at all frequencies (0.25-8.0 kHz). Three patients (cases TX 2, TX3 and NTX 6) were unable to complete audiometry because of poor co-operation.

Of the 10 cases that were able to perform audiometry at baseline, 4 cases were in the treatment group (TX 1, TX4, TX5 and TX 6) and 6 cases in the no-treatment group

(NTX 1-5 and NTX 7). Patient NTX5 had no measureable hearing on the left ear and this data was excluded from all further analysis.

Audiometry (or screening audiometry) in the treatment group revealed normal hearing thresholds in 3 cases; a mild unilateral sensorineural hearing loss on the right in one case, a mild bilateral sensorineural hearing loss in one case and a case with a bilateral sensorineural hearing loss which was moderate-severe in the high frequencies (Table 3.1, Appendix 2, section 9.1).

Table 5-9 Mean (± SEM) hearing threshold levels measured at baseline (t=0) for the right and left ears in dB HL

Group	*Frequency (kHz)*	*Right Ear*		*Left Ear*	
		Mean	Std.Err.	Mean	Std. Err.
Treatment (n=4)	0.25	16.7	4.4	13.3	1.7
	0.50	15.0	5.0	16.7	1.7
	1	18.3	6.7	20.0	2.9
	2	23.3	11.7	30.0	12.6
	4	13.3	6.0	20.0	10.0
	8	8.3	4.4	40.0	15.0
No treatment (n=6)	0.25	20.0	5.7	18.0	4.6
	0.50	19.0	5.6	20.0	3.9
	1	15.0	3.2	18.0	5.6
	2	11.0	3.7	12.0	2.5
	4	11.0	2.4	15.0	3.5
	8	8.0	4.1	15.0	5.0

At baseline in the no-treatment group, 4 cases had normal hearing bilaterally, one had a mild unilateral conductive hearing loss on the right ear, another case had a bilateral mild moderate conductive loss and one case had a profound sensorineural hearing loss on the left ear (Table 3.1, Appendix 2, section 9.1). In summary, in the no-treatment group, 3/7 cases had a hearing loss and 3/6 had a hearing loss in the treatment group. The mean hearing thresholds (in dB HL) as measured at baseline are compared

in Table 5.9 for both groups. For illustrative purposes we have also constructed individual audiometric profiles for each participant. The right and left ear data for the treatment group are shown in Figure 5.5 and the data for the non-treatment group are shown in Figure 5.6.

Of the 10 cases that were able to perform audiometry at month 12, 4 cases were in the treatment group (TX 1, TX4, TX5 and TX 6) and 6 cases in the no-treatment group (NTX 1-5 and NTX 7). Mean hearing thresholds (in dB HL) as measured at month 12 are compared below in Table 5.10 for both groups.

There was very little change in audiometric hearing thresholds in either group. In the treatment group, one case showed a remarkable improvement of 10 dB. This meant that the sensorineural hearing loss 'recovered' slightly on the right side. Another case that had previously shown a conductive hearing loss also improved and was within normal levels (Table 3.1, Appendix 2, section 9.1).

Table 5-10 Mean (± SEM) hearing threshold levels measured at month 12 (t=1) for the right and left ears in dB HL (n=10).

Group	*Frequency (kHz)*	*Right Ear*		*Left Ear*	
		Mean	Std. Err.	Mean	Std. Err.
Treatment (n=4)	0.25	15.0	5.0	13.8	4.3
	0.50	12.5	1.4	16.3	5.2
	1	13.8	4.3	15.0	5.0
	2	15.0	7.4	18.8	12.1
	4	10.0	6.1	15.0	10.0
	8	11.3	4.3	22.5	12.0
'No treatment' (n=6)	0.25	18.3	5.7	16.7	5.1
	0.50	16.7	4.9	17.5	5.6
	1	15.0	3.4	15.0	4.5
	2	10.8	1.5	12.5	1.2
	4	9.2	2.0	10.8	1.5
	8	6.7	2.1	14.2	2.7

Only 9 cases underwent audiometry at month 24: one case withdrew from the treatment group (TX 4) so that the data presented in Table 5.11 represents hearing threshold levels from three participants only (n=3). Six cases remained in the no-treatment group (NTX 1-5 and NTX 7).

Table 5-11 Mean (± SEM) hearing threshold levels measured at month 24 (T=2) for the right and left ears in dB HL (n=10).

Group	*Frequency (kHz)*	*Right Ear*		*Left Ear*	
		Mean	Std.Err.	Mean	Std. Err.
Treatment (n=3)	0.25	15.0	7.6	13.3	8.8
	0.50	13.3	6.0	16.7	7.3
	1	8.3	4.4	10.0	2.9
	2	6.7	1.7	8.3	4.4
	4	8.3	1.7	18.3	13.3
	8	10.0	2.9	23.3	18.3
No treatment* (n=6)	0.25	19.0	3.7	29.0	5.1
	0.50	18.0	5.1	25.0	4.5
	1	16.0	4.0	21.0	2.9
	2	14.0	2.9	16.0	1.9
	4	17.0	4.6	16.0	2.9
	8	13.0	4.4	15.0	4.2

*On combined therapy for 12 months only as part of the extension study.

If we examine the data from the treatment group (as presented in Figure 5.5) on a case-by-case basis, we see that there is a wide range of hearing thresholds for each case. Audiometry performed on patient TX1, in Figure 5.5, revealed a mild bilateral SNHL (bone conduction levels not shown) at baseline. By month 12, there was a clinically significant improvement in the overall hearing threshold levels, with both sides now within normal limits. By month 24, there was little change in the overall hearing thresholds and hearing was normal bilaterally (Table 3.1, Appendix 2, section 9.1).

The audiometric data for patients TX 2 and TX 3 is not shown in Figure 5.5. These were the two youngest participants in the clinical trial and were unable to

complete a full audiogram. Both participants had their hearing screened using otoacoustic emissions (See Chapter 3). These data showed the presence of OAEs bilaterally, consistent with normal cochlear outer hair cell function. This is consistent with hearing thresholds with normal-near normal hearing thresholds (<35 dB HL). There was no observable change in peripheral hearing sensitivity in either case when at follow up.

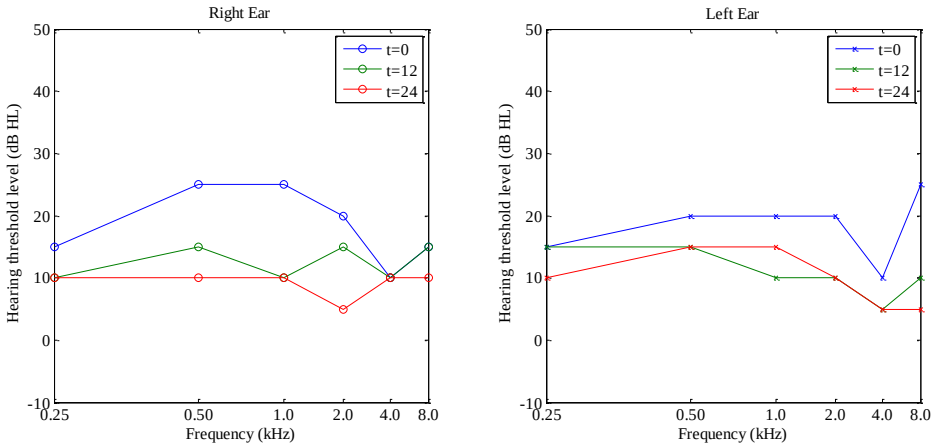
Patient TX 4 withdrew from the clinical trial after month 12 – therefore no data is available for month 24. The audiometric profile of this participant is shown in Figure 5.5. At baseline, a mild-moderate high frequency SNHL was recorded bilaterally. This showed a small improvement of 10 dB at 2 kHz only on the right ear (Table 3.1, Appendix 2, section 9.1).

Pure tone audiometry revealed a mild low frequency hearing loss on the right ear in patient TX 5 at baseline. This is shown in Figure 5.5. A moderate-severe high frequency SNHL was recorded on the left. At month 12, hearing threshold levels had decreased by 10 dB. At month 24, this participant showed deterioration in hearing thresholds on both sides (Table 3.1, Appendix 2, section 9.1).

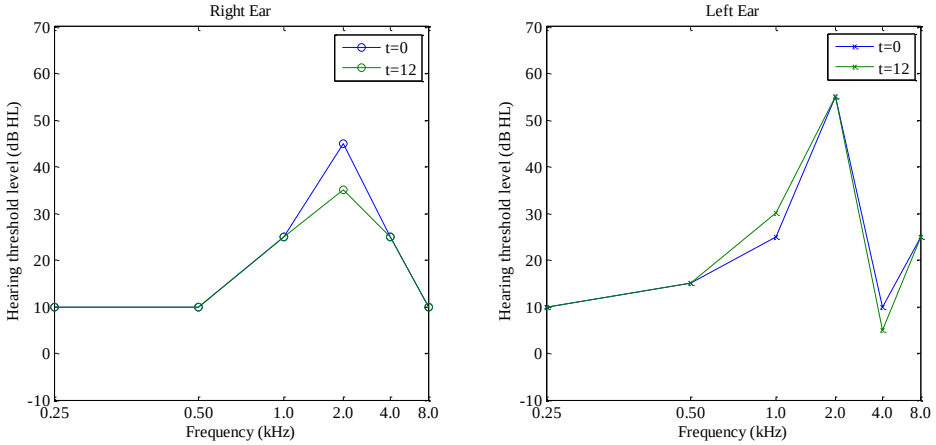
We were unable to record audiometry at baseline (t=0) in patient (TX6). However hearing was screened using a hand-held warbler system and conditioned play audiometry performed in the sound field (dashed blue line) in Figure 5.5. Hearing thresholds were within normal limits, at least for one ear (Table 3.1, Appendix 2, section 9.1). No change in hearing thresholds was seen at either the month 12 or month 24 follow-up.

Figure 5-5 Individual audiometric data from treatment group shown as measured at three time points (baseline, month 12 and month 24). Normative PTA data is shown in Appendix 2, Section 9.2.1 for comparison.

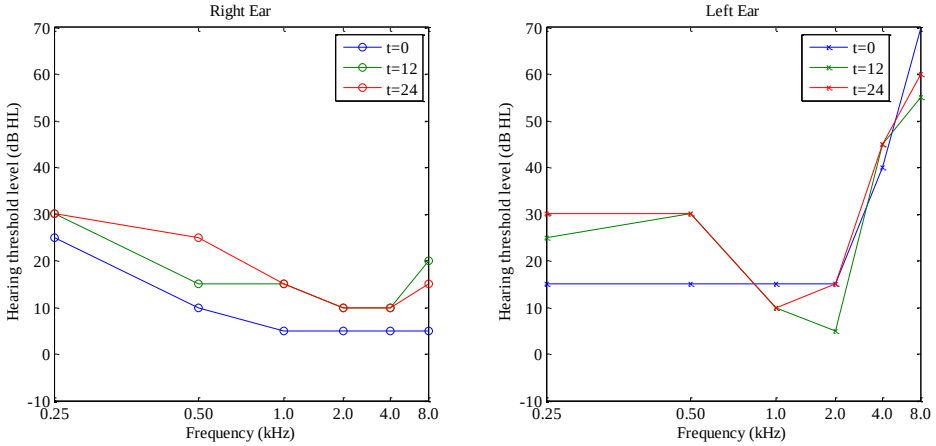
TX1



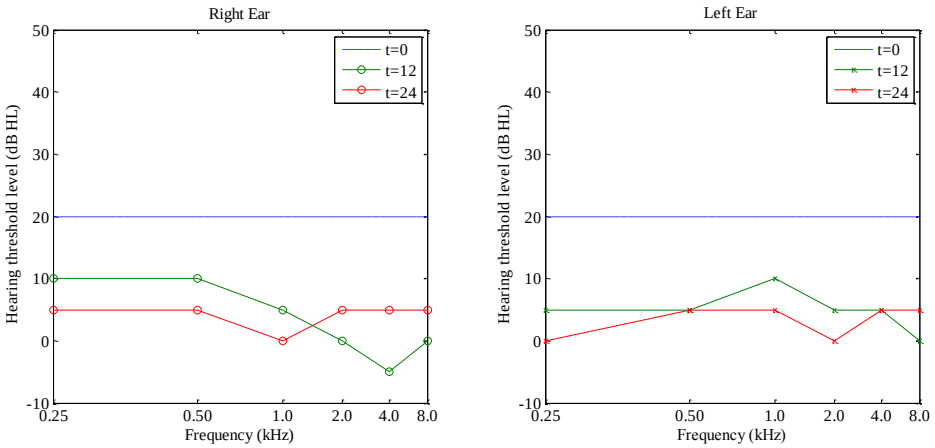
TX4



TX5



TX6

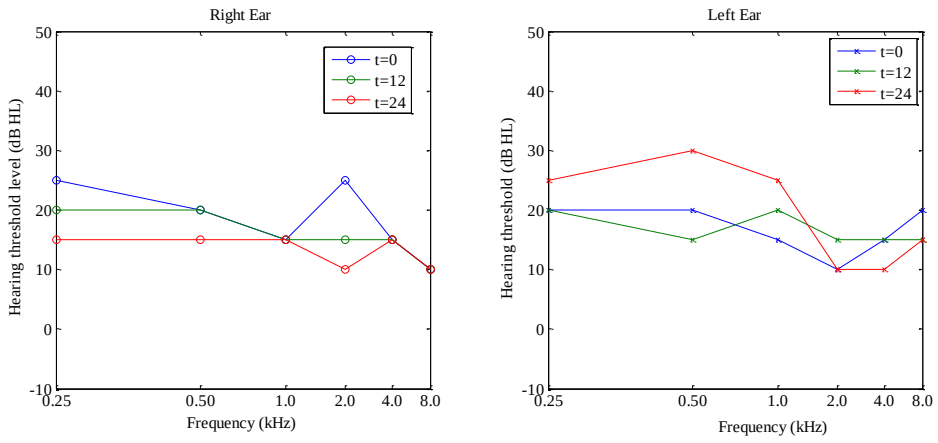


By 24 months, the total number of participants with a hearing loss (in at least one ear) had increased from 6/13 at baseline to 8/11 (72%) of all GD3 participants. However, the hearing loss in the majority of these of these cases was the result of a conductive impairment such as impacted wax or otitis media.

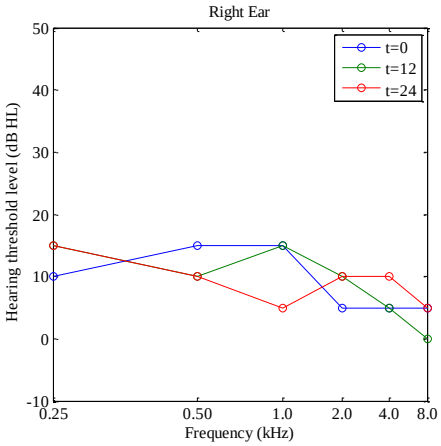
If we examine the data from the no-treatment group (as presented below in Figure 5.6) on a case-by-case basis across the 24 month interval, we see that there is a wide variation in the hearing thresholds for each participant but there is no obvious effect of treatment or no-treatment.

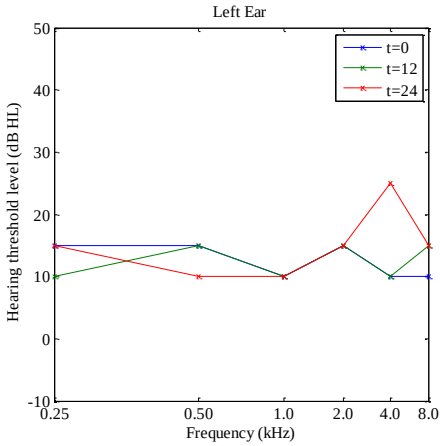
Figure 5-6 Individual audiometric data from the non-treatment group shown as measured at three time points (baseline, month 12 and month 24). Normative PTA data is shown in Appendix 2, Section 9.2.1 for comparison.

NTX 1

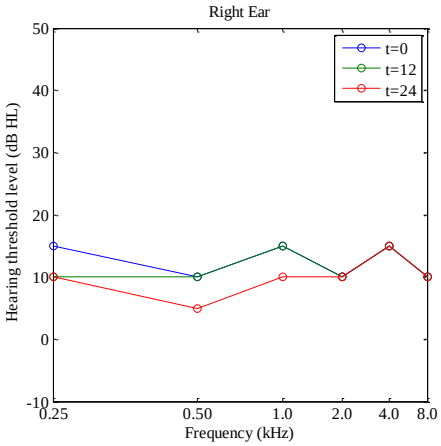


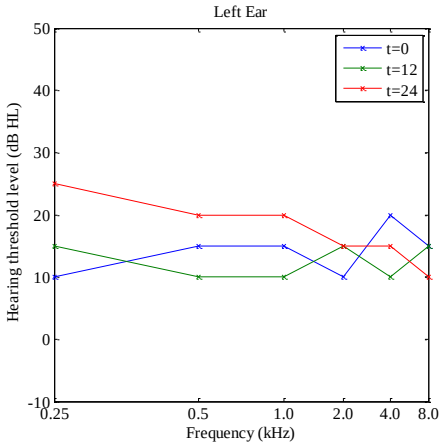
NTX 2



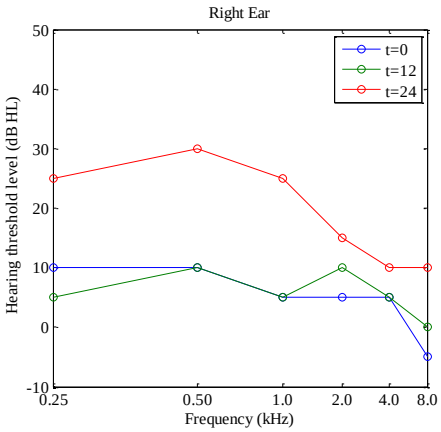


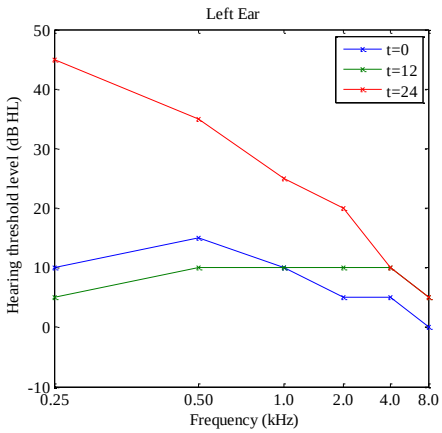
NTX 3



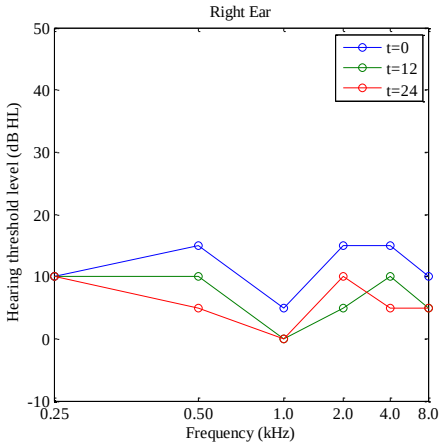


NTX 4

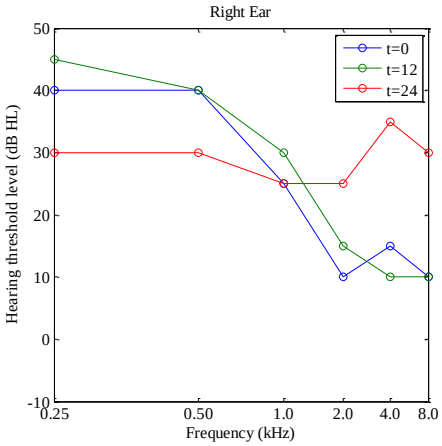


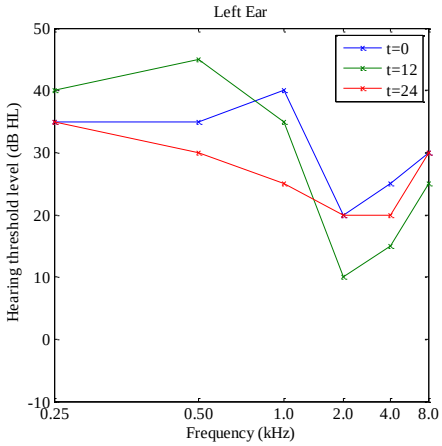


NTX 5



NTX 7





For example, patient (NTX1) had a mild conductive hearing loss on the right ear only (bone conduction thresholds are not shown) at baseline. The hearing thresholds are within normal limits for the left side (Table 3.1, Appendix 2, section 9.1). At month 12 (t=12), we observe a small improvement of 10 dB at 2 kHz on the right ear only but no change on the left side. At month 24 (t=24), the hearing on the right ear has normalised but the left ear has a mild low frequency hearing loss.

Two cases (NTX2 and NTX3) had normal hearing thresholds for the right ear over the entire 24 month period. However, the hearing thresholds in their left ears showed some fluctuation over the same time scale (Table 3.1, Appendix 2, section 9.1). Patient (NTX4) had normal hearing thresholds bilaterally at baseline which showed

little change at month 12 (Table 3.1, Appendix 2, section 9.1). However, by month 24, there was a marked deterioration in hearing thresholds, particularly in the low frequencies on the left.

Patient (NTX5) had a profound sensorineural hearing loss on the left but normal hearing thresholds on the right (Table 3.1, Appendix 2, section 9.1). There was little change in the overall hearing during the clinical trial. The data for case NTX6 is limited and is not shown in Figure 5.6. This patient withdrew from the trial after month 12. Hearing thresholds were screened and shown to be within normal limits for this case.

The final participant (NTX7) had a mild-moderate mixed hearing loss bilaterally. A slight decline in hearing thresholds of 10 dB at 0.5 kHz and a slight improvement in the high frequencies by 10 dB on the left ear was seen at month 12. There was no observable change in these thresholds on the right side. By month 24, the hearing had shown some improvement but the high frequencies still showed a mild loss bilaterally (Figure 5.6) (Table 3.1, Appendix 2, section 9.1).

5.3.3.2 Brainstem auditory evoked potentials

We present the absolute latencies and amplitudes for the ABR for each patient in the treatment group in Tables 5.12 and 5.13 respectively. An arbitrary value of 0 was assigned as an amplitude value when a waveform was absent. A summary of the presence or absence of waves (I, III and V) for individuals in the 'treatment' group is presented in Table 5.14. There is no clear pattern across the group particularly when we examine individual patients' performances at baseline, month 12 and month 24.

At baseline, only wave I was seen on both ears for patient TX 1. However at month 12, there was a remarkable improvement in ABR morphology and amplitude with waves III on the right ear and wave V clearly identifiable on the left ear. A small decrease in amplitude in both ears for wave I was seen at 12 months (Table 5.13). However, at month 24, only wave I was recordable on both ears (Table 5.12).

Table 5-12 Individual latency data for the treatment group

Subject	Timepoint (t)	Latency (ms) Right Ear			Latency (ms) Left Ear		
		Wave I	Wave III	Wave V	Wave I	Wave III	Wave V
TX1	0	1.78	-	-	1.7	-	-
	12	1.73	3.82	-	1.8	-	5.4
	24	1.8	-	-	1.63	-	-
TX2	0	1.68	-	-	1.73	-	-
	12	1.68	-	-	1.73	-	-
	24	1.66	3.41	5.88	1.8	-	-
TX3	0	1.73	3.5	-	1.8	3.67	-
	12	1.61	3.43	-	1.63	3.67	-
	24	1.7	3.48	-	1.78	-	-
TX4	0	1.58	-	-	-	-	-
	12	1.58	-	-	-	-	-
	24	W	W	W	W	W	W
TX5	0	1.51	-	-	-	-	-
	12	1.58	-	5.4	-	-	-
	24	1.66	-	5.3	1.58	4.61	-
TX 6	0	-	-	5.98	1.82	4.06	5.86
	12	1.82	-	5.66	1.8	-	5.81
	24	1.54	3.82	5.47	1.56	3.55	5.57

Table 5-13 Individual amplitude data for the treatment group
Note: Amplitude values recorded in µV, where a wave is absent a value of 0 has been inserted.

Subject	Timepoint (t)	Amplitude (µV) Right Ear			Amplitude (µV) Left Ear		
		Wave I	**Wave III**	**Wave V**	**Wave I**	**Wave III**	**Wave V**
TX1	**0**	0.18	0	0	0.25	0	0
	12	0.17	0.22	0	0.12	0	0.35
	24	0.23	0	0	0.31	0	0
TX2	**0**	0.45	0	0	0.27	0	0
	12	0.35	0	0	0.27	0	0
	24	0.65	0.15	0.17	0.21	0	0
TX3	**0**	0.34	0.17	0	0.24	0.04	0
	12	0.35	0.23	0	0.41	0.31	0
	24	0.48	0.32	0	0.31	0	0
TX4	**0**	0.4	0	0	0	0	0
	12	0.33	0	0	0	0	0
	24	W	W	W	W	W	W
TX5	**0**	0.3	0	0	0	0	0
	12	0.21	0	0.31	0	0	0
	24	0.25	0	0.36	0.08	0.08	0
TX 6	**0**	0	0	0.45	0.14	0.06	0.23
	12	0.29	0	0.47	0.24	0.24	0.37
	24	0.19	0.07	0.28	0.24	0.04	0.28

Only wave I was identifiable on the right and left side in Patient TX 2 at baseline (Table 5.14). At 12 months, there was little change in the ABR except for a small decrease in wave I amplitude on the right (Table 5.13). At month 24, there was a marked improvement on the right side, with all waveforms now visible but on the left, only wave I was evident at a prolonged latency (Table 5.12).

Table 5-14 Presence/absence ABR data for the treatment group

Subject	Timepoint (t)	Right Ear			Left Ear		
		Wave I	**Wave III**	**Wave V**	**Wave I**	**Wave III**	**Wave V**
TX1	**0**	N	AB	AB	N	AB	AB
	12	N	N	AB	N	AB	N
	24	N	AB	AB	N	AB	AB
TX2	**0**	N	AB	AB	N	AB	AB
	12	N	AB	AB	N	AB	AB
	24	N	N	N	N	AB	AB
TX3	**0**	N	N	AB	N	N	AB
	12	N	N	AB	N	N	AB
	24	N	N	AB	N	AB	AB
TX4	**0**	N	AB	AB	AB	AB	AB
	12	N	AB	AB	AB	AB	AB
	24	W	W	W	W	W	W
TX5	**0**	N	AB	AB	AB	AB	AB
	12	N	AB	N	AB	AB	AB
	24	N	AB	N	N	N	AB
TX6	**0**	AB	AB	D	N	D	D
	12	N	AB	N	N	N	D*
	24	N	N	N	N	N	N

Abbreviations: N – normal (present at normal peak latency); D– delayed (waveform is present but delayed latency); AB – absent (waveform is absent); W – withdrew from clinical trial (*) Waveform is delayed but the delay has improved compared with baseline latency measurement (ms)

Waves I and III were present bilaterally at baseline for patient TX 3. Wave V was absent on both ears at this time. By month 12, there were no major changes in the

ABR morphology for either side, except a mild improvement in peak waveform amplitude on the left (Table 5.13). At month 24, a similar pattern emerged on the right, with no change in the overall ABR – waves I and III were still present. However, the left ear had shown some deterioration by month 24 and only wave I was evident (Table 5.14).

The ABR for Patient TX 4, at baseline, only demonstrated wave I in the right ear and there was little change by month 12. Only a small decrease in amplitude was observed (Table 5.13). This patient withdrew from the clinical trial so no data was available for month 24.

Patient TX 5 was the oldest participant recruited onto this trial and the only patient to have received BMT. Only wave I was identifiable on the right side of the ABR. There was no clear observable waveform on the left side. This may be a reflection of the high frequency SNHL that was measured on this ear (Figure 5.5). By month 12, a significant improvement in the ABR was evident, with a clear wave V. Significant improvement in the ABR with wave V clearly identifiable on the right ear. At the final measurement, month 24, waves I and V were present on the right side and wave I and III were seen on the left.

The final patient enrolled in the treatment group, patient TX 6, showed wave V only on the right but all ABR waves were present on the left ear. An overall improvement in the ABR was seen at month 12, with wave I clearly identifiable on the right ear. There are also improvements in the absolute latencies in waves V on the right ear and waves III and V on the left (Table 5.13). At month 24, the ABR was normal on

both sides, with all waveforms showing a remarkable improvement in latency (Table 5.12).

Table 5-15 Individual latency data for the NO-treatment group

Subject	Timepoint (t)	Latency (ms) Right Ear			Latency (ms) Left Ear		
		Wave I	Wave III	Wave V	Wave I	Wave III	Wave V
NTX1	0	1.68	-	-	1.82	3.79	5.66
	12	1.66	3.6	5.69	1.79	3.72	-
	24	1.63	3.5	-	1.68	-	-
NTX2	0	1.54	-	5.47	1.54	3.12	5.42
	12	1.58	3.65	5.57	1.61	3.34	5.47
	24	1.61	-	5.47	1.54	-	5.5
NTX3	0	1.51	3.55	5.83	1.58	3.5	5.66
	12	1.58	-	5.69	1.63	3.36	5.54
	24	1.56	3.74	5.81	1.61	-	5.47
NTX4	0	1.63	3.65	5.76	1.63	-	5.81
	12	1.73	3.67	6	1.54	-	5.81
	24	1.68	3.67	6.02	1.66	3.98	5.93
NTX5	0	1.82	3.94	-	-	-	-
	12	-	-	-	-	-	-
	24	1.66	-	5.54	-	-	-
NTX 6	0	-	-	-	-	-	-
	12	-	-	-	-	-	-
	24	W	W	W	W	W	W
NTX7	0	-	-	-	1.8	-	-
	12	-	-	-	1.8	-	-
	24	1.9	3.22	6.05	1.73	-	5.76

We present the absolute latencies and amplitudes for the ABR for each patient in the no-treatment group in Tables 5.15 and 5.16 respectively. An arbitrary value of 0 was assigned as an amplitude value when a waveform was absent. A summary of the

presence or absence of waves (I, III and V) for individuals in the 'treatment' group is presented in Table 5.17. Visual inspection of the data shows no clear pattern across the group particularly when we examine individual patients' performances at baseline, month 12 and month 24.

When we compare the wave amplitudes for the treatment group with the non-treatment group at 24 months, we find no evidence to support a difference in the ABR amplitude for wave I (U=13, n.s, z= -0.365), wave III (U=13.5, n.s, z= -0.281), or wave V (U=10, n.s, z=-0.921) for the right ear. There was no significant difference in the amplitude between the treatment groups for wave I (U=5, n.s, z= -1.834), wave III (U=12, n.s, z= -0.699), or wave V (U=13, n.s, z= -1.409) on the left side.

A summary of the presence or absence of waves (I, III and V) for individuals in the 'no-treatment' group is presented in Table 5.17. There is no clear pattern across the group particularly when we examine individual patients' performances at baseline, month 12 and month 24.

For patient NTX 1 only wave I was present on the right ear but all waveforms were present on the left side. At 12 months, a marked improvement was observed for waves III and V on the right ear, but deterioration in wave V on the left ear. At month 24, waves I and III were identifiable but there was marked deterioration in the overall morphology of the ABR on the left side and only wave I was observed (Table 5.17).

Patient NTX 2 had a normal ABR bilaterally at baseline. At the 12 month follow-up, a small decrease in amplitude of wave I on both ears was evident (Table 5.16). A worsening in waveform amplitude morphology in wave III and V on the right side was also seen (Table 5.16). The deterioration is independent of the peripheral

hearing, which has remained essentially normal. A mild improvement in wave III amplitude on the left ear was also seen at month 12 but at month 24 wave III was absent again (Table 5.17).

Table 5-16 Individual amplitude data for the NO-treatment group

Subject	Timepoint (t)	Amplitude (µV) Right Ear			Amplitude (µV) Left Ear		
		Wave I	**Wave III**	**Wave V**	**Wave I**	**Wave III**	**Wave V**
NTX1	**0**	0.41	0	0	0.77	0.08	0.28
	12	0.42	0.12	0.23	0.49	0.2	0
	24	0.21	0.32	0	0.44	0	0
NTX2	**0**	0.5	0	0.41	0.2	0.35	0.56
	12	0.39	0.05	0.36	0.62	0.36	0.44
	24	0.1	0	0.32	0.34	0	0.18
NTX3	**0**	0.39	0.19	0.42	0.41	0.08	0.28
	12	0.36	0	0.14	0.31	0.22	0.28
	24	0.52	0.04	0.66	0.66	0	0.29
NTX4	**0**	0.33	0.2	0.44	0.35	0	0.44
	12	0.18	0.12	0.32	0.31	0	0.35
	24	0.62	0.06	0.24	0.39	0.08	0.28
NTX5	**0**	0.25	0.22	0	0	0	0
	12	0	0	0	0	0	0
	24	0.46	0	0.23	0	0	0
NTX 6	**0**	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	W	W	W	W	W	W
NTX7	**0**	0	0	0	0.31	0	0
	12	0	0	0	0.33	0	0
	24	0.2	0.09	0.29	0.34	0	0.28

Table 5-17 Presence/absence ABR data for the NO-treatment group

Subject	Timepoint (t)	Right Ear			Left Ear		
		Wave I	**Wave III**	**Wave V**	**Wave I**	**Wave III**	**Wave V**
NTX1	**0**	N	AB	AB	N	N	N
	12	N	N	N	N	N	AB
	24	N	N	AB	N	AB	AB
NTX2	**0**	N	N	N	N	N	N
	12	N	AB	N	N	N	N
	24	N	N	N	N	AB	N
NTX3	**0**	N	AB	N	N	N	N
	12	N	N	N	N	N	N
	24	N	N	N	N	AB	N
NTX4	**0**	N	D	AB	AB	AB	AB
	12	AB	AB	AB	AB	AB	AB
	24	N	N	N	N	N	N
NTX5	**0**	N	N	N	N	AB	D
	12	N	N	D	N	AB	D
	24	N	AB	N	AB	AB	AB
NTX 6	**0**	AB	AB	AB	AB	AB	AB
	12	AB	AB	AB	AB	AB	AB
	24	W	W	W	W	W	W
NTX7	**0**	AB	AB	AB	N	AB	AB
	12	AB	AB	AB	N	AB	AB
	24	N	N	N	N	AB	AB

Patient NTX 3 had a normal ABR on the left and only wave III was absent on the right side. A significant improvement for waves III on the right ear was observed at month 12 but wave I and wave V showed a small decrease in absolute amplitude at this time (Table 5.16). There was a marked improvement in waveform amplitude

morphology in wave I on the left side. At the 24 month follow-up, wave I and V were present on both sides and wave III was absent bilaterally (Table 5.17).

At baseline, on the ABR only waves I and III were identifiable in patient NTX 4. At the 12 month follow-up, a small decrease in amplitude of wave I on the right ear was evident (Table 5.16). Interestingly, at month 24 on the right ear, wave I and V were recorded and wave III was absent (Table 5.17). The ABR was not recorded on the left ear in this patient because of the presence of a profound SNHL that was measured on audiometry.

The ABR for patient NTX 5 was normal on the right ear and only wave III was absent on the left. No change in the ABR was seen on the left ear at the follow up, 12 months later but an increase in the absolute waveform latency of wave V on the right side was evident (Table 5.15). The deterioration is independent of the peripheral hearing which has remained normal. A small decrease in waveform amplitude and overall morphology was also seen in both ears (Table 5.16). At month 24, all waves were present on both ears, although wave V was prolonged on the right side (Table 5.15).

The ABR was only measured at month 12 and at baseline in patient NTX 6 because they withdrew from the clinical trial. At baseline, there were no observable responses in the ABR for either ear. There was no change in the ABR at month 12 (Table 5.17).

At baseline, there were no clear waveform components on the ABR for the right ear and only wave I was seen on the left in patient NTX 7[214]. No change in the ABR was seen at the 12 month follow up, except for slight improvement in the amplitude of wave I (Table 5.16). At month 24, all waveforms were seen on the right side, although

the latency of wave V was prolonged (Table 5.15). On the left ear, wave I and V were seen (Table 5.17).

Table 5.18 presents a table summarising the overall results of the ABR. We compared the ABR waveform data at baseline with that at month 24. We considered an improvement in the ABR had occurred if a wave is not present at baseline and is now present. Conversely, we defined a deterioration is said to have occurred if a wave is present at baseline and is now absent. No change is said to have occurred if a wave is absent at both baseline and at month 24.

Table 5-18 Summary of the changes in the ABR based on the presence/absence of the ABR for each group.

	Treatment (TX) group						No-treatment (NTX) group					
	Right			**Left**			**Right**			**Left**		
	I	**III**	**V**	**I**	**III**	**V**	**I**	**III**	**V**	**I**	**III**	**V**
Improved	1	2	3	2	2	1	1	4	2	1	1	1
No change	4	3	2	3	2	3	5	1	4	4	2	3
Deteriorating	0	0	0	0	1	1	0	1	0	1	3	2
N	5	5	5	5	5	5	6	6	6	6	6	6

No notable differences were observed between the two treatment groups for the presence or absence data when we compared the groups from baseline to month 24. The most striking feature of the data was the variability in the ABR, which was not evident in our longitudinal studies using the ABR in patients receiving ERT alone.

5.3.4 Discussion

5.3.4.1 Baseline audiometry

Our application of pure tone audiometry in this clinical trial has revealed two important findings. First our data constitutes the first longitudinal study of pure tone audiometry in children with neuronopathic disease. Our data over the 24 months has

shown that peripheral hearing loss is more common in these children than previously thought and may emerge as a concern later in this condition. We found at baseline that 6/13 cases had a hearing loss. Four of these cases (31%) had a sensorineural hearing loss, consistent with damage to the cochlea. None of these cases had previously shown any hearing loss. By 24 months, the total number of participants with a hearing loss had increased to 8/11 cases (2 withdrawals). More than half of these cases were the result of a conductive impairment such as impacted wax or otitis media.

The unveiling of hearing loss in GD may have been signalled earlier in our studies in Chapter 4, as evidenced by the lower amplitude TEOAEs in the GD3 group. This finding may reflect a subclinical cochlear lesion and not be detected on routine audiometry. Several studies have shown that normal audiograms can have 'hidden hearing loss' or cochlear 'dead' regions. Indeed, studies have shown that up to 30% of the OHC population may be damaged *before there is any audiometric evidence* in the quarter octaves pure tone audiometry from 0.125 kHz to 16 kHz. (Ceranic et al., 1998).

Such findings could reflect widespread loss of afferent nerve terminals and degeneration of the cochlear nerve, which may also provide a partial explanation of the variability seen in our ABR studies (see 5.3.2.2). Damage to these inner ear structures, can result in perceptual abnormalities such as the speech-in-noise difficulties ('noisy') as described on the listening profile by 7/11 GD3 patients (Table 4.8). Future drug trials should consider the inclusion of more fine-grained audiometric assessments including TEOAEs or the TEN HL test.[41]

[41] The TEN HL test is a simple audiometric test recently developed to measure cochlear dead regions in the inner ear.

Hearing loss is prevalent in a number of other related LSD such as Fabry's disease (Brady and Schiffmann, 2004, Desnick, 2004, Whitfield et al., 2005) and Niemann-Pick type C (Aisen et al., 1985, Pikus, 1991, Higgins et al., 1992, Lossos et al., 1997, Patterson et al., 2007). This suggests that these storage disorders may be more predisposed to developing cochlear hearing loss or VIIIth nerve damage (see Chapter 4, section 4.7.1 for further discussion of this topic).

The data further underscores the importance of measuring hearing thresholds in all children, particularly those undergoing ABRs as a part of monitoring the disease process. There are few studies that have investigated the hearing loss in older adults with GD and it may be that hearing loss becomes more prevalent with age (Schiffmann et al., 1997, Bamiou et al., 2001, Grasso et al., 2006). Alternatively, in the era of new therapies, which have extended the life expectancy in these conditions, it is entirely conceivable that we are beginning to see the emergence of manifestations that were not observed prior to the introduction of these treatments. Clearly this is an area that warrants further study as only GD has been thoroughly investigated to date.

Our second finding is related to the application of pure tone audiometry in clinical trials. We encountered a number of difficulties in implementing this behavioural measure in such a young population and, in interpreting the data for a clinical trial, this test presented a number of challenges. For example, what can be considered a clinically significant improvement or deterioration in audiometric thresholds? Perhaps the greatest barrier lies in the lack of any standardised method of reporting hearing loss. For example, there are no international guidelines for describing the configuration or degree of hearing impairment.

Our studies throughout this thesis, have clearly flagged the importance of correctly measuring peripheral hearing. In the absence of this data, interpretation of

other audiological measures such as the ABR is less meaningful. Future studies urgently need to consider the importance of accurately reporting this often overlooked but vital test data.

5.3.4.2 ABR findings

Over the 24-month study period there was remarkable diversity in the BAEP responses at both recordings including absent waves I, III and/or V. When present, latencies were prolonged in many, but not all, cases. The majority of patients in each group (treatment vs non-treatment) showed no little or no change from baseline to last value with respect to the presence or absence in the right ear and left ear of waves I, III and V.

Comparison between baseline and month 24 in the treatment revealed no change in the overall ABR in the majority of waveform components (17/30); an improvement in 11/30 waveform parameters and deterioration in only 2/30 peak waveforms. In the no-treatment group, comparison between baseline and month 24 ABR parameters reveals a similar trend with the majority of patients showing no evidence for change (19/36), deterioration in 7/36 and 10/36 an overall improvement in their ABR. We found no significant difference in the waveform amplitude (I, III or V) between the treatment and non-treatment participants.

It is difficult to account for the reversal in ABR abnormalities in some cases of GD3, especially in the absence of a placebo group (i.e. a group not receiving any treatment). The improved ABR morphology was evident in some cases in both groups

but it is important to remember that by month 24, both groups had received some degree of combination therapy.

Interpretation of the data is further complicated by the possible irreversible nature of the neurological deficits in GD3. For example, if the critical cells (and/or neural circuits) involved in generating a synchronous onset ABR response have suffered irreparable damage during the disease process, then the best outcome we would expect is that treatment would prevent further progression and the ABR parameters would remain stable over time or show no change at all. The improvement in the ABR data in some cases suggests partial or incomplete damage to auditory brainstem circuits.

To the best of our knowledge, only 6 studies have undertaken serial ABR measures (Table 5.2). Two of these studies (n=16) have examined the effect of combined therapy on the ABR. Interestingly, the uncharacteristic variability that we observed in the treatment and non-treatment groups is not restricted to our study. Similar data has recently been published in Benko et al., (2011).

They reported serial ABR data from 12 cases diagnosed with GD3. The ABR showed remarkable variability, even within the same subject, similar to our own findings. For example, 3/12 showed an improved ABR, 4/12 deterioration and 5/12 reported no change in the ABR on the right ear. Similar data was presented for the left ear (3/12 improvement in the ABR; 2/12 deterioration on one or more ABR parameters and no change in 7/12). Unfortunately, latency and amplitude data was not shown and it is entirely unclear which patients were on combined treatment (ERT and Miglustat) although the authors comment that 10 of the patients had received combination therapy during the study interval (4 years).

Such variation in the ABR is not typical in studies of normal hearing controls (Robinson and Rudge, 1978, Chiappa et al., 1979, Tusa et al., 1994). Indeed, ABR peak latencies and inter-peak latencies have been shown to be highly reproducible, even when measured over a 2 year interval (see Section 5.2.2.1 for further discussion). Nor was this variability evident in our longitudinal ABR studies of ERT in GD2 and GD3.

Studies in MS have reported a highly variable ABR in 'active' disease but not in healthy controls or 'clinically stable' patients (Robinson and Rudge, 1978). These studies point to a number of possible causes for fluctuations in the ABR conduction velocity. For example, it might be due to the sensitivity of the ABR to extrinsic factors such as small changes in temperature, or non-pathologic characteristics such as hormone fluctuations or altered calcium/phosphate levels in patients, an important consideration in GD patients or methodological issues such as sampling rate of patient testing (i.e. how often were patients tested, time of day assessed etc) (Table 3.7) (Hall et al., 1988, Jerger and Johnson, 1988, Dehan and Jerger, 1990, Rodriguez et al., 1995, Rodriguez et al., 1999, Hall, 2007). These data could also be explained by sub-clinical changes in the peripheral auditory system (see earlier in section 5.4.3.1). Further study exploring the potential influence of each of these factors is essential.

Alternatively, we could attribute the variability to the disease state, but the neural origin is unknown (i.e) due to the natural history of the disease. Or it may simply be the case that some individuals have a more positive response to treatment.

We could not clearly show evidence that Miglustat had an influence on the progression of ABR abnormalities, in part because there was little change in ABR parameters over the observed period. These results differ significantly from previously

reported case studies (Cox-Brinkman et al., 2008) (Table 5.2). The failure to detect treatment effects on the ABR may have resulted from multiple factors. For example, our trial may have been too short and lacked statistical power to detect any significance. Tusa et al., (1994) has suggested a *minimum* of 7 patients in each group (control and treatment) to detect a statistically significant difference of 0.20ms in the I-III and III-V IPL and a minimum sample size of 10 to detect a statistically significant change in the I-V IPL[42] (Tusa et al., 1994). Other possibilities that could explain the lack of an effect include a marginal inhibitory effect on glucosylceramide synthesis of Miglustat compared with the concentration in the brain.

In summary, the 24-month trial of Miglustat – the first randomised, controlled study of a drug treatment in patients with GD3 – did not show significant differences on the ABR. These results must be interpreted cautiously because of the design of the study including a small sample with widely different baseline characteristics and a lack of a placebo control. Our findings also indicate that the ABR may provide important diagnostic information; but their role in monitoring disease progression has not been established. Larger scale trials, with rigorous methodology and systematic test application need to be undertaken.

5.3.4.3 Comparison of audiological assessment in other treatment trials

A number of clinical studies have advocated the use of eye movements and/or auditory studies as biological markers for monitoring GD (Harris et al., 1999, Vellodi et al., 2001, Baldellou et al., 2004, Grabowski et al., 2004, Accardo et al., 2005a, Pensiero

[42] The sample size is calculated on two measures in each subject, a power of 80% and a two-tailed $\alpha = 0.05$.

et al., 2005, Cox-Brinkman et al., 2008, Vellodi et al., 2009, Accardo et al., 2010) and in other LSD (Bembi et al., 2006, Patterson et al., 2007).

One study that systematically measured SEM in siblings diagnosed with GD3, showed that that quantitative eye movement studies were able to identify subtle neurological changes in these patients. Moreover, they documented an improvement in SEMs in one case (Accardo et al., 2010). The ABR has also been shown to be a useful marker in studies of neuronopathic GD (Cox-Brinkman et al., 2008, Accardo et al., 2010, Benko et al., 2011). More recently, Benko et al., (2011) reported a significant correlation between saccadic eye movements and ABR latency in nGD.

However, the use of eye movements has also been strongly criticised. Kraoua et al. (2011) measured eye movements in 6 patients with neuronopathic GD. They observed that the eye movement deficits occurred early in the disease process in nGD but argued that eye movements were not a sensitive outcome measure for monitoring disease progression in GD. They argued that the presence or absence of atypical eye movement signs was as

"erratic in treated patients as in untreated patients making it a poorly reliable marker of the efficacy of ERT on other neurological signs of the condition" (p1).

Eye movement studies in clinical trials have also been heavily criticised for selecting inappropriate saccadic measures. One small study of children with Tay Sachs disease (and other GM2) showed that saccadic latency was a more robust and sensitive serial measure in LSD (Roos et al., 2011). The authors strongly condemn earlier clinical trials (Patterson et al., 2007, Schiffmann et al., 2008, Benko et al., 2011) that have used markers such as duration, amplitude and peak velocity to quantify neurological impairment.

Remarkably, the use of audiology and eye movement studies in clinical trials has also received criticism for failing to measure cortical function. Roos et al., (2011) argued that these markers of brainstem function fail to reveal anything about global cortical function or produce any data that could be used to measure therapeutic efficacy. The censure of these measures simply because they are not measures of cortical function is unreasonable. These measures are selected because *they do* measure brainstem disease.

So what are the characteristics of an ideal marker of neurological disease? Cox (2009) argues that these markers need to be simple, quick to administer, inexpensive and sensitive. The techniques that we have advocated in this thesis, meet many of these requirements. However, we do not believe that the application of these techniques, particularly the audiological measures have demonstrated sufficient sensitivity for GD to use as a 'stand-alone' or as a 'primary outcome measure'. There is a wide variation in the ABR – across parameters – as demonstrated by our application in a clinical trial.

Outcome measures of the efficacy of any treatment for neuronopathic disease may require years of follow-up. Thus, there is an urgent need to find means of monitoring the disease burden. It is possible that ocular movements and auditory testing may provide such a way. However, too few patients and lack of standardised measures represent significant hurdles that need to be overcome.

5.4 Conclusions

In the first experiment of this chapter we showed that the ABR in neuronopathic GD patients continues to deteriorate even during treatment with ERT, suggesting that ERT therapy alone does not halt the decline in the neurological manifestations of GD.

In the second experiment of this chapter we examined whether the ABR can be a useful both as a longitudinal biomarker of disease progression and as a tool for objectively assessing the efficacy of new drug treatments for GD. We recorded the ABR in patients participating in a clinical trial for a new therapy for GD – Miglustat. We found that, over a 24 month period, the ABR varied in its abnormality within and between subjects but found no significant difference between the treatment and non-treatment groups. We believe that the ABR has most application when considered as part of a battery tests to assess brainstem function and neurological deficits.

Chapter 6
Audiological profile of Dancing Eye Syndrome

6.1 Introduction

In the preceding experimental chapters, we investigated the use of combined audiological and oculomotor assessment in GD, a neurodegenerative disorder that is characterised by SIF and slow saccades. We identified a number of auditory abnormalities, originating within the auditory brainstem pathways. These findings raise an interesting question – *if the auditory brainstem system is impaired in disorders with slow saccadic function, is it also impaired in disorders seen at the other end of the eye movement spectrum?* For example, is there evidence of auditory dysfunction in disorders with hyper-excitable eye movements, such as opsoclonus, one of the distinguishing features of Dancing Eye Syndrome (DES)?

DES is an extremely rare disorder that is characterised by a jerky ataxia, shivering movements (myoclonus), and 'chaotic' saccadic oscillations (opsoclonus). In the majority of cases, initial signs and symptoms appear in very young children, typically aged between 10 months and 3 years, who have never displayed any evidence of neurological disease. While a number of the acute neurological signs that are characteristic of this disease resolve quickly, approximately 80% of all cases are left with residual neurological deficits (Wilson, 2006, Klein et al., 2007, Brunklaus et al., 2011b).

Many unanswered questions remain about which brain structures are involved in DES. Previous eye movement and auditory studies have implicated both the cerebellum (Mezey and Harris, 2002) and the brainstem (Horikawa et al., 1993) as probable candidates. Whatever the underlying cause, it is crucial that we identify which parts of the brain are affected by the disease.

In this chapter, we apply all that we have previously learned from chapters 4 and 5, and investigate the utility of auditory measures in advancing our understanding of pathophysiology in DES. It is possible that application of the ABR (in addition to eye movement studies) could provide valuable new insights into the neuroanatomical structures that may be involved in DES. Furthermore if we can show that these studies are sensitive markers of any brainstem abnormalities in DES, they may offer a unique opportunity to facilitate earlier diagnosis, identify neural structures involved in the disease process and could potentially prove useful as longitudinal (prognostic) tests. Auditory studies in DES could also be particularly important in the acute phase because many of the tests used require no overt responses.

We begin this chapter by presenting a synopsis of the signs and symptoms that are seen in DES. We then present a summary of previous eye movement and auditory studies that are pertinent to this thesis.

6.2 Dancing eye syndrome

Dancing Eye Syndrome (DES) was initially reported by Kinsbourne (1962), who described a striking combination of symptoms in six children. These signs included opsoclonus, myoclonus, ataxia and extreme irritability (Kinsbourne, 1962). Since then, a number of publications have described the same disorder – albeit with a wide variety of different names. We have summarised these various synonyms in Table 6.1. In order to avoid confusion we will use the term DES throughout the remainder of this thesis.

Table 6-1 A summary of the various names by which DES is known in the scientific literature*

Description	Reference
Myoclonic encephalopathy of infants (or childhood)	(Martin and Griffith, 1971, Leonidas et al., 1972, Senelick et al., 1973, Brandt et al., 1974, Delalieux et al., 1975)
Dancing eyes	(Chiba et al., 1970, Kalmanchey and Veres, 1988, Matthay et al., 2005)
Dancing feet	(Dyken and Kolar, 1968, Imtiaz and Vora, 1999, Senanayake and Sumanasena, 2004, Badaki and Schapiro, 2007)
Infantile polymyoclonia or polymyoclonus syndrome	(Dyken and Kolar, 1968, Moe and Nellhaus, 1970, Manson, 1973, Fowler, 1976, Kumta et al., 1976, Sugie et al., 1992, Horikawa et al., 1993)
Kinsbourne syndrome	(Corrias et al., 1985, Papini et al., 1992, Rodriguez-Barrionuevo et al., 1998)
Opsoclonus, body tremulousness and benign encephalitis	(Cogan, 1968, Aikawa et al., 1984)
Syndrome of ocular oscillations and truncal myoclonus	(Baringer et al., 1968, Bhatt et al., 1982)
Encephalopathy associated with occult neuroblastoma	(Berg et al., 1974)
Opsomyoclonus	(Nickerson and Hutter, 1979, Warrier et al., 1984, Warrier et al., 1985, Hiyama et al., 1994, Tejeda and Kaplan, 1994, Honnorat et al., 1997)
Opsoclonus-myoclonus	(Battaglia et al., Leder, 1981, Rosenberg, 1984, Araki et al., 1989, Bataller et al., 2003, Armstrong et al., 2005, Koul et al., 2007, Burke and Cohn, 2008, Brunklaus et al., 2011b)
Opsoclonic cerebellopathy	(Nausieda et al., 1981, Bachman, 1982)
Acute cerebellar encephalopathy	(Bray et al., 1969, Bray, 1972, Telander et al., 1989, Liebling et al., 1993)
Syndrome of rapid irregular movements of eyes and limbs in childhood	(Pampiglione and Maia, 1972)

*Adapted from Pranzatelli (1992)

DES has been reported in both children (Korfei et al., 2005, Badaki and Schapiro, 2007, Klein et al., 2007, Krasenbrink et al., 2007, Catsman-Berrevoets et al., 2009, Brunklaus et al., 2011b, a) and adults (Anderson et al., 1988, Garg et al., 1996, Baets et al., 2006, Brunklaus et al., 2011b, Groiss et al., 2011). The disease is often observed following an apparent viral infection or with neuroblastoma in children (Sheth et al., 1995, Turkel et al., 2006, Rothenberg et al., 2009, Sakuma et al., 2010, Brunklaus et al., 2011a). In adults, this constellation of symptoms exists much more rarely in association with carcinoma (Scholz et al., 1994, De Luca et al., 2002, Baets et al., 2006) or viral infection (Markakis et al., 2008, Scott et al., 2009).

DES is extremely rare, with prevalence estimates of 10 new cases diagnosed in the UK each year (Dale, 2003)[43]. Males and females appear to be equally affected (Boltshauser et al., 1979, Pohl et al., 1996, Tate et al., 2005) with most cases typically presenting before a child's third birthday (Dale, 2003).

6.3 Clinical signs and symptoms in DES

Clinical signs and symptoms seen in DES have two phases: acute and chronic. Symptoms observed in the acute phase are typically more intense. For example, at the peak of the illness, an affected child will have difficulty sitting or standing. They may have trouble speaking clearly and sleep patterns are also frequently disturbed. Many children will experience extreme bouts of disruptive behaviour, e.g.rage attacks, and extreme irritability. Others demonstrate a desperate need to be held, almost constantly (Mitchell et al., 2002, Turkel et al., 2006). Table 6.2 presents a summary of the more common signs and symptoms reported in DES.

Table 6-2 Common presenting signs and symptoms of DES

Signs and Symptoms	
Primary CNS involvement	**Other**
Opsoclonus Myoclonus Ataxia Dysphasia Neuroblastoma Learning difficulties and cognitive impairment	Lethargy Irritability Sleep disturbances Developmental sequelae are common including motor, speech and language deficits

Neuroblastoma is common in DES, although its reported prevalence is highly variable across studies, ranging from 40% (Brunklaus et al., 2011b, a) to 85% (Mitchell

[43] Other studies have reported a much lower incidence of 0.18 cases per million total population per year Pang KK, de Sousa C, Lang B, Pike MG (2010) A prospective study of the presentation and management of dancing eye syndrome/opsoclonus-myoclonus syndrome in the United Kingdom. Eur J Paediatr Neurol 14:156-161..

et al., 2005). Such variability may reflect an improvement in imaging methods. However, others have argued that a low-grade neuroblastoma is present in all cases with DES but may have spontaneously regressed before detection (Pranzatelli, 1992).

Relapses are common. The acute neurological symptoms often return during periods of illness, fever, stress or discontinuation of immunotherapy. Interestingly, those cases who experience a monophasic disease course, appear more likely to recover without residual cognitive and motor deficits (Mitchell et al., 2005).

6.3.1 Acute neurological signs

In this section, we describe the acute neurological signs and symptoms that are generally seen over a relatively short time frame, typically a few days or weeks (Klein et al., 2007). These include opsoclonus, myoclonus, ataxia and a disruption to normal behaviour patterns. We have summarised typical and atypical features in Figure 6.1.

Opsoclonus consists of a series of involuntary, arrhythmic, chaotic, multidirectional saccades, with horizontal, vertical, and torsional components. We have previously described the features of opsoclonus earlier in Chapter 2. These characteristic eye movements often occur in ‘spells’ or ‘bursts of activity’ in DES. Several studies have reported that opsoclonus is occasionally seen within the two days of the onset of the disease (Pranzatelli, 1992, Mitchell et al., 2002, Dale, 2003, Matthay et al., 2005, Mitchell et al., 2005, Pang et al., 2010). It has also been reported as

Figure 6-1 Flow chart summarising the distinctive motor and behavioural characteristics that are seen in DES.

Figure has been removed due to copyright restrictions

appearing later in the course of the disease – after the onset of motor symptoms such as ataxia (Chiba et al., 1970). We will discuss the eye movement signs in DES later in this chapter (section 6.4.2).

Children with DES also experience profound dysfunction in other movement systems (i.e.) myoclonus and ataxia. The degree of myoclonus seen in DES is highly variable – ranging from tremulous polymyoclonia to coarse multi-focal jerks (Baringer et al., 1968, Bhatt et al., 1982). These shaky movements are usually exacerbated when a child is trying to move or because of increased emotional distress. The cause of the myoclonus is unclear but EMG studies have implicated the brainstem (Gwinn and Caviness, 1997). This finding is also supported by sporadic case studies that describe exaggerated startle responses to unexpected auditory stimuli during myoclonic episodes (Maeoka and Maegaki, 1998, Yonekawa et al., 2011). These studies suggest that the pontine brainstem may be involved in DES.

The ataxia observed in DES can have an acute or sub-acute onset. In most cases, the child shows signs of significant motor regression – they are often no longer able to walk independently or sit unsupported (Pang et al., 2010). Speech and language abilities are also critically impaired in children with DES but systematic studies examining this aspect of the disease are sorely lacking (Brunklaus et al., 2011b).

DES is also characterised by abrupt changes in mood and behaviour. Turkel and colleagues (2006) describe a wide range of psychiatric symptoms including disruptive behaviour, affective dysregulation, severe irritability, impulsivity, cognitive impairment,

and poor attention[44]. However, other children with DES may present with malaise, apathy, a loss of social interaction and a general unwillingness to engage in age-appropriate play (Turkel et al., 2006).

6.3.2 Chronic neurological signs

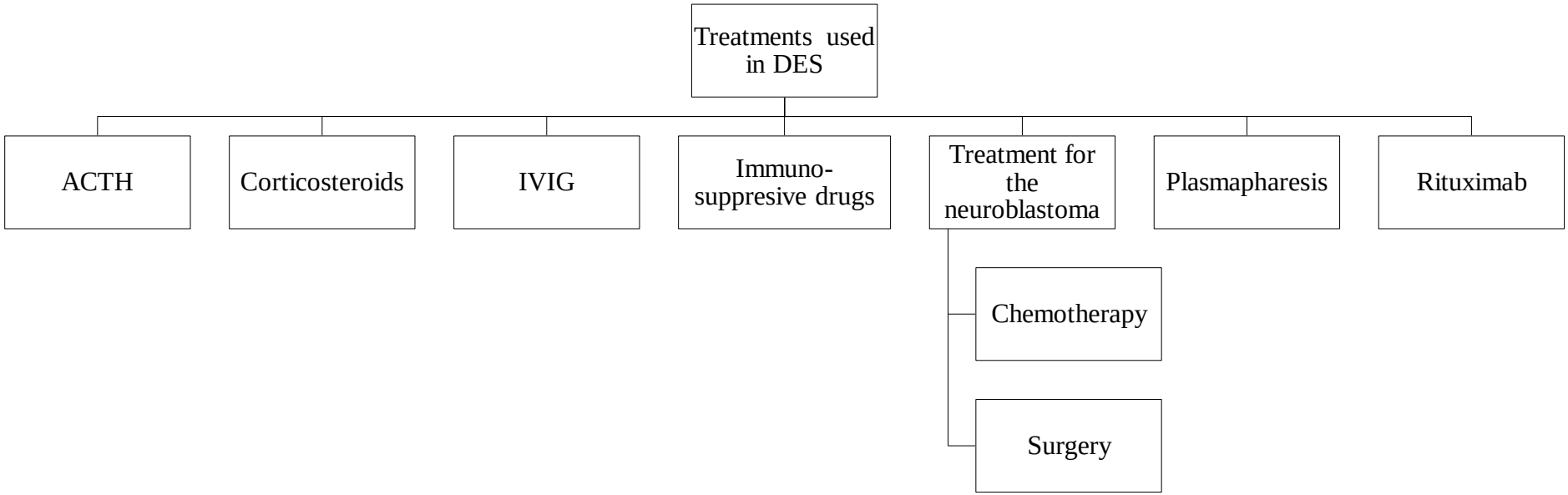
Longitudinal studies have shown that DES is a chronic debilitating illness. Many affected children experience lifelong neurologic sequelae that impair motor, cognitive, language, and behavioural development (Mezey and Harris, 2002, Dale, 2003, Brunklaus et al., 2011b). A recent retrospective review of 101 cases reported residual motor problems in 60% cases; atypical speech patterns in 66% cases, learning disability in 51%, and behaviour problems in 46% (Brunklaus et al., 2011b).

Although there is mounting evidence that indicates that both humoral and cell mediated immune mechanisms are involved in DES, treatment options remain limited. Surgical resection of the neuroblastoma has failed to resolve many of the neurological symptoms (Tate et al., 2005). Short term, high dose steroid therapy is generally the most commonly reported treatment used but with mixed results, particularly in view of the associated side effects (Pranzatelli, 1992, Burke and Cohn, 2008, Catsman-Berrevoets et al., 2009). Figure 6.2 is a presents a summary of commonly used therapies used to treat DES.

A number of different treatment strategies have been advocated in the literature. However, the efficacy of these individual therapies is questionable as there is little data

[44] Affective dysregulation describes a severe and persistent inability to control one's mood states. These children will demonstrate an impaired ability to filter and process the sensory information presented to them. This is often accompanied by explosive and unpredictable episodes of out of control behaviour.

Figure 6-2 A summary of the different possible treatments that are currently used in DES.
Abbreviations: ACTH – Adrenocorticotropic hormone; IVIG – intravenous immunoglobulin



available from randomised clinical trials. Further discussion of treatments is beyond the scope of this thesis but see (Battaglia et al., Oguma et al., Pranzatelli, 1992, 1996, Veneselli et al., 1998, Yiu et al., 2001, Armstrong et al., 2005, Matthay et al., 2005, Mitchell et al., 2005, Pranzatelli et al., 2005b, Bartos, 2006, Blaes et al., 2008, Burke and Cohn, 2008, Ertle et al., 2008, Wilken et al., 2008, Pang et al., 2010) for an excellent review of this topic.

6.3.3 Immunopathogenesis

Earlier in Chapter 2, we stated that while opsoclonus and myoclonus can occur separately, the combination of both signs is extremely rare. These specific clinical signs signal the possibility of a tumour or a viral infection (Pranzatelli, 1992). Indeed, the well-documented presence of a tumour (typically a neuroblastoma) and/or infection in so many cases of DES has given rise to a number of theories which have attempted to reconcile how a tumour or a viral infection could manifest as opsoclonus or myoclonus.

The most widely accepted theory is that DES is an auto-immune disorder in which the brain is,

"an innocent bystander which is caught in the cross-fire between the immune system and the tumour or virus, which it is trying to destroy" (Pranzatelli, 1992, p 187).

This hypothesis has received significant support from clinical studies that have shown marked improvement in symptoms following treatment with immune modulating therapies (Dale, 2003, Glatz et al., 2003, Armstrong et al., 2005, Kirsten et al., 2007, Fuhlhuber et al., 2009, Gorman, 2010). However, the exact immunopathogenesis of the disease is not known and no common antibody–antigen complexes have been consistently identified (Antunes et al., 2000).

6.4 Diagnosis of DES

Diagnosing DES is fraught with difficulty – particularly as many of the clinical features of DES have an early onset. Furthermore, recent data suggests that children with a severe initial presentation, particularly in those who are very young at disease onset, and those who are untreated are more likely to develop long-term sequelae (Brunklaus et al., 2011b).

Misdiagnosis is common. Tate and colleagues reported an average delay to correct diagnosis of almost 3 months, with a maximum delay of 26 months. Another study has shown that children are erroneously diagnosed with post-infectious acute cerebellar ataxia of childhood (Tate et al., 2005, Pang et al., 2010). In fact, until opsoclonus appears, the conditions are clinically similar, and acute cerebellar ataxia of childhood is far more common. Other misdiagnoses have included common childhood illnesses such as otitis media or more infrequently labyrinthitis, and Guillain-Barre syndrome.

The difficulty in accurately diagnosing DES is further compounded by a lack of any objective marker of this disease. Conventional neuroimaging studies such as MRI or CT have provided little evidence of abnormalities or overt damage. Two case reports have described focal inflammatory lesions in the pons and cerebellar vermis (Araki et al., 1989). Conversely, Dale and colleagues (2007) found no evidence of any anomalies in the brain MRI of 10 cases of DES children. Other studies such as EEG have also been reported as normal (Mukherjee and Chakrabarty, 2004, Aguilera Albesa et al., 2009).

These problems underscore the need for early diagnosis and treatment. Clearly novel techniques are urgently required to facilitate timely diagnosis (Gorman, 2010).

Throughout this thesis, we have argued that audiology and oculomotor studies may offer a way forward. In the following sections, we begin to review the application of these tests in DES.

6.4.1 Eye movement signs in DES

We discussed the aetiology and possible pathophysiological mechanisms of opsoclonus at some length, earlier in Chapter 2. Here we *briefly* review the relevant studies that have examined opsoclonus in DES. Opsoclonus is evident when the saccadic system becomes 'unstable'. This rare pattern is the hallmark of DES, although the onset in the acute phase of the disease is highly variable with some studies reporting the onset of opsoclonus as late as 18 months after the start of other clinical signs (e.g. ataxia) (Pang et al., 2010).

Although opsoclonus is a dramatic condition in which the eyes appear to 'jump wildly', misdiagnosis is frequent, particularly as the abnormal eye movements can be fleeting or incorrectly diagnosed as *nystagmus* (Cassidy et al., 2000a). Formal eye movement recording is essential and can be used to differentiate between the two abnormal eye movements[45]. However measuring eye movements in the acute phase of the disease is challenging and quantitative data remains limited (Pranzatelli, 1992).

Ocular flutter has also been widely reported in DES, particularly in patients with resolved DES (Pranzatelli, 1992, Mezey and Harris, 2002, Matthay et al., 2005). Ocular flutter consists of back-to-back saccades which are confined to the horizontal plane.

[45] Formal eye movement recordings will show burst of back-to-back saccades with no inter-saccadic interval if opsoclonus is present. It will not show the rhythm or slow phases of nystagmus (see Cassidy et al., 2000 for a review).

Both of these saccadic abnormalities can result in significant visual disturbance including oscillopsia, although systematic study examining the potential effects of these secondary disturbances has not been undertaken (Leigh and Zee, 1999b, Leigh and Kennard, 2004, Matthay et al., 2005, Ramat et al., 2005).

An important consideration is whether opsoclonus truly resolves. Opsoclonus can reappear – even after apparent complete resolution – during a relapse or if medication is altered or reduced. One study suggests that that minimal opsoclonus can still be elicited on clinical examination[46] (Matthay et al., 2005). Even in the absence of opsoclonus, abnormalities of smooth pursuit eye movements and hypometric saccades are commonly seen even years after treatment (Shawkat et al., 1993, Mezey and Harris, 2002, Mitchell et al., 2002).

Interestingly, one study that formally examined eye movements in 6 children diagnosed with DES reported that smooth pursuit was more adversely affected than the saccadic eye movements. They also reported that vertical saccades were more impaired than the horizontal saccades but that vertical saccadic function showed some degree of improvement when the target stimuli was slower (Mitchell et al., 2002).

In direct contrast, Shawkat et al (1993) reported normal smooth pursuit and OKN in 5 children with DES, when they measured their eye movements using EOG. The most consistent finding in each case was overshoot dysmetria which lead the authors to propose that the origin of the eye movement deficit was in the cerebellar fastigial nuclei.

[46] Matthay et al., (2005) describes a series of clinical exams in which the child is asked to repeatedly re-fixate from near to far, or asking a child to partially squeeze their eye-lids shut, then the clinician partly opens them.

There is considerable controversy surrounding the possible neuroanatomical structures that are involved in DES and the combined symptoms of opsoclonus and ataxia suggest a regional involvement of the brainstem and cerebellum neural circuits (Shawkat et al., 1993, Mezey and Harris, 2002, Helmchen et al., 2003a, Helmchen et al., 2003b, Helmchen et al., 2003c).

In the following section, we examine the scientific literature to determine whether auditory studies, particularly the ABR, show any evidence for brainstem involvement in DES.

6.4.2 Auditory signs in DES

There is limited evidence of auditory involvement in DES. Peripheral hearing loss is described in a single adult case study (Rosenberg, 1984) however it is not clear whether the hearing loss was the result of ototoxic treatment (i.e. chemotherapy regime) or whether it constituted part of the natural history of the disease.

Only six studies have measured the ABR in patients diagnosed with DES. These studies have shown auditory electrophysiological abnormalities in children (Kalmanchey and Veres, 1988, Horikawa et al., 1993, Maeoka and Maegaki, 1998, Sakuma et al., 2010) and adults (Araki et al., 1989, Bartos, 2006). We have summarised these studies in Table 6.3. Of the 10 cases that have been reported, 5 have shown abnormalities on the ABR (4 children and 1 adult). Delayed wave III and V, and prolonged inter-peak intervals I–III and III–V were the most commonly reported findings. These data suggest

Table 6-3 Summary of all previous studies comparing the ABR in DES in the last 30 years.

Author	*Age at time of assessment*	*Age on onset*	*Disease course*	*Treatment*	*Hearing*	*Recording method*						*Comments*
						Stimulus	Intensity(dB)	Polarity	Rate (sec)	Filters (Hz)	Control Data?	
Kalamanchey & Veres (1988)	Serial ABRs recorded in 3 cases[1]	15month old female; 21month old female; 48 month old female	Relapsing	Corticosteroids ACTH	NR	Click	60 & 80	NR	12.8	150-3600	Y	ABR abnormalities worse in acute stage of disease; less marked in remission. Prolonged III-V interval in 1 case and I-III interval in 2/3 cases
Araki et al. (1989)	ABR recorded in a single case. Age of subject at assess NR	41 yr old female;	Monophasic	NR	NR	NR	NR	NR	NR	NR	Y	Prolonged I-III interval
Horikawa et al. (1993)	Serial ABRs recorded in a single case[2]	15month old male	Relapsing	Prednisolone ACTH	NR	Clicks	80 dB	Alt	10	NR	Y	Prolonged I-III interval bilaterally
Maeoka and Maegaki (1998)	ABR recorded in a single case. Age of subject at assess NR	13 month old female*	Relapsing	Tumour resection	NR	NR	NR	NR	NR	NR	NR	ABR normal
Bartos (2006)	ABRs recorded in 1 adult case. Age of subject at assess NR	29 yr old female	Relapsing	Prednisolone ACTH Clonazepam	NR	NR	NR	NR	NR	NR	NR	ABR normal
Sakuma et al. 2010	ABR recorded in 3 cases. Age of subject at assess NR	23 month old male; 8 month old male; 21 month old male	Relapsing	IVIG Prednisolone	NR	Click	70	NR	10	NR	NR	ABR normal in all cases – data not shown in the study

Abbreviations: *neuroblastoma associated, ** ganglioneuroma associated. Age at time of assessment: Serial –[1]ABR measured at [2]ABR measured at month intervals (15,16,17,18,19,20,21,22,23,25,28,32,39 months), Disease course: monophasic: symptom control within 5 months, no relapses; rl: multiple relapses; >14, 24 months, respectively: no remission of opsoclonus and myoclonus during that time on various medications.NT – not tested.

that the auditory brainstem may be impaired in DES although it is not clear why some cases have normal ABR responses and others show abnormalities.

Other studies have described abnormalities in the auditory startle reflex circuit. Yonekawa and colleagues described an exaggerated startle response to unexpected auditory stimuli during an episode of myoclonic status in a 3 year old male. Similar findings have also been reported in adults with DES (Wirtz et al., 2002). These studies lend support to the theory that the pons is damaged in DES. Other reports of auditory hypersensitivity have also been described. One parent described their daughter's behaviour as,

"…..very distressed when around loud noises and sudden movements. Moving traffic and car journeys made her even worse" (Caunter, 2010, p29).

Clearly further systematic studies investigating the involvement of auditory brainstem pathways are needed.

We now turn to our experimental data and present two studies. In our first study, we present our preliminary findings from a parental questionnaire that was administered to families of DES throughout the UK. Our core goal in this experiment was to determine whether the hyper-excitability expressed in the eye movement system, as opsoclonus, is mirrored in the auditory system, i.e. *do children diagnosed with DES demonstrate an unusual hypersensitivity to sound?*

In our second study, we present for the first time a systematic audiological evaluation of DES. Here, we were chiefly interested in identifying whether children with DES have auditory deficits, and if so, whether there is any evidence of longer-term brainstem disease, particularly in the absence of any overt eye movement abnormalities.

6.5 Experiment 1: Do children with DES have hyperacusis?

6.5.1 Introduction

Several studies have argued that DES is the result of hyper-excitable neural activity in independent but neighbouring structures within the pontine tegmentum (Hattori et al., 1988, Araki et al., 1989, Maeoka and Maegaki, 1998, Yonekawa et al., 2011). This hyper-excitability is evident in both the motor (opsoclonus and myoclonus) and behavioural responses (e.g. irritability, lack of sleep). This theory is supported by the apparent efficiency of ACTH in reducing some of the cardinal symptoms in DES symptoms, consistent with the role of ACTH in reducing cerebral blood flow and thus neuronal excitability. If this 'pontine' hypothesis is correct, then we could speculate that the hyper-excitability displayed in other sensory domains could have an audiological analogue, i.e. *hyperacusis,* which presents as an unusual hypersensitivity to acoustic stimuli.

Hyperacusis is a subjective phenomenon that is used to describe an unusual hypersensitivity or discomfort induced by exposure to sound (Baguley, 2003). Hyperacusis may be due to either peripheral or central causes and is well documented in patients with Williams's syndrome (WS) (see Baguley, 2003 for an in-depth review). Central excitability, increased central gain and the failure of the nervous system to habituate to the startle response are all theories regarding the mechanism by which hyperacusis is thought to be produced (Baguley, 2003). Interestingly, a few case studies have described an exaggerated startle response in both children (Maeoka and Maegaki, 1998, Yonekawa et al., 2011) and adults (Wirtz et al., 2002).

Measuring hyperacusis is problematic – there are no tests that are able to verify or quantify hyperacusis. As such, measurement of hyperacusis relies solely on self-

reported assessment and questionnaires (Klein et al., 1990, Anari et al., 1999). However, evaluating the prevalence of hyperacusis in children is difficult as few questionnaires have been specifically developed for use in a younger population (Baguley, 2003), although it is clear from the limited published data that hyperacusis is relatively common in young children (Klein et al., 1990, Oen et al., 1997, Rosenhall et al., 1999, Khalfa et al., 2004, Coelho et al., 2007). For example, one study of 506 children (aged 5-12 years) reported that the prevalence of hyperacusis was 3.2% in this group and that 42% were 'bothered by sounds'. Phonophobia, defined as a fear of sound, was experienced by 9% of the children (Coelho et al., 2007). Table 6.4 summarises the studies that have examined the issue of hyperacusis in children.

Table 6-4 Studies that have been used to assess the prevalence of hyperacusis in children.

Author	Questionnaire detail	Key findings
Klein et al., (1990)	Parental questionnaire to families of children with Williams syndrome (WS) (n=65). Control group (n=65).	Prevalence of 95% hyperacusis in WS; 12% in control group.
Oen et al., (1997)	Oral interview and parental questionnaire to families of children diagnosed with spina bifida (n=50). Control group (n=19)	Prevalence of 50% hyperacusis in spina bifida; 10.5% in control group.
Rosenhall et al., (1999)	Hyperacusis was defined as intolerance to broadband click at 80 dB HL. Study was undertaken in a group of children diagnosed with autism (n=111). Control group (n=57)	Prevalence of 95% hyperacusis in ASD; 12% in control group.
Khalfa et al., (2004)	Hyperacusis was defined as LDL < 80 dB HL. Study was undertaken in a group of children diagnosed with autism (n=11). Control group (n=11)	Prevalence of 18% hyperacusis in ASD; 0% in control group.
Coelho et al., (2007)	Hyperacusis was defined as lowered LDL associated with an abnormal annoyance to sound. Questionnaires for parents and children, interviews and estimates of LDLs were used in 506 school-aged children aged between 5 and 12 years old.	Prevalence of 3.2% hyperacusis in school aged-children.

Abbreviations: ASD – Autistic spectrum disorder; LDL – Loudness discomfort levels; WS – Williams Syndrome

In this study we present our preliminary findings from a parental questionnaire that was administered to families of DES throughout the UK. Our goals in this study were (a) to determine the prevalence of hyperacusis and (b) to determine if we could better understand DES by investigating whether the 'hyper-excitability' of DES has an audiological analogue, such as hyperacusis?

6.5.2 Methodology

6.5.2.1 Subjects

An invitation letter explaining the study and a hyperacusis questionnaire (see 6.5.2.2) was sent to parents (or guardians) who were all members of the UK DES association. A copy of the invitation letter is shown in Appendix 3. Of the initial 87 postal questionnaires that were sent, 50 questionnaires (58%) were completed and returned by post. However, 2/87 (2%) were returned 'addressee unknown' and 4/87 (4%) were received after the data analysis was completed and were not used. The remaining 31/87 (36%) did not respond for unknown reasons.

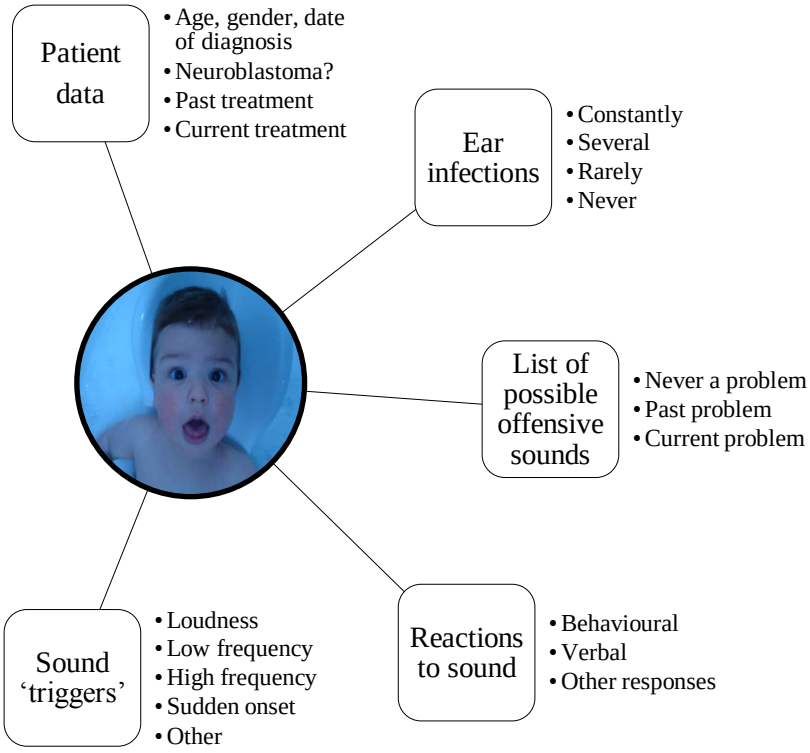
The returned questionnaires represent data from 31 females and 19 males, with an age range of 2.5 to 21 years (mean 9.5 years). Table 6.5 presents the individual responses for each participant to the medical and treatment segment of the questionnaire. An equal number of age-and-gender matched children (n=50; 31 female, age range 2-22 years, mean 10 years) recruited using convenience sampling techniques and with no known predisposing factors for hearing loss were used as controls (n=50). Local ethical committee approval was obtained for the study and informed consent was

obtained from the parents of the younger children and from the older subjects themselves.

6.5.2.2 Hyperacusis Questionnaire

The questionnaire was adapted from Klein et al., (1990). This questionnaire was originally designed to obtain prevalence data of hyperacusis and otitis media with effusion (OME) in Williams Syndrome (WS) (Klein et al., 1990). The questionnaire consists of eight questions relating to the occurrence of hyperacusis, the nature and characteristics of the sounds that are considered offensive, the reaction of the child to these offensive sounds, previous counselling on hyperacusis and the occurrence of otitis media and hearing loss. The complete questionnaire is shown in full in Appendix 3. Figure 6.3 shows a summary of the key points of the questionnaire and self-assessment questions that were asked. The same questionnaire was administered to the control group. Parents were also asked to provide additional information regarding the history of their child's illness including treatment and outcome.

Figure 6-3 Flow diagram summarising the key characteristics of the hyperacusis questionnaire used.



6.5.2.3 Data analysis

The following descriptive statistics were presented for continuous variables: number of values, mean, standard deviation (SD), median, minimum, and maximum. For categorical data, frequencies and percentages were to be presented. Associations between categorical variables were evaluated using Pearson χ^2 or Fisher exact tests as appropriate. For all correlations, Spearman's correlation coefficient was used due to non-normality of the DES data. Two-tailed statistics were used throughout, and $p < 0.05$ was considered significant. All statistics were computed with SPSS (version 14) statistical software (SPSS, Chicago, IL).

Table 6-5 Summary of the medical, diagnostic and treatment regimes for individual respondents of the hyperacusis questionnaire (n=50)

Patient	*Sex*	*Age (yr)*	*Age at presentation (months)*	*Neuroblastoma*	*Past treatment*	*Current treatment*	*DES Improved?*	*Improved at what age? (years)*	*Hyperacusis*
1	F	2.5	7	Y	SURG,CHEMO	Viagram	Y	2	Y
2	F	2.5	28	Y (ganglioneuroblastoma)	SURG , IV IMG	N	Y	2.7	Y
3	F	2.5	22	Y (Adrenal gland)	SURG	Pred	Y (slow)	3	Y
4	F	3	U	U	U	U	U	U	Y
5	F	3	13	Y (Adrenal gland)	None	N	Y	2	Y
6	M	3	U	U	U	U	U	U	Y
7	F	3	18	N	ACTH	Steroids	Y	U	Y
8	F	3.5	24	Y(Adrenal gland)	CHEMO, STEROIDS, SURG	Pred	Y	3	Y
9	F	3.5	12	Y	SURG, STEROIDS	Pred	Y (slow)	-	Y
10	F	3.5	11.5	Y (Thoracic)	SURG, STEROIDS	N	Y	3	Y
11	F	3.5	11	Y	SURG,CHEMO	Pred, IMG infusion	Y	4.25	Y
12	F	3.5	U	U	U	U	U	U	N
13	M	3.5	U	U	U	U	U	U	N
14	F	4	12	Y (Left kidney)	SURG,CHEMO	Pred, IMG infusion	Y	2	Y
15	M	5	18	N	N/A	N	Y	1.8	Y
16	F	5	U	U	U	U	U	U	Y
17	M	5	23	N	STEROIDS	N	Y	5	Y
18	M	5	18	Y	SURG, STEROIDS	N	Y	4.5	Y
19	F	5	23	Y	SURG	Pred, Azathioprine	Y	5	N
20	F	6	21	N	STEROIDS	N	Y	6.2	Y
21	M	6	U	U	U	U	U	U	Y
22	M	6	21	N	STEROIDS	N	Y	5	Y
23	F	6.5	2	N	N/A	N	Y	6	Y
24	F	8	18	Y (Adrenal gland)	SURG, STEROIDS	N	Y	4	Y
25	M	8	28	N	STEROIDS	N	Y	7.5	Y
26	F	8	23	N	N/A	N	N	-	Y

Patient	*Sex*	*Age (yr)*	*Age at presentation (months)*	*Neuroblastoma*	*Past treatment*	*Current treatment*	*DES Improved?*	*Improved at what age? (years)*	*Hyperacusis*
27	F	8	16	N	STEROIDS (2.5 yrs)	N	Y	4	N
28	M	8	U	U	U	U	U	U	UNCLEAR
29	M	9	18	N	N/A	N	Y	3	Y
30	F	10	13	N	N/A	N	Y	5	UNCLEAR
31	F	12	U	U	U	U	U	U	Y
32	F	12	U	U	U	U	U	U	Y
33	F	12	13	N	ACTH	N	Y	7	Y
34	F	13	18	Y	SURG, STEROIDS	N	Y	3	N
35	M	13	U	N	N	N	N	-	N
36	M	14	21	N	ACTH	N	Y	8	Y
37	F	14	U	U	U	U	U	U	Y
38	M	15	U	U	U	U	U	U	Y
39	M	15	U	U	U	U	U	U	Y
40	F	15	18	Y	SURG, STEROIDS	N	Y (slow)	-	N
41	F	16	13	N	STEROIDS	N	Y	14	Y
42	M	16	U	U	U	U	U	U	N
43	F	17	17	N	STEROIDS	N	Y (slow)	-	Y
44	M	17	24	N	STEROIDS	N	Y	5	N
45	F	18	U	U	U	U	U	U	N
46	F	19	U	U	U	U	U	U	N
47	M	20	15	N	N/A	N	Y	8	Y
48	M	20	20	N	NONE	N	Y	8	N
49	F	20	20	Y (Adrenal)	STEROIDS	N	Y (slow)	-	UNCLEAR
50	M	21	U	U	U	U	U	U	Y

Abbreviations: ACTH - , F – female, IMG – Immunoglobulin treatments, M – male, N – No, N/A – Not Applicable, Pred – Prednisolone, SURG – Surgery, U – unknown, Y - Yes

6.5.3 Results

6.5.3.1 Study characteristics

The mean age of the children at the time of this questionnaire was 11 years for males (n=19) and 8.4 years for females (n=31). The average age of onset of the disease was 18 months (range 7 – 28 months; n=33). Disease onset was not reported in 17 cases. The majority of respondents reported that they had never been diagnosed with a neuroblastoma (n=19, 10 males). Fifteen respondents (1 male) reported that they had been previously diagnosed with a neuroblastoma. A significant association between gender and the presence of neuroblastoma was seen in our study. Neuroblastoma was more significantly more common in females than males in our study population (χ^2, $p < 0.01$) however this finding should be interpreted with caution as data was unavailable in 16 cases (8 male).

Several respondents reported a significant delay in the diagnosis of DES. The initial diagnosis was often reported as an inner ear infection (18%) or ataxia (24%). Medical, diagnostic and treatment information is summarised in Table 6.6.

Thirty six percent of respondents received immunosuppressant medication, in various dosages, schedules and combinations. The most commonly prescribed immunotherapies were ACTH or prednisolone. Other treatments included surgery, chemotherapy or a combination (Table 6.6). Sixty four percent of our sample reported a significant improvement in the signs and symptoms of DES. Only 3 cases (case 5, 35 and 48) reported receiving no treatment. Interestingly, two of these cases reported a significant improvement in their overall symptoms. At the time of completing this survey, 8 cases (16%) were still receiving treatment. Only 4% cases reported no overall improvement in the disease course (Table 6.6).

Table 6-6 Summary of the diagnostic and treatments in the study population (n=50)

		Males (n=19)	Females (n=31)
Mean age (years)		11	8.4
Mean length of time with DES (years)		9.4	6.1
Mean age DES first diagnosed (months)		19	17
Neuroblastoma reported?	Yes	1	14
	No	10	9
	Unknown	8	8
Past treatments	None	2	1
	Steroids only	9	9
	Surgery and chemotherapy	0	1
	Chemotherapy and steroids	0	2
	Surgery and Steroids	1	10
	Unknown	7	8
Currently receiving treatment?	Yes	0	8
	No	11	15
	Unknown	8	8
DES improved?	Yes	10	22
	No	1	1
	Unknown	8	8
Hyperacusis?	No	6	9
	Past problem?	8	8
	Current problem?	5	14

6.5.3.2 Hyperacusis

Of the 50 questionnaires returned, 35 (70%) respondents described having difficulties with hypersensitivity to sound. Of the 35 respondents who reported hyperacusis, 19 (5 males) reported this as a current problem (5 males) despite most parents reporting a marked improvement of DES symptoms (Table 6.6). Sixteen respondents reported that although hyperacusis had been a problem, it was no longer a concern (8 males). There was no significant difference between the percentage of males and females with hypersensitivity (χ^2, $p > 0.05$). Thirty percent (15/50) reported that they had never been frightened or bothered by certain sounds. In the age-and gender

matched control group, only 4% (2/50) reported a problem with hyperacusis (χ^2, $p > 0.05$). We could find no evidence to suggest a significant relationship between those reporting hyperacusis and those children diagnosed with neuroblastoma (χ^2, $p > 0.05$). Despite the high occurrence of hyperacusis, only one parent (2.5%) reported ever having received counselling by a professional about dealing with the hypersensitivity problem. They were advised to "avoid loud noises".

Respondents were asked to indicate which sounds disturbed their child (if any). Figure 6.5 shows the sounds that parents considered most offensive to their children. The types of sounds that provoked the discomfort were variable in intensity and frequency. Sounds that children found most disturbing were fire crackers in 80% cases; electric drills in 66% and motor cycles in 63%.

The prevalence of hyperacusis in our control population was 4%, which compares favourably with the data published in Coelho et al., (2007) but is slightly lower than control groups reported in previous studies (see Table 6.4) (Klein et al., 1990, Oen et al., 1997, Rosenhall et al., 1999, Khalfa et al., 2004).

Parents were also asked to describe their child's reactions to 'offending' sounds and the general type of sound that triggers the adverse reaction. Of the 70% of DES children who indicated that they had hyperacusis, 80% reported problems with sudden sounds and 71% with loudness (71%). Figure 6.6 shows a graphical representation of the data across gender. Regardless of the sound characteristic, females consistently showed more distressed responses across all categories. This was particularly evident when the sound was considered as having a sudden onset or was more intense (louder). Low frequency sounds, were reported to be the least disturbing (3%) in the DES group and not at all a concern in males.

Behavioural responses to these sounds are shown graphically in Figure 6.6. The most commonly reported reactions were verbal responses (51 % reporting ‘I don’t like it’) and the most common behavioural response was children covering their ears with their hands in 69 % or cringing in 54%.

Figure 6-4 Stacked bar chart showing the total percentage of respondents who indicated that the sound was offensive to their child (n=35).

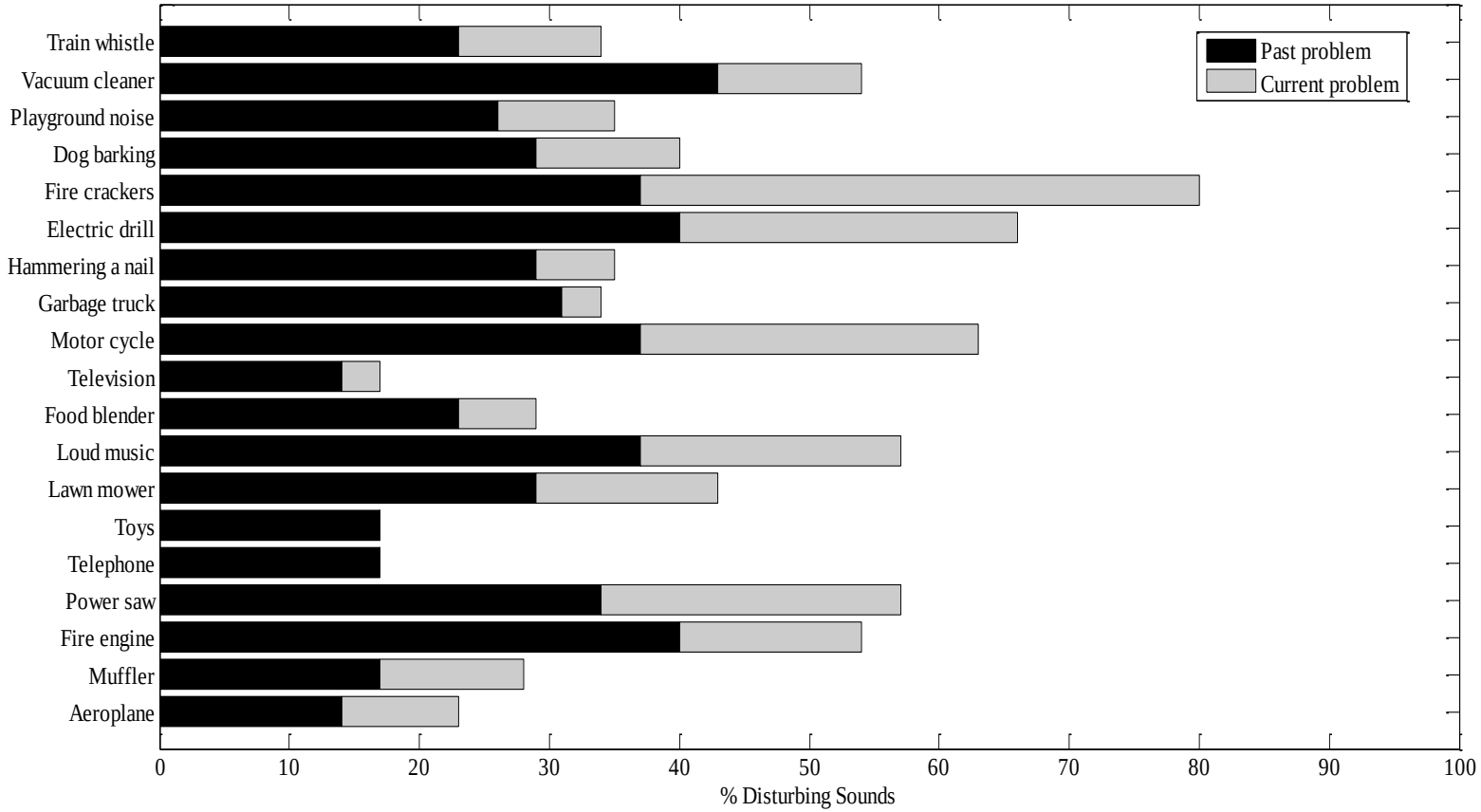


Figure 6-5 Stacked bar chart showing the sound characteristics that triggered an adverse reaction (%) in children with DES (n=35).

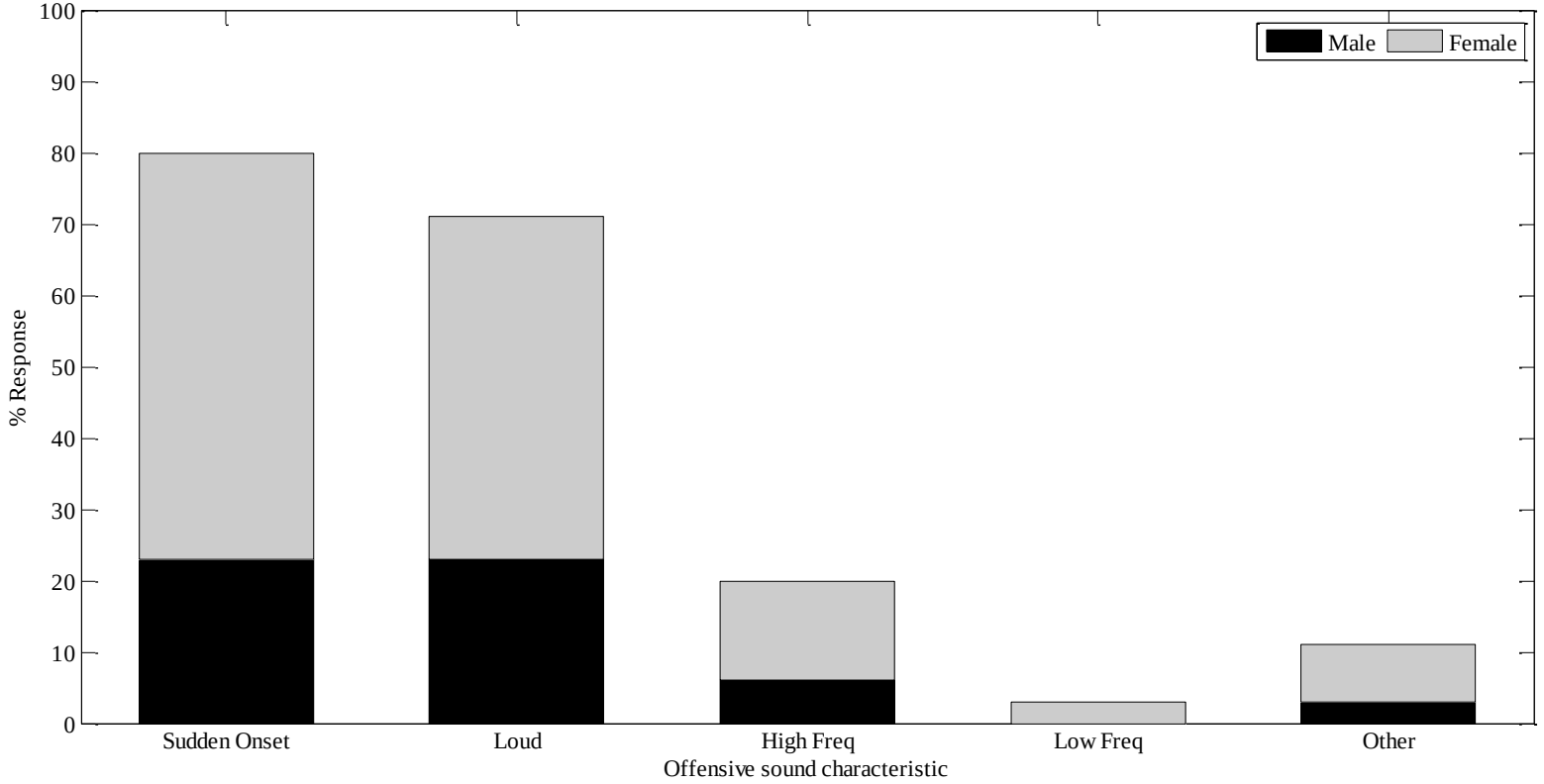
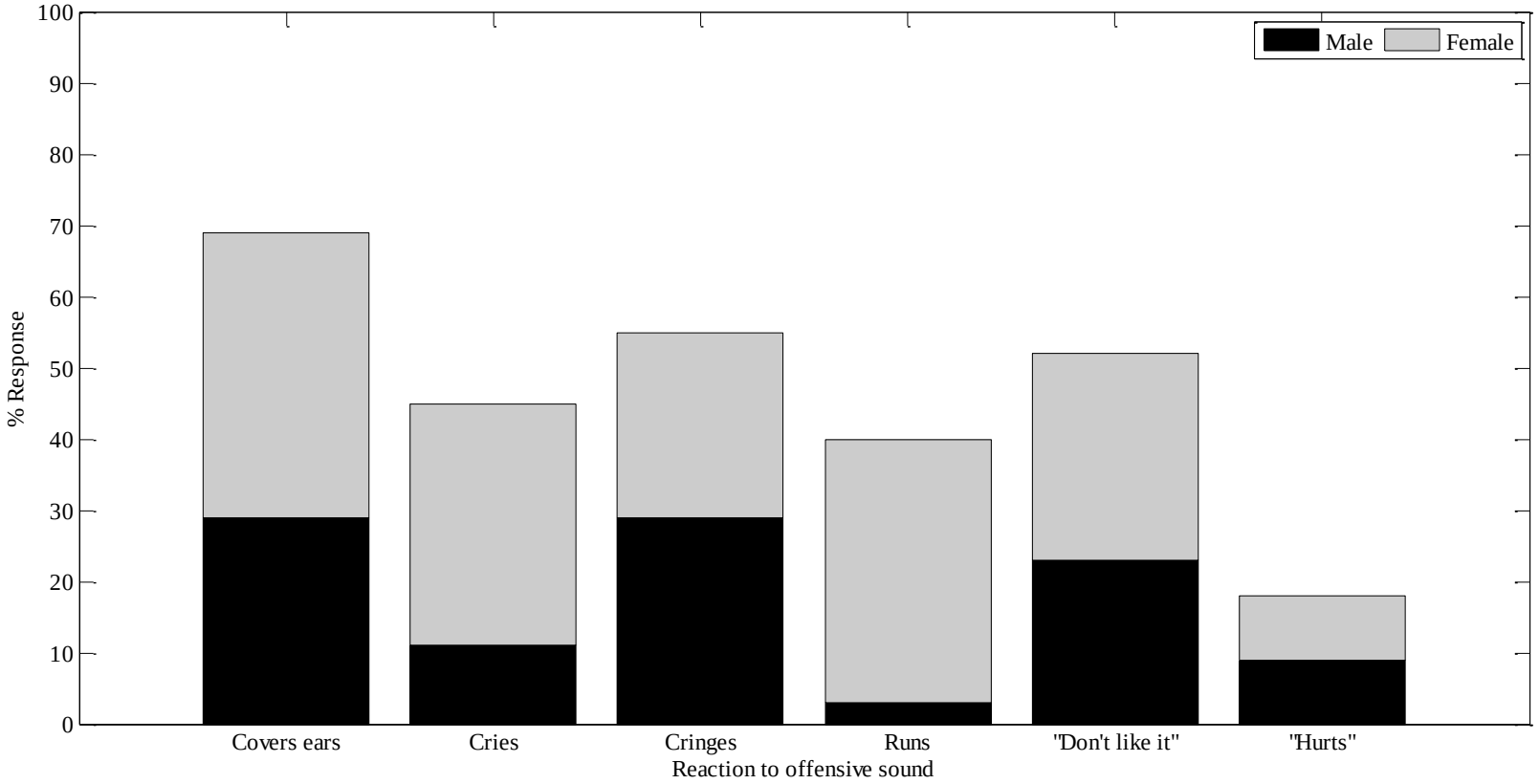


Figure 6-6 Stacked bar chart showing the reaction (behavioural and verbal) to sounds offensive to the DES respondents (n=35).

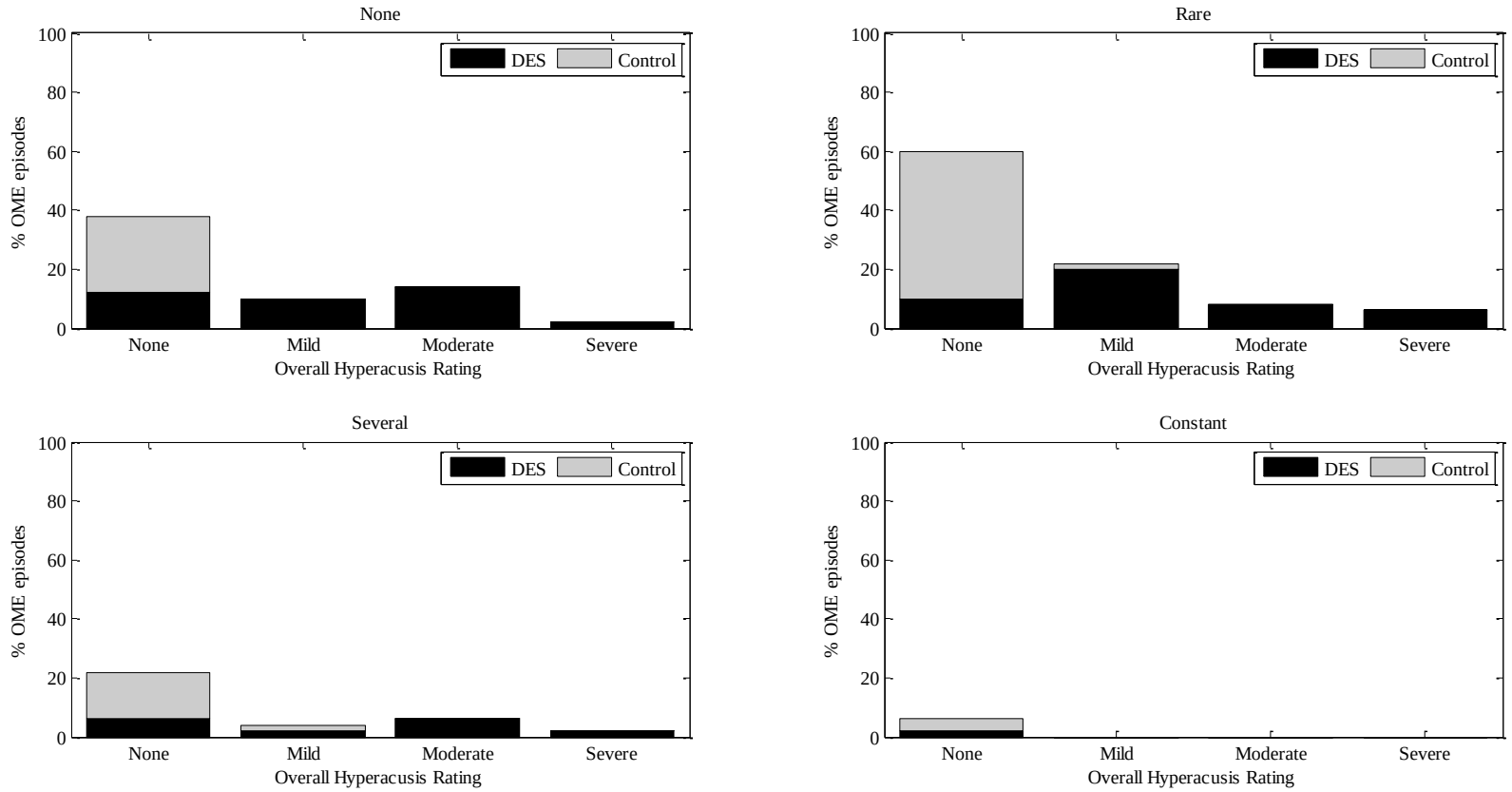


6.5.3.3 Otitis Media

Episodes of otitis media were reported as 'never or rare' in 90% of females and 68% in males. There was no significant difference between males and females for episodes of OME in the DES children (χ^2, $p > 0.05$). Only one case was diagnosed with a unilateral sensorineural hearing loss following chemotherapy.

Each participant was assigned an overall hyperacusis rating (OHR), which was a number from 0 to 3 (0 - never a problem to 3 – severe problem). A 0 was assigned to those who had never had problems with hypersensitivity to sound; a rating of 1 ('mild') was given to those children who had a 'mild' problem with sound as indicated by reacting to 1-7 offensive sounds; a rating of 2 ('moderate') was given to those children who had a 'moderate' problem with sound as indicated by reacting to 8-14 offensive sounds and a rating of 3 ('severe') was given to those children who had a 'severe' problem with sound as indicated by reacting to at least >14 offensive sounds. Figure 6.7 shows the percentage of children with DES and controls with reported number of episodes of OME. The Spearman rank correlation coefficient between frequency of otitis media and OHR was not significant ($r_s = 0.022$, $p < 0.44$).

Figure 6-7 Stacked bar chart showing the reported episodes of otitis media in the DES group and in controls.



6.5.4 Discussion

In this study, we have shown for the first time, that hyperacusis is a common problem in children diagnosed with DES. Seventy percent of parents who responded to this study, reported an abnormal sensitivity to ordinary environmental sounds in their children. Furthermore, of the 35 respondents who described hyperacusis as a problem, 19 cases (54%) described this as an on-going concern.

A number of caveats need to be noted regarding the present study. Firstly, although the overall response rate was satisfactory (50/81), 31 parents/guardians failed to return the questionnaire, raising the problem of responder bias. It is possible that those who returned the questionnaire had different profile characteristics than those who did not. Because invitations were sent by an intermediate party, in this case, the DES UK support trust, we do not have any information about the non-responders. However, our data still lead us to conservatively conclude that hyperacusis is present in *at least* 43% (35/81) of children diagnosed with DES.

Another limitation of our study is the poor completion of some categories of questions. The reason for this is not clear. One possibility is the complicated therapeutic regime that many of the children receive. This was particularly the case where respondents had to indicate whether or not their child had been diagnosed with a tumour. Our study found that significantly more females with DES were diagnosed with neuroblastoma. Other studies fail to report any significant gender differences (Tate et al., 2005). We could find no significant relationship between the presence of a tumour and hyperacusis. However, because of the high levels of missing data we must interpret these results with caution.

Finally, our study could be criticised for being too simplistic. We did not re-validate the original questionnaire tool, a study design error common to a number of earlier studies published in this area (Klein et al., 1990). Studies investigating hyperacusis in children are hampered by a lack of well-validated instruments and a non-uniform description of hyperacusis (see Table 6.4) (Baguley, 2003). It is essential that future work in this area in undertaken to overcome these measurement difficulties.

The high prevalence of hyperacusis documented in other neurological diseases, such as Williams syndrome (WS) has led to the suggestion that the abnormal auditory sensitivity could be the result of a higher incidence of OME and the associated conductive hearing loss in these populations (Klein et al., 1990, Marriage and Barnes, 1995, Katzenell and Segal, 2001). However, episodes of otitis media were reported as rare or infrequent in the majority of our study population and we could find no evidence to support a relationship between hyperacusis and otitis media.

The pathophysiology of hyperacusis is poorly understood (Baguley, 2003). At present, the causes of hyperacusis are typically divided into: 1) clinical conditions which affect the peripheral auditory system (e.g. Bell's palsy, stapedectomy, Ramsay Hunt syndrome, and noise induced hearing loss); 2) conditions involving the CNS (e.g. depression, headache, head injury, learning disabilities, temporal lobe lesions and WS); and 3) hormonal and infectious diseases such as Addison's disease or Lyme disease. In many cases, no underlying cause has been identified (Katzenell and Segal, 2001).

Several studies have speculated that hyperacusis may be the result of altered serotonin function (i.e) reduced serotonin levels within the CANS. (Marriage and Barnes, 1995, Katzenell and Segal, 2001, Attri and Nagarkar, 2010, Mazurek et al., 2010a, Mazurek et al., 2010b). Although Marriage et al., (1995) has also provided

marginal evidence to show that excessive serotonin has also been linked to hyperacusis. Further experimental support for this theory has been reported in rats investigating the role of 5-HT in sensory modulation (specifically auditory startle) (Davis, 1980, Davis et al., 1980a, Davis et al., 1980b). These animal studies examined whether there was a specific association between sensory oversensitivity and 5-HT using the behavioural acoustic startle response in rats. They demonstrated that the normal startle response to 90 ms burst of white noise at 115 dB is modified by changes in serotonin levels. The startle response is depressed when serotonin levels in the forebrain are raised and showed that serotonin inhibits auditory input in the forebrain.

Interestingly, an exaggerated startle reflex to has been reported in an adult patient (Wirtz et al., 2002) and in children with DES (Yonekawa et al., 2011). In these studies these abnormal 'hyper-excitable' responses were elicited using unexpected and intense stimuli; both characteristics identified as 'offensive' in 80% and 71% respectively in our study.

Serotonin abnormalities have also been closely linked to a number of human myoclonic disorders (Pranzatelli, 1994) and in cases of opsoclonus (Pranzatelli, 1992). Altered serotonin activity has also been report in DES (Pranzatelli, 1992, Pranzatelli et al., 1995, Pranzatelli et al., 2005a). Abnormal levels of serotonin metabolites were reported in the CSF in DES patients. Concentrations were 30-40% lower in patients with DES compared to control subjects. Patients with the lowest values were less than 4 years old, and a subgroup had extremely low levels. His data suggests a disturbance and possible altered ontogeny of serotonin or dopamine neurotransmission in a subpopulation of children DES (Pranzatelli et al., 1995). Based on these findings, they speculated about the possibility of different 'phenotypes' based on biochemical data.

Such studies are interesting and may be used to predict longer term outcomes and also explain why some cases are more 'pre-disposed' to neurological sequelae.

An alternative view is that the hyperacusis results from a failure of the CNS to inhibit the non-classical auditory pathways. As we mentioned previously in Chapter 2, auditory information ascends through the brainstem to the cortex in two parallel pathways, known as the classical and the non-classical ascending auditory pathways. The importance of the non-classical auditory pathway for hearing in humans is unknown but recent data has underscored the functional importance of these pathways in loudness perception in children, but not in adults (Moller and Rollins, 2002).

The non-classical pathways have a number of important anatomical and functional links between the subcortical auditory system and the limbic structures which are thought to have an important modulatory role in hyperacusis. The limbic system, which regulates instinctive behaviour and emotions, is linked to the auditory system via the medial geniculate body (amygdala). The hypothalamus, which is the integrative centre of the endocrine and autonomic systems, is linked to the auditory system via the inferior colliculus. The reticular system, which is focused on the behaviour pattern of attention and excitement, projects serotonergic fibres to all pathways of the auditory system, ranging from the cochlea to the auditory cortex (Mazurek et al., 2010a).

It is plausible then that damage to this pathway could provide an explanation of the behavioural disturbances (irritability, needing to be held all the time) experienced during the acute phase in DES. However, it is also possible that the irritability and desire to be held arise because the child's balance and visual world are so unstable and further studies are required to assess the vestibular and proprioceptive input in DES.

Several studies have shown that hyperacusis can have a detrimental effect on an individual's lifestyle, as a result of decreased sociability and inability to spend time with family and friends due to sound intolerance. Our results from the behavioural reactions of our respondents certainly support earlier studies, although we could find no clear pattern regarding which acoustic feature evoked a negative response. Previous studies have also failed to identify any common acoustic features that evoke an adverse reaction in children with hyperacusis (Klein et al., 1990, Baguley, 2003).

One issue that emerges from our novel finding is whether therapy, either modified tinnitus retraining or cognitive behavioural therapy (Jastreboff, 1990, Mattox et al., 1997) (Andersson & Lyttkens, 1999), would be beneficial in children diagnosed with DES. Other treatments that could be considered include the fitting of noise generators or even a hearing aid, in more severe cases. The role of some drugs involved in the metabolism of the serotonin, for example, serotonin reuptake inhibitors may also offer the potential for a new treatment approach.

In conclusion, the results of this research support the idea that hyperacusis is prevalent in DES. These preliminary data suggest that the auditory pathways, in particular, the auditory brainstem may be involved in DES. However, we cannot exclude the possibility that there may be other lesions in the cerebellum, cochlea or the VIIth nerve that may cause hyperacusis (Marriage and Barnes, 1995). However, we believe that our preliminary findings provide sufficient ground to examine this issue further. In the following experiment we will undertake detailed experimental investigation to further clarify the anatomical origins of these abnormalities.

6.6 Experiment 2 – The audiometric profile of children diagnosed with DES

6.6.1 Introduction

The distinctive dyskinesia pattern that characterises DES strongly suggests neural dysfunction in cerebellar and brainstem circuits. However, a more accurate neuroanatomical localisation has not been possible, because of the lack of post-mortem studies. One approach that has been used to investigate possible brainstem involvement in children and adults diagnosed with DES is the ABR. The literature examining this issue is sparse. A systematic review of the literature found only six studies published over a 30 year period (see Section 6.5.3 and Table 6.3 for a summary of these studies). These studies represent the results from a total of 10 individual subjects (2 adults; 8 children; 6 female). Collectively, these studies have described abnormalities in the ABR in half of the cases studied (Table 6.3).

The first report documenting the utility of the ABR in DES measured the ABR at different phases of the disease (in the acute, chronic and remission stages) in 3 children. All of the cases had abnormal ABRs. The degree of abnormality was dependent on the phase of the disease – ABR abnormalities were worse in the acute stages. Interestingly, mild pontine abnormalities were still evident even when the disease was in remission (Kalmanchey and Veres, 1988).

Another study measured the ABR longitudinally over a two year interval in a single case (Horikawa et al., 1993). Despite the improvement in the opsoclonus and other clinical symptoms, serial ABR measurements showed that the I-III interval remained prolonged and showed no evidence of physiological shortening with maturation (Horikawa et al., 1993).

This limited research into the ABR and DES is also full of inconsistencies. Three studies have shown ABR abnormalities in all of their cases, most notably in the I-III interval (Kalmanchey and Veres, 1988, Araki et al., 1989, Horikawa et al., 1993). This is thought to reflect the conduction time from the intracanalicular segment of the VIIIth nerve across the subarachnoid space through the VCN to the caudal pontine tegmentum. Conversely, three studies have reported no abnormalities on the ABR (Maeoka and Maegaki, 1998, Bartos, 2006, Sakuma et al., 2010). Further study is clearly warranted.

In this study, we systematically measured auditory brainstem function in greater detail in a larger group of DES patients. Our aim in this study was to examine whether patients with DES have auditory brainstem abnormalities as indexed by the ABR. We were chiefly interested in identifying whether children with DES have auditory deficits, and if so, whether there was any evidence of longer-term brainstem disease, particularly in the absence of any overt eye movement abnormalities.

6.6.2 Methodology

6.6.2.1 Subjects

Subjects were ten patients diagnosed with DES (age range 5 – 22 years; mean age 10.4 years; 5 males); diagnosis of DES had been previously made by Neurologists at the local hospitals. Subjects with DES were recruited through the DES association following the success of the questionnaire study. The only criterion for inclusion in the study was a definitive diagnosis of DES. Participant numbers were capped at 10 because of the limited travel funds available[47]. Table 6.7 shows the clinical characteristics for

[47] This pilot study was supported by a small pump priming grant awarded by the DES Association, UK. The grant met all of the expenses for the DES participants (eg. Travel and accommodation costs etc) which is why the participant numbers were capped at 10.

each of the individuals enrolled in this study. The age of onset of DES ranged from 12-30 months in this group, which is typical in this disease (Pang et al., 2010). Neuroblastoma was reported in 3 of our study group (cases 5 – 6, 9). Seven participants complained of hyperacusis, as assessed by our earlier questionnaire study (see Section 6.5).

Full-scale IQ data was available only in 5 cases and ranged widely from 54 – 110 (case 3). This data was not available in the remaining 5 cases. The participants in our study group had a number of additional problems including speech difficulties and dysarthria in 3 cases; ataxia and poor-co-ordination in 3 cases and behavioural difficulties in 3 cases. Learning difficulties were common in our study group (8/10 cases), with 3 children attending special needs school. Only one case (case 3) showed no long term deficits following the onset of DES.

An equal number of age-matched children were enrolled on the study (age range 6 – 21 years; mean age 10 years; 4 males). Age was matched to within 9 months of the age of individual DES participants. Controls were recruited using convenience sampling techniques from a larger normative data study (n=90) which was also being undertaken by the author (PC) to determine the normative response to audiometric measures in children (Appendix 2, Section 9.2). All controls had normal hearing threshold levels (normal pure tone audiogram and tympanometry) with no known predisposing factors for hearing loss. Ethical Committee approval was obtained for the study with informed consent obtained from each subject.

6.6.2.2 Procedures

All subjects had standard baseline audiological assessment as previously described in Chapter 3. Audiometric results were obtained from all subjects in the same

Table 6-7 Summary of individual clinical characteristics and treatments.

Case	Age at onset of disease (mo)	Gender	Age at time of assessment (yr)	Neuroblastoma	Hyperacusis?	Full Scale IQ	Other
1	30	M	11	N	N	NT	Dyslexia LTM deficits Dysarthric Learning difficulties
2	15	M	22	N	Y	74	Dysarthric Learning difficulties Behavioural difficulties
3	23	M	8	N	Y	110	No concerns
4	13	F	12	N	N	NT	Learning difficulties
5	14	F	15	Y	Y	48	Learning difficulties Still unsteady on her feet Attends a special needs school
6	12	F	6	Y	Y	73	Learning difficulties Attends a mainstream school
7	18	M	8	N	Y	NT	Learning difficulties Still unsteady on her feet Attends a special needs school
8	12	F	5	N	Y	NT	Learning difficulties Behavioural difficulties Attends a special needs school
9	13	F	5	Y	Y	NT	Separation anxiety Speech delay Behavioural difficulties
10	24	M	6	N	N	54	Stroke in the cerebellum Ataxic and very clumsy Learning difficulties

Special school: school for learning-disabled children

session in which electrophysiological tests were performed. The ABRs were recorded to clicks (100 μs in duration) of alternating polarity presented at a rate of 11.1/s at an intensity of 80 dB nHL using TDH-49 headphones. Eye movements were examined clinically by an experienced clinician. The findings are summarised in Table 6.8. It was not possible to perform a clinical exam in 3 cases (cases 1, 9 – 10) because of poor co-operation. In the remaining 7 cases, eye movement abnormalities were observed in 6 cases – opsoclonus was observed in one case and ocular flutter and/or hypometric saccades in 5 cases. The prevalence of eye movement abnormalities is consistent with previously reported studies (see Section 6.4.2).

6.6.2.3 Statistical Analysis

The following descriptive statistics were presented for continuous variables: number of values, mean, standard deviation (SD), median, minimum, and maximum. For categorical data, frequencies and percentages were to be presented. Distribution normality and equality of variance between groups were assessed by one-sample Kolmogorov-Smirnov test (with Lilliefors correction) and Levene's tests, respectively.

Differences between groups and among conditions were tested for significance using a repeated measure ANOVA using SPSS (version 19.0). Significance tests on within factors and interactions were made using the Greenhouse-Geissler correction for effects with significant Mauchly test for sphericity. Two-way ANOVAs were used to test between groups when a significant interaction was found. In all instances, the reported *p* values were two-tailed since the direction of possible group differences was not known. A p value < 0.05 was considered significant.

Table 6-8 Clinical eye movements in the study group (n=10)

Case	Pupils	EOMs	Smooth pursuit	Doll's head	Saccades (H &V)	Other
1	Sluggish	Full	Normal	Normal	?? fast	Vergence Manual spinning – high frequency VN
2	NT	NR	NT	NT	NT	Data not available
3	Normal	Full – some jitter in the lateral gaze	Normal	Normal	Normal	Eyes steady under closed lids Manual spinning – ok with no observable flutter 3^{o} left esotropia
4	Normal	Full	Normal	Normal	Normal	Normal Vergence; Downward saccades – hypometric? Manual spinning – ok No intention tremor
5	Normal	Full – endpoint nystagmus in right gaze	Horizontal – normal Vertical - low gain	Normal	Normal	Manual spinning – ok with no observable flutter Excellent vergence Possible flutter observed under closed eye-lids
6	Normal	-	Some SP	Unable to tolerate	Saccades with flutter	Right esotropia (from 2yrs old)
7	Normal	Full	Normal	?some flutter	Normal	-
8	Normal	Full	Normal	Vigorous	Saccades present but hypometric	Query opsoclonus with closed lids Some vergence but difficult to elicit Saccades present but hypometric Intention tremor
9	NT	NT	NT	NT	NT	Poor co-operation
10	NT	NT	NT	NT	NT	Not possible to perform a clinical exam. When quiet frequent flutter episodes were observed.

Abbreviations: Ab – abnormal; H – horizontal; N – no; Norm – normal; NT – not tested; V – vertical; Y – yes;

6.6.3 Results

6.6.3.1 Baseline Audiometric Tests

Pure tone audiometry was completed by all the DES subjects at all frequencies (0.25-8.0 kHz). Audiometric thresholds were within the normal range for all DES participants. Mean hearing thresholds (in dB HL) are compared below in Table 6.9 for both groups.

Table 6-9 Mean (± SEM) hearing threshold levels for the right and left ears in dB HL (n=10).

Group	*Frequency (kHz)*	*Right Ear*		*Left Ear*	
		Mean	Std.Err.	Mean	Std. Err.
DES	0.25	16.5	1.5	17.5	1.3
	0.50	17.0	1.9	15.5	0.90
	1	13.0	1.3	12.5	1.3
	2	7.0	1.6	7.5	1.7
	4	8.0	2.0	6.5	1.3
	8	10.5	2.2	9.5	2.0
Controls	0.25	9.5	1.8	10.5	1.4
	0.50	8.0	1.5	10.0	2.0
	1	5.5	2.2	6.5	2.0
	2	8.0	1.1	8.0	1.3
	4	11.0	1.8	6.0	1.5
	8	8.0	1.3	8.0	1.3

Overall mean thresholds are plotted in dB HL for the DES group and the control subjects (±SD) are shown in two groups in Figure 6.8 (upper panel). Although the hearing threshold levels are within the normal range for both groups, plotting these separately revealed a distinct trend, with the DES group showing an increase in hearing threshold levels in the lower frequency range (0.25, 0.5 and 1.0 kHz) compared with the control group.

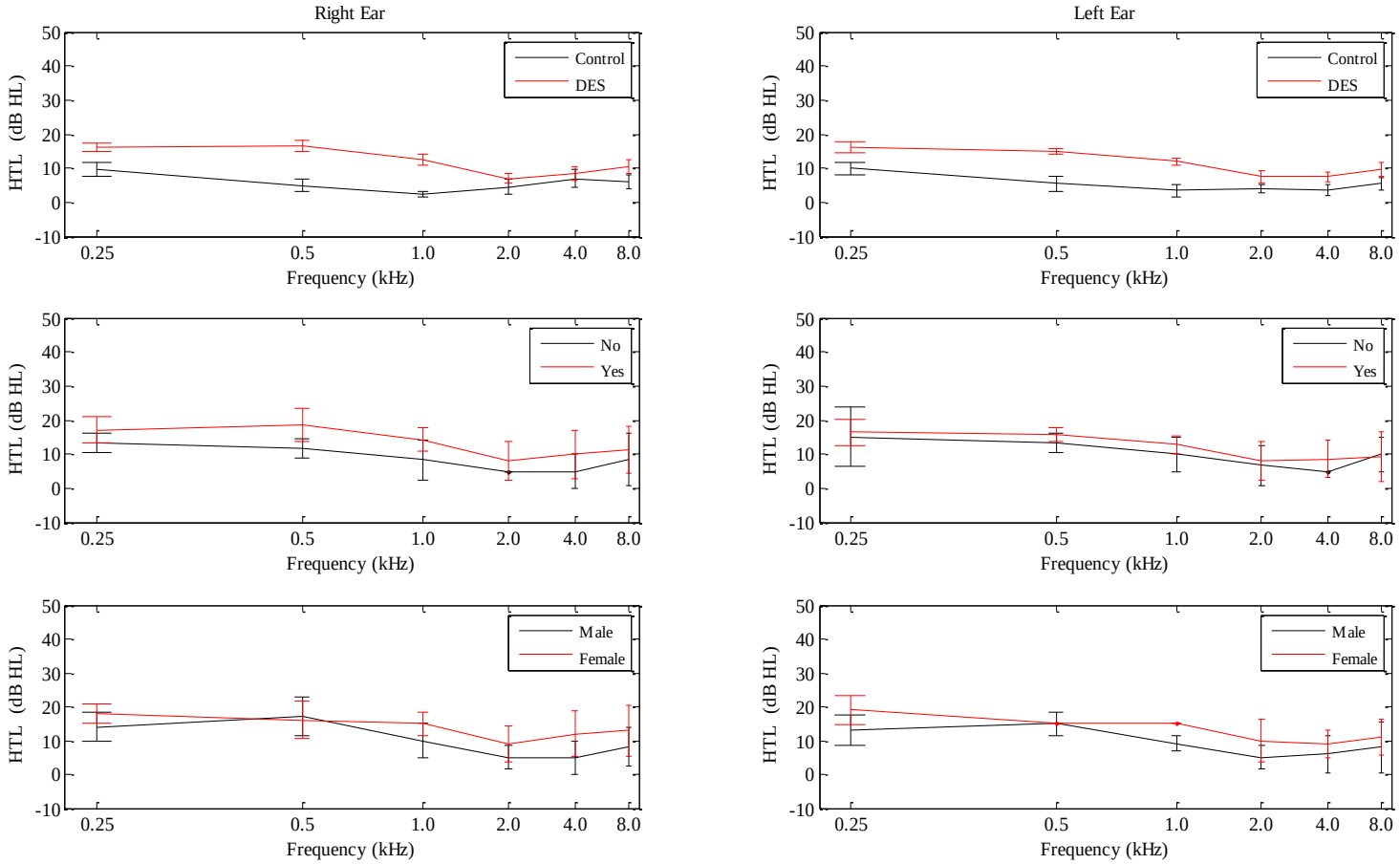
When we plot mean audiometric thresholds across all frequencies for DES subjects who reported hyperacusis (Figure 6.8 middle panel) compared with DES

subjects without hyperacusis, we see that subjects who have had a problem with hyperacusis also have worse audiometric thresholds on average, particularly in the right ear. However, we observed no difference in audiometric thresholds across gender in the DES group (lower panel, Figure 6.8).

A repeated measure ANOVA was performed to examine whether there were any differences within groups. Within subject factors were frequency (levels: 0.25, 0.5, 1, 2, 4, 8 kHz) and ear (levels: left, right). In the DES group, thresholds were significantly different depending on frequency ($p<0.001$) with a clear U-shaped function; there was no significant difference between ears ($p=0.49$).

A two-way ANOVA (group x frequency) revealed a significant difference between the groups ($p<0.01$) with a significant interaction between groups and frequency ($p<0.05$). Post-hoc analysis using Tukey's least significant difference test showed significant differences on hearing threshold levels at 0.25 kHz ($p<0.01$), 0.5 kHz ($p<0.01$) and 1 kHz ($p<0.05$) for both ears.

Figure 6-8 Top panel: Mean (± SD) air conduction thresholds for the right and left ear for the DES and normal control groups (n=10); Middle panel: Mean (± SD) air conduction thresholds for DES subjects complaining of hyperacusis symptoms (n=7) compared with those reporting no hyperacusis symptoms (n=3); Bottom panel: Mean (± SD) air conduction thresholds for male DES subjects (n=5) compared with female DES subjects (n=5).



All of the DES subjects underwent tympanometry. Three data measures were collected: ear canal volume (ECV); middle ear pressure (MEP) and compliance. Table 6.10 shows the mean and standard error of the middle ear analysis for the right and left ear in the DES group and the control group. All DES subjects had normal middle ear function compared with the BSA (1992) normative data values and ranges (see Table 3.2 and Appendix 2, section 9.3) and the middle ear pressure was comparable between the groups.

Table 6-10 Mean and standard error of the mean of the tympanometry values in normal control subjects and in patients with DES.. Abbreviations: ECV – ear canal volume.

Group	*Tympanometry measure*	*Right Ear*		*Left Ear*	
		Mean	Std.Err.	Mean	Std. Err.
DES	ECV	0.94	0.08	0.93	0.06
	MEP	-17.00	19.34	-25.00	20.03
	Compliance	0.65	0.05	0.66	0.07
Controls	ECV	0.91	0.06	0.90	0.06
	Pressure	-15.00	10.80	-32.50	16.52
	Compliance	0.82	0.10	0.89	0.13

6.6.3.2 Transient evoked otoacoustic emissions

Transient evoked otoacoustic emissions were recorded in all DES participants. The TEOAE amplitude responses were comparable across right and left ears, although slightly reduced on the left ear in both groups. The mean TEOAE amplitudes (± SD) in the DES group was 15.4 ± 4.0 (95CI=12.5-18.3) dB SPL for the right ear and 13.7 ± 5.2 (95CI=11.1-17.2) dB SPL on the left ear compared with 14.9 ± 3.3 (95CI=10-17.4) dB SPL for the right ear and 12.4 ± 2.9 (95CI=10.3-14.5) dB SPL for the control group. An independent-t test revealed no significant difference in the TEOAE amplitude between the two groups for the right ear (p=0.753) or left ear (p=0.498).

6.6.3.3 Acoustic reflex response

The acoustic reflex response (ARTs) was measured using ipsilaterally at 0.5, 1, and 2 kHz for both ears in all of our DES subjects. Table 6.11 shows the mean ART and standard error mean values for the DES and the control group. ARTs were normal with ipsi-and-contralateral stimulation across 0.5, 1.0 and 2.0 kHz in all DES participants (range 80 – 100 dB) (Cohen and Prasher, 1988, 1992) (Appendix 2). Interestingly, our control group showed marginally elevated ART responses compared with the DES group for both ipsilateral and contralateral stimulation.

Table 6-11 Mean (± SEM) ART (dB HL) for the right and left ears in dB (n=10).

Reflex frequency (Hz)	GROUP	RIGHT EAR		LEFT EAR	
		Mean ART (dB HL)	Std.Err.	Mean ART (dB HL)	Std.Err.
Ipsi500	DES	84.2	2.4	82.5	1.7
	Control	83.8	2.2	87.5	3.2
Ipsi1k	DES	85.0	2.8	86.7	2.1
	Control	87.5	3.2	85.0	3.5
Ipsi2k	DES	85.0	1.9	81.5	2.2
	Control	90.5	1.4	87.5	4.8
Contra500	DES	87.8	4.5	88.3	3.7
	Control	86.8	3.3	93.8	4.2
Contra1k	DES	84.2	1.8	89.2	2.0
	Control	87.5	1.4	90.0	2.8
Contra2k	DES	84.2	2.1	86.7	3.2
	Control	87.5	2.5	91.2	2.4

6.6.3.4 The auditory brainstem response

The ABR was measured in all subjects across both ears. The absolute latencies of waves I, III, and V and the inter-peak intervals I-III, III-V, and I-V for each individual case are shown in Table 6.12. All of the peak and interpeak waveform components were inspected for each individual case. The ABR was considered abnormal when latency or IPL were >2SD outside the normal controls (i.e.) peak latency on the ABR was considered abnormal when wave I, III or V latency were respectively greater than 1.97 ms, 3.98 ms and 5.86 ms on the right ear and ABR was considered abnormal when wave I, III or V latency was greater than 1.96 ms, 3.86 ms and 5.81 ms on the left. Table 6.13 shows the mean latency values of wave I, III, and V in the DES group and the age-gender matched control group.

Interpeak latencies were considered abnormal when the I-III, III-V and I-V intervals were respectively greater than 2.26 ms, 2.08 ms and 4.17 ms on the right ear and 2.18 ms, 2.07 ms and 4.09 ms on the left. Table 6.14 shows the mean latency values of wave I, III, and V in the DES group and the age-gender matched control group.

All peak waveforms were present in the DES group but 9/10 DES children (90%) showed abnormal peak latency or inter-peak latency values (Table 6.12). Wave III was delayed in 1/10 (case 7) on the left ear and wave V was delayed in 2/10 cases on the right ear (cases 4 and 7). The inter-peak intervals showed a greater number of abnormalities with a delayed I-III interval in 3/10 cases on the left, a prolonged III-V interval in 4/10 cases on the right ear (cases 2, 4, 7 and 9) and in 4/10 cases on the left ear (cases 3, 4, 6 and 9). The I-V interval was also delayed in 4/10 cases on the right (cases 4, 5, 7 and 9) and in 7/10 cases on the left (cases 3-6, 8-10). Only one subject (case 1) showed no evidence of any prolongation of peak and inter-peak latency measures.

Table 6-12 Individual ABR peak latency data for both ears .*> 2SD above the control mean.

Case	Right Ear						Left Ear					
	I	*III*	*V*	*I-III*	*III-V*	*I-V*	*I*	*III*	*V*	*I-III*	*III-V*	*I-V*
1	1.68	3.82	5.66	2.14	1.85	3.98	1.63	3.79	5.69	2.16	1.90	4.06
2	1.63	3.65	5.74	2.02	2.09*	4.10	1.68	3.67	5.59	1.99	1.92	3.91
3	1.61	3.73	5.62	2.12	1.89	4.01	1.59	3.67	5.81	2.08	2.14*	4.22*
4	1.68	3.62	5.88*	1.94	2.26*	4.20*	1.68	3.62	5.78	1.94	2.16*	4.10*
5	1.63	3.84	5.86	2.21	2.02	4.23*	1.68	3.72	5.78	2.04	2.06	4.10*
6	1.56	3.67	5.71	2.11	2.04	4.15	1.56	3.60	5.69	2.04	2.09*	4.13*
7	1.63	3.82	5.98*	2.18	2.16*	4.34*	1.70	3.96*	5.69	2.26*	1.73	3.98
8	1.58	3.77	5.66	2.18	1.87	4.06	1.63	3.77	5.74	2.14	1.97	4.10*
9	1.63	3.67	5.86	2.04	2.19*	4.23*	1.51	3.62	5.74	2.11	2.12*	4.23*
10	1.78	3.76	5.84	1.98	2.08	4.06	1.61	3.72	5.78	2.11	2.06	4.17*

For the right ear, the mean peak latency for the DES group was 1.64 ms (95CI= 1.60-1.69) for wave I; 3.74 ms (95CI= 3.68-3.79) for wave III and 5.78 ms (95CI= 5.70-5.87) for wave V. For the left ear, the mean peak latency for the DES group was 1.63 ms (95CI= 1.58-1.67) for wave I; 3.71 ms (95CI= 3.63-3.79) for wave III and 5.73 ms (95CI= 5.68-5.78) for wave V (Table 6.13).

A repeated measure ANOVA was performed to examine whether there were any differences within groups using latency in ms units. Within subject factors were latency (levels: wave I, wave III and wave V) and ear (levels: left, right). There was no significant difference between ears (p=0.63).

Table 6-13 Mean (± SEM) peak latency for the right and left ears in milliseconds (n=10).

Group	*Amplitude*	*Right Ear*		*Left Ear*	
		Mean	Std.Dev	Mean	Std.Dev
DES	Wave I	1.64	0.02	1.63	.02
	Wave III	3.74	0.03	3.71	.03
	Wave V	5.78	0.04	5.73	.02
Controls	Wave I	1.73	0.12	1.76	.10
	Wave III	3.76	0.11	3.74	.06
	Wave V	5.50	0.18	5.51	.15

Because there were no observable differences, we collapsed the latency data across both ears. Differences across the two groups were then examined for statistical significance by using a two-way ANOVA. There were significant differences between the groups ($p<0.01$). In addition, there was significant interaction between groups and waves ($p<0.05$). To test for group differences at each specific wave, simple main-effect comparisons were made using Tukey's LSD test. We could find no evidence to support any difference between the control group and the DES group for wave I (p=0.51) or wave III (p=0.48), but a significant difference was evident for wave V ($p<0.05$).

For the right ear, the mean IPL for the DES group was 2.09 ms (95CI= 2.03-2.16) for wave I-III; 2.05 ms (95CI= 1.94-2.15) for wave III-V and 4.12 ms (95CI=

4.03-4.20) for wave I-V. For the left ear, the mean IPL for the DES group was 2.09 ms (95CI= 2.02-2.15) for wave I-III; 2.02 ms (95CI= 1.92-2.11) for wave III-V and 4.10 ms (95CI= 4.03-4.17) for wave I-V. Eight of 10 DES subjects had I-V IPLs longer than 2 SD above the control mean at 80 dB nHL (Table 6.12).

Table 6-14 Mean (± SD) interpeak latency for the right and left ears in milliseconds (n=10).

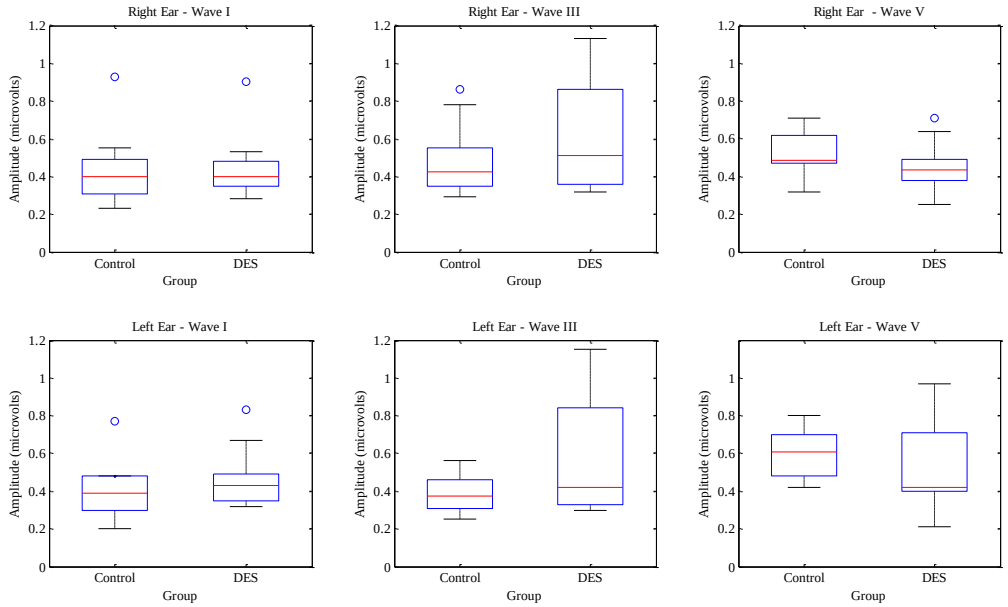
Group	*Amplitude*	*Right Ear*		*Left Ear*	
		Mean	Std.Dev	Mean	Std.Dev
DES	Wave I-III	2.09	0.09	2.08	0.09
	Wave III-V	2.05	0.14	2.02	0.13
	Wave I-V	4.12	0.12	4.10	0.10
Controls	Wave I-III	2.04	0.11	1.98	0.10
	Wave III-V	1.74	0.17	1.77	0.15
	Wave I-V	3.77	0.20	3.75	0.17

As compared with the values of the control group, I-V, and III-V inter-peak latency values were significantly prolonged in the DES group (p<0.05). The prolonged III-V interval for the DES group is in line with previously reported cases (Kalmanchey and Veres, 1988, Horikawa et al., 1993).

A repeated measure ANOVA was performed to examine whether there were any differences within groups using inter-peak latency in ms units. Within subject factors were IPL (levels: I-III, III-V and I-V) and ear (levels: left, right). There was no significant difference between ears (p=0.47).

A two-way ANOVA revealed significant differences in IPL between the groups (p<0.01). To test for group differences at each specific wave, simple main-effect comparisons were made using Tukey's LSD test. There was no significant difference for the I-III IPL (p=0.62) but there were significant differences was evident for the III-V and the I-V IPLs (p<0.05).

Figure 6-9 Mean ABR wave I, III and V amplitudes at 80 dB nHL for control and DES subjects Vertical bars represent ±2 SEM (n=10) Error bars show the 95% confidence interval of the mean at each peak latency.



Amplitudes were measured for all peak waveforms. For illustrative purposes we also constructed a series of whisker and box plots of the amplitudes which are shown above in Figure 6.9.

A repeated measure ANOVA was performed to examine whether there were any differences within groups using amplitude in µV units. Within subject factors were wave amplitude (levels: I, III and V) and ear (levels: left, right). There was no significant difference in amplitude ($p=0.23$) or between ears ($p=0.06$). A two-way ANOVA revealed no significant difference in amplitude between the groups ($p=0.16$).

One interesting trend that we observe from the amplitude data is that wave III is more variable with greater amplitude than seen for the other two components (I and V). Previous studies have reported greater wave III amplitudes in patients displaying hyper-excitable behaviour (Knott et al., 1994). However, in our data, there does not seem to be any relationship between the incidence of self-reported hyperacusis and larger wave III

amplitude. For example, the mean wave III amplitude in hyperacusis cases (n=7) is 0.42 µV (range 0.23-0.92 µV) on the right ear and 0.36 µV (range 0.24-0.83 µV) on the left which is smaller when compared with the amplitude data from the 3 DES cases who reported no hyperacusis (cases 1, 4 and 10) (right ear: mean wave III amplitude of 0.64 µV; range 0.32-1.13 µV and right ear: mean wave III amplitude of 0.58 µV; range 0.3-1.15 µV).

6.6.4 Discussion

6.6.4.1 Peripheral hearing in children with DES

Our data from this study indicates that peripheral hearing is normal in children with DES as measured on pure tone audiometry, tympanometry and otoacoustic measures. To the best of our knowledge, there are no other reports of audiometry in children with DES. Indeed, we could find only one case report identifying hearing loss in an adult case in the literature (Rosenberg, 1984). This is a surprising finding, particularly in view of the extensive chemotherapy treatments that many of these children receive.

Interestingly our group showed significantly poorer hearing thresholds in the low frequency range (0.25-1 kHz) compared with an age-and-gender matched control group. Hearing thresholds in the low frequency range were also mildly elevated in those participants who had complained of hyperacusis compared with those who did not. However, these results must be interpreted with caution as our sample size is extremely small.

6.6.4.2 Tests of auditory brainstem function in DES

We undertook two audiometric tests of brainstem function in children with DES: the acoustic reflex threshold and the ABR. The acoustic reflex thresholds were normal across a wide frequency range regardless of whether the ipsilateral or contralateral pathway was stimulated. We are not aware of any other study that has examined this pathway in DES.

This finding of completely normal ART in all of our patients is interesting. Recent studies in children with Williams syndrome (WS), who have well documented hyperacusis, reported an absent ART in 62-86% (Attias et al., 2008). However, it is not clear whether this result is due to a genuine deficit in the stapedial reflex pathway or is an artefact – the result of associated with a high-frequency hearing loss resembling the configuration of noise-induced hearing loss. Furthermore, the authors only investigated 1-4 kHz so we have no information regarding low frequency ART. This is an important consideration for future studies.

We identified a number of mild abnormalities on the ABR although the overall morphology of our ABRs was typically unaffected. We were able to record peak and interpeak latencies for all the major waveform components – no waveform component was absent in any of our recordings. The ABR showed abnormalities on one or more latency parameters in 9/10 cases. These slightly prolonged waveforms are still evident in children who are in remission from the disease, consistent with one case reported in Kalmanchey and Veres (1988). Only one case (case 1) showed no evidence of any abnormality.

This prolongation of peak and interpeak latencies is consistent with a delay in the conduction velocity along the auditory brainstem pathways. In our study, three parameters were particularly affected: wave V, the III-V IPL and I-V IPL. Our data

seems to reflect a delay in conduction between the upper pons and the lower midbrain pathways of the auditory brainstem. Our finding is supported by a single case from Kalmanchey and Veres (1988) but contradicts other studies that have previously shown that investigating the ABR in DES which have shown that the abnormalities are typically in the I-III IPL. Previous studies have failed to measure peripheral hearing so it is plausible that the abnormal I-III responses are a reflection of hearing loss and concomitant disruption in the auditory nerve.

An alternative explanation for our findings is that the ABR is sensitive to learning difficulties. Numerous studies in the children with learning difficulties population have shown that individuals with LD have normal click-ABRs (Mason and Mellor, 1984, Jerger et al., 1987, Grontved et al., 1988a, b, Lauter and Wood, 1993, Purdy et al., 2002). However, this does not explain the abnormal ABR finding in the single case that did not have any learning problems.

Another interesting finding in our study was the variability shown in the amplitude of wave III. Although not statistically significant, wave III amplitude reported in this study was highly variable especially when compared with control data. The magnitude of wave III amplitudes has also been reported as abnormal in patients with behavioural disorders such as panic disorder (Knott et al., 1994). This had led to a number of authors hypothesising that this is a marker of brainstem hyper-excitability in these patients.

However, when we examined possible differences in wave III amplitude with the incidence of self-reported hyperacusis, we found no evidence to show that wave III amplitude was any greater in cases with self-reported hyperacusis compared with the group who had no complaint of hyperacusis. Indeed, the mean wave III amplitude was

smaller on both ears in the hyperacusis cases. This may be related to poorer neural synchrony within the cochlear nucleus complex in DES subjects with hyperacusis but such an assertion is purely speculative at this stage. Obviously, these data must be interpreted cautiously as our sample size is extremely small but it would be valuable to re-examine this issue in a much larger sample.

It is not clear from our study whether the changes that we have measured represent an irreversible alteration to auditory brainstem function or whether it is an effect of treatment. It is not clear at present, what role (if any) that treatments such as ACTH may play in the measurement of the ABR and we cannot exclude that our results may reflect a treatment effect. Such questions can only be answered with longitudinal studies with larger sample sizes, strict selection criterion and well-documented treatment protocols.

6.6.4.3 Pathophysiology

The pathophysiological mechanisms of DES are unclear although a number of studies have provided conflicting evidence for the involvement of both the brainstem and the cerebellum. Our data provides the first systematic audiological study of children diagnosed with DES who are in the chronic phase of the disease. Our data clearly shows that the brainstem pathways are affected in DES – 9/10 cases had a delay in their ABR waveforms.

These findings support the view that some of the abnormalities seen in DES could be consistent with a lesion in the brainstem (Kalmanchey and Veres, 1988, Harris, 1997). There is growing support for brainstem involvement in DES including recent studies which have described an exaggerated startle response in a patient with DES

(Yonekawa et al., 2011). The acoustic startle response is thought to be generated in the medial pontine tegmentum although the precise location of the neural substrates in humans is not clear (Davis et al., 1980b). The case study reported by Yonekawa et al., (2011) argue that the combination of the startle response and the myoclonus are due to augmented excitability in the brainstem. They argue that hyperexcitability in co-located but independent structures within the pontine tegmentum could explain many of the signs and symptoms seen in DES.

Our findings of hyperacusis and prolonged ABR latency would also lend support to these observations, however we cannot rule out damage to the cerebellum, which could also explain the abnormal eye movements in DES. These findings in addition to the limited literature published in this field, may be useful in providing a framework for future study.

Our findings of an abnormal ABR also raise the possibility that there may be a manifestation of a wider 'problem' in the CANS not detected by pure-tone audiometry. There is some evidence (Chapter 2) that a deficit in the brainstem could affect the still developing pathways in the auditory cortex. These findings may suggest that the aberrant function in auditory brainstem could be partly responsible for some of the longer term learning difficulties seen in DES, particularly as the onset of DES occurs between 10-36 months when the auditory brainstem is still maturing. This hypothesis should be tested in further studies.

6.6.4.4 A link between auditory signs and eye movement signs in DES?

Three main theories have been proposed to explain the striking eye movements that characterise DES. These have included: 1) damage to cerebellar circuits could

result in disinhibition of the saccadic system from the FN (Wong, 2007); 2) dysfunction of the OPN cells in the PPRF, which inhibit the BN that drive ocular motor neurons to create saccades (Hattori et al., 1988, Pranzatelli, 1992) and 3) damage to the BN which results in increased excitability of these cells in the PPRF (Yonekawa et al., 2011).

Yonekawa et al., (2011) speculated that the clinical signs observed in DES are due to neural hyper-excitability in brainstem pathways. They argued that cerebellar lesions due to various aetiologies do not usually alone cause OMS symptoms and that the clinical signs seen in DES is a direct result of increased excitability of BN.

What can our audiological findings contribute to our understanding of the abnormal eye movements in DES? Our data from our initial experiment – *that some children with DES may have hyperacusis* – presented here may provide some interesting clues which have a direct bearing on the interpretation of the eye movement data.

The mechanism responsible for hyperacusis is unclear. Several theories have been advocated although empirical data supporting each is tenuous (Baguley, 2003). One theory is that hyperacusis may reflect a disturbance in serotonin levels (Marriage and Barnes, 1995, Katzenell and Segal, 2001). Interestingly, *excessive* serotonin in the brainstem has been shown to inhibit the OPN cells – therefore their inhibition *should* lead to excessive saccadic activity (Schenck et al., 1992, Armitage et al., 1995, Boulos et al., 2011). Moreover, there is limited evidence to support a possible serotonergic link between opsoclonus in the rat brain and chlordecone (Pranzatelli, 1992). However animal data (Boulos et al., 2011) and computational models (Leigh and Kennard, 2004, Ramat et al., 2005, 2007) suggest otherwise – showing that dysfunction in OPN results in a slowing of saccades – such as we saw for GD earlier in Chapters 4 and 5.

Other theories that have been proposed to explain hyperacusis include alterations in central gain[48] and startle reflex – both of these mechanisms explain hyperacusis in terms of neural hyperexcitability. Our findings would not exclude either of these theories and they could be used to explain the 'hyper-excitability' seen across a number of sensory circuits and behaviours.

We presented data in our second empirical study which is suggestive of specific damage in the pons. We identified that the ABR was abnormal in the majority of the cases that we examined, even after the resolution of the primary 'acute' symptoms following disease onset. The prolongation of wave V and delay in interpeak latencies (III-V and I-V) are consistent with a deficit auditory processing across multiple levels in the auditory brainstem including the cochlear nucleus, SOC, and lateral lemnisical pathways. These findings are consistent with the 'pontine' hypothesis posited by Yonekawa et al., (2011) and support earlier studies (Kalmanchey and Veres, 1988, Ramat et al., 2005). These data when interpreted alongside the findings of an exaggerated startle reflex also seem to strongly suggest that the cochlear nucleus complex is involved in DES.

While our data is not able to differentiate between the potential damage to eye movement circuits in the brainstem (i.e.) whether the BN or OPN are affected in DES, it does provide strong support for the pontine hypothesis. Our data however, does not preclude additional lesions in the cerebellum. It is possible that damage in the pons affecting the lateral lemnsicus could also affect ponto-cerebellar connections (Kalmanchey and Veres, 1988).

[48] This alteration in gain has recently been discussed using the thalamocortical dysrhythmia model. This model considers the reciprocal circuits between thalamus and cortex to be fundamental to a range of positive and negative clinical signs and symptoms (e.g. hyperacusis, tinnitus)

The prolonged wave I-V pattern in the ABR that we have reported in DES is also evident in a number of cases diagnosed with GD (see Chapter 4 and Chapter 5). This would indicate a similar auditory deficit in the auditory brainstem pathways in both of these conditions but clearly the eye movements are distinctly different. The delayed conduction velocity in the ABR seen GD is mirrored in the 'slow' saccadic speeds seen in the oculomotor system of nGD patients. As such, we could anticipate that the auditory abnormalities seen in DES would echo the chaotic back-to-back saccadic disturbances evident in opsoclonus (i.e.) we might predict that conduction velocity in the ABR would be abnormally 'fast'. So how do we explain these results? At this juncture we refer to the limitations of the ABR measurement that we previously discussed in Chapter 2 (section 2.4.2.2).

As a far-field recording, the ABR findings may indicate the level or severity of the lesion within the brainstem in these disorders but they *do not* accurately identify the exact site as there is no point by point relationship between the ABR waveforms and the anatomical structures (Hall, 2007). Moreover, it is unclear whether ABR activity is based on activity from the nerves and fibre tracts or in nuclei. Thus although eye movements centres and auditory pathways are undoubtedly affected in both of these disorders, the poor correlation between the site of the lesion studies and the ABR, and the complexity of the CANS preclude us from drawing any further parallels between the two systems.

6.7 Conclusion

In this chapter we presented data from two studies. In our first study we showed that hyperacusis is present in at least 43% of children with DES. Our data lends support

to the theory of hyper-excitability in brainstem pathways, specifically within the pontine tegmentum area. In our second study, we showed that there were subtle abnormalities in the auditory brainstem as shown by the ABR. The most common deficit was seen as a prolongation of the wave V, the III-V and I-V interpeak interval. This novel finding, even in children with 'resolved' DES, indicates that the auditory brainstem pathways are affected in this disease.

Chapter 7
The offset ABR

7.1 Introduction

Our earlier experiments have considered how the *onset* of a stimulus is represented within the auditory brainstem pathways. In this final experimental chapter we are concerned with the first stages of understanding the importance of the "offset" of sound, i.e. establishing how the brain encodes sound when it disappears. Such study is critical in developing our understanding of how the brain identifies the boundaries in speech.

By its very nature, speech is composed of complex sound waves that have rapid spectral and temporal changes which are separated by discrete 'non-acoustic or silent' intervals. For example, consider the distinction that the auditory brain is required to make between two words – 'stay' and 'say' – in which a short, transient difference occurs within the syllable of the word.

Thus, there is significant evidence that the cessation of sound (or 'the sound of silence') is an important acoustic cue in consonant identification (Pind, 1998), discriminating sound duration (Schlauch et al., 2001) and in the acoustic startle reflex (Ison and Allen, 2003). Furthermore, the existence of offset-type neurons at all levels of the auditory tract suggests that detection of stimulus cessation may play an important role in central auditory processing (Palmer, 1987, Phillips et al., 2002a, Scholl et al., 2010). The functional role of these offset-type neurons is still vague, and there has been relatively little study of the offset response. Moreover, the origins of the offset response, particularly in the auditory brainstem are still controversial (Brinkmann and Scherg, 1979, Van Campen et al., 1997, Phillips et al., 2002a).

There is extensive literature supporting the representation of stimulus onsets throughout the CANS (see Phillips et al., 2002 or Scholl et al., 2010 for a

comprehensive review of this topic). However, the available evidence regarding the offset response is extremely limited. 'Offset' responses have been described in electrophysiological studies of the auditory brainstem in humans (Brinkmann and Scherg, 1979, Perez-Abalo et al., 1988, Van Campen et al., 1997) and animals (Kodera et al., 1977a, Henry, 1985a, b, 1986, Henry and Lewis, 1988). Cortical responses to sound offset have also been reported, using a variety of techniques including auditory evoked potentials (Takahashi et al., 2004) and imaging studies (Gutschalk et al., 2002, Harms et al., 2005). However, the study of sound offsets is still controversial with some researchers arguing that the paucity in offset responses in the CANS reflects the functional

> *"asymmetry in the neurophysiological and perceptual processing of stimulus onsets and offsets: sound onsets have a more elaborate neurophysiological representation, and receive a greater perceptual weighting"* (p192).

This view has recently been challenged with new evidence supporting the existence of separate auditory pathways for encoding the onset and offset of tones in the rat (Scholl et al., 2010). Clearly the study of sound offsets merits closer study as it may provide important clues in our understanding of hearing and speech processing.

One approach that has been used to probe how the brainstem encodes sound offsets is the 'offset ABR' (Kodera et al., 1977a, Henry, 1985a, b, 1986, Henry and Lewis, 1988, Van Campen et al., 1997). This component is visualised as a second response – with a similar morphology to the onset waveform component – which is evoked at stimulus offset. It was first described by Kodera et al., (1977) in both cats and humans. Subsequent studies of the offset-ABR have been plagued by a number of technical challenges and the origin of the offset ABR as reported in Kodera et al., (1977) remains contentious (Brinkmann and Scherg, 1979).

In this chapter, we begin by presenting an in-depth review of all 'offset-ABR' studies undertaken in the last 30 years. We then present offset-ABR data from a series of different experiments, employing a wide range of frequencies (and rise-fall times) and polarity (rarefaction, condensation) in normal hearing participants. This work replicates and extends previous studies (Van Campen et al., 1997). Finally, we present, for the first time, a clinical application of the offset ABR. This application involves recording the offset ABR in our two eye movement disorders (GD and DES) that have been the subject of our previous chapters.

7.2 Experiment 1: The offset ABR in normal control subjects

7.2.1 An overview of the 'offset' ABR response

Since the publication of the seminal study of Kodera et al., (1977), a modest number of papers have appeared describing AEPs generated by the offset portion of the stimulus in cats (Kodera et al., 1977a, Laukli and Mair, 1985); mice (Henry, 1985a, b, 1986); gerbils (Henry, 1986); rats (Henry and Lewis, 1988) and humans (Kodera et al., 1977b, Brinkmann and Scherg, 1979, Elfner and Barnes, 1983, Perez-Abalo et al., 1988, Van Campen et al., 1997).

These studies have ascribed different functional properties to the onset and offset brainstem components. For example in the mouse, these responses differed significantly in thresholds, slope intervals of the amplitude-intensity functions and tuning curves (Henry, 1985a, b). We have summarised the available literature on the offset response in the brainstem undertaken in animals in Table 7.1 and in humans in Table 7.2.

Author	Year	n	Stimulus				Key findings
			Frequency	Rise-fall times	Intensity	Polarity	
Kodera et al.	1977	4 cats	1 kHz	Varying durations (1, 3, 5, 10, 25 and 55 ms) and rise-fall times (0.25, 2, 4, 6, 8 & 10 ms) in cats and rise-fall times of 0.25,1, 2.5, 5 and 10 ms in humans	40-90 dB peSPL in cats	A	Different morphologies for latency and amplitude responses for the offset compared with onset responses.
Henry	1985	41 mice	4-64 kHz	Duration of 10ms. Rise and fall time of 200 µs.	40-100 dB SPL	NR	Similar morphology and inter-peak latencies for onset an offset responses. Offset responses were present at all frequencies if the stimulus was sufficiently long enough (> 7.5 ms). Increased stimulus duration resulted in increased latency for offset response but no change in the onset ABR was observed. Offset audiogram did not parallel the onset ABR audiogram.
Henry	1985b	10 mice in expt. 1 33 mice in expt. 2	8 or 32 kHz (in the presence of cont. masking tone) ABR TC at 8,12,16, 20, 24, 32 & 40 kHz	Rise and fall times of 0.3 ms Plateau – NR	Masker held constant at 60 dB SPL	NR	At low and high frequencies, onset and offset responses have similar thresholds. At mid-frequencies, the offset responses have higher thresholds. The offset response was very different from the onset response depending on the masking profile applied. Offset response at mid-frequencies was more sensitive to continuous masking by bands of frequencies above and below the probe stimulus frequency. Offset TC response was more resistant to continuous masking at the probe stimulus frequency. Offset TC peak was more finely tuned than the tip of the onset TC.

Laukli and Mair	1985	4 cats	0.5-4 kHz Masking WN	Plateau constant at 10 ms. Varying rise and fall times of 0.3, 2, 4 and 8 ms	10-60 dB RL	A	Varying plateau lengths (from 5-20ms) resulted in an increase in the off-response latency. The waveform morphology and amplitude of the offset response was related to the frequency specificity of the onset response (greatest for the 4Kz tone and smallest for the WN tone burst). Threshold of the 0.5 kHz response was lower than the onset response and the amplitude did not increase with increased stimulus level.
Henry	1986	17 mice & 12 gerbils	8, 12, 16, 20,24, 32 & 40 kHz	Rise and fall times of 0.3 ms Varying duration of 5-15ms	60 dB SPL	NR	Onset and offset responses were abolished with cochlear lesions suggesting a peripheral origin for the offset response. Simultaneous masking enhanced offset responses suggesting that the cochlea may also encode transients in different ways
Henry	1988	Rats, gerbil, guinea pig and mouse	8-20 kHz	NR	65 dB SPL	NR	Examined the offset ABR masking tuning curve. Extended previous studies across 'new animal models' and found similar tuning curves across all of these species. Also showed that lesions to the contralateral ear, OCB or the outer ear do not alter the properties of the offset tuning curve.

Table 7-1 Summary of previous studies investigating the offset responses in animals.

Abbreviations: A: alternating; D: duration; I-A: intensity-amplitude; I-L: intensity level; NR: not reported; RL: relative to the VDL of the ABR; TC: Tuning curves; VDL: visual detection threshold level; WN: white noise.

Table 7-2 Summary of previous studies investigating the offset ABR response in humans

Author	Year	n	Stimulus				Key findings
			Frequency (kHz)	Rise-plateau-fall time (ms)	Intensity	Polarity	
Kodera et al.	1977	10 adults	1 kHz	Varying rise-fall times of 0.25,1, 2.5, 5 and 10 ms in humans.	0-60 dB SL	A	On and offset responses recorded in humans for the first time Offset responses were similar to onset responses at lower intensity levels; however the I-L and I-A was elevated in human subjects at louder intensities. Increased rise times resulted in decreased amplitude and a broadening of the offset response for the onset response. Offset responses were not seen with rise times > 2.5-10ms.
Brinkmann and Scherg	1979	3 adults	Gated WN 2kHz gated sinusoids	WN gated with Gaussian-shaped envelopes of 2ms half-width. 2kHz stimuli was recorded using Gaussian-shaped envelopes of 0.2, 1,2,3,4,and 6 HW and linear rise times of 0, 1, 2.5, 5 and 10 ms. Rise and fall times were kept electrically identical within each measurement.	80-85 dB SPL	Varied	Offset potentials were only observed at levels > 55 dB SPL with smaller amplitudes than those seen in the onset response. The polarity of the offset potential was the opposite of the onset response to WN stimuli. Systematic decrease in latency as a function of increased rise time. Offset potentials amplitude increased with shorter fall envelopes and no polarity reversal when recorded with 2 kHz tone.
Elfner and Barnes	1983	4 adults	2 kHz	1 ms rise-fall time but stimulus duration varied 2, 3, 4, 5, 6, 7 and 8 ms which were randomly presented	60 dB SL	NR	Onset potential was reliably recorded for all durations and showed little change in latency with increased duration. The offset potential varied significantly with duration (i.e an increased latency was observed with increasing duration of the signal). The offset was not observed with stimulus duration <5ms.
Perez-Abalo et al.	1988	7 adults	0.5 kHz	Rise-fall times of 2 ms with a variable plateau of 2, 4, 6 and 8 ms	100 dB SPL	A	Authors report a linear dependence on plateau time (i.e. increased plateau duration showed an increase in latency). The offset response less affected by noise compared to onset response.
Van Campen et al.	1997	40 adults	0.5 and 2.0 kHz	Stimulus duration of 10ms Rise and fall times of 0.5, 1, 2.5 and 5 ms	103 dB peSPL	A	Offset response reliably recorded in the absence of any acoustic ringing. Offset ABR was sensitive to rise and fall times and was optimal when recorded using a 500 Hz stimulus.

Human studies of the offset ABR have shown that, although the morphology of the response is similar to the onset response, the offset response is characterised by a number of differences. For example, the threshold of the onset-response was 20 dB SL and 30-40 dB SL for the offset response; i.e. the offset response has a higher (or poorer) threshold (by 10-20 dB) (Kodera et al., 1977b). A similar finding was reported by Brinkmann and Scherg (1979) who observed that the 'offset' response had smaller amplitudes (1/3 to 1/5 smaller compared with the 'on' response). The authors commented that offset thresholds were elevated (55 dB SPL) as they were not easily discernible because of the decrement in amplitude. We have summarised the differences between the onset and offset ABR, which have been published, in Table 7.3 below.

Table 7-3 Functional properties of the offset ABR as compared to the onset ABR

Characteristics of the offset ABR	Reference
Smaller amplitude	(Kodera et al., 1977b, Brinkmann and Scherg, 1979, Henry, 1985a)
More sensitive to changes in rise-fall time	(Kodera et al., 1977b, Brinkmann and Scherg, 1979, Henry, 1985a, Laukli and Mair, 1985)
Responds to intensity changes in a non-monotonic pattern	(Kodera et al., 1977b, Henry, 1985a, Laukli and Mair, 1985)
Higher threshold	(Kodera et al., 1977b, Henry, 1985a, Laukli and Mair, 1985)
More resistant to effects of masking	(Henry, 1985b, 1986, Henry and Lewis, 1988, Perez-Abalo et al., 1988)
May be generated from apical cochlear areas	(Henry, 1985b, Henry and Lewis, 1988, Perez-Abalo et al., 1988)

Kodera et al., (1977) also described the effect of changing rise-fall times on both the onset and offset ABR. They found that an increase in rise time caused a decrease in amplitude and broadened the waveform of the onset response. The increased rise time also resulted in an increased latency for onset responses. Rise and fall times also had an effect on the offset response: with short rise-fall times of 0.25-1ms offset responses were still recorded but not if these were >2.5-10ms.

Elfner and Barnes (1983) examined the effect of duration on the onset-offset ABR. They reported that duration had little effect on the onset response but that a prolongation of the latency and an increase in amplitude was observed in the offset response, with increasing stimulus duration. There was no discernible offset for stimuli with a shorter duration (<5ms). Collectively, these studies suggest that on and off responses may have different generators, however the offset response is often overlooked and, as we show in or next section, the origin of the response is fiercely debated (Phillips et al., 2002a, Scholl et al., 2010).

7.2.2 The generator of the offset response

There is little agreement regarding the mechanisms involved in the generation of the offset response. Several studies published after Kodera et al., (1977) have argued that what is thought to be an offset response is, in fact, a stimulus artefact, produced by acoustic transducer 'ringing' that follows stimulus onset (Brinkmann and Scherg, 1979, Elfner and Barnes, 1983). Support for the 'artefact' theory came from early work published by Brinkman and Scherg (1979) that showed that the offset response 'reversed' in polarity. They argued that the response was elicited by the oscillation of the headphone diaphragm which 'rings' at its resonant frequency, activating neuronal activity. Brinkman and Scherg (1979) successfully argued that the offset is really "*an 'onset' response evoked by new spectral components occurring at stimulus termination*" (Van Campen et al., 1997, p36). As a result the offset ABR received little further study.

The issue of whether the offset response was an authentic neural response or an artifact was re-examined by Van Campen et al., (1997). They showed that with careful stimulus selection (including stimuli characterised by no ringing artefact) it was possible to record a reliable ABR for the offset of tonal stimulation (Van Campen et al.,

1997) (Van Campen, Hall and Grantham, 1997). Although Van Campen et al., (1997) clearly demonstrated reliable offset ABR responses for "*all degrees of acoustic ringing*" (p35), the number of recordable offset responses was much poorer in the no-ringing condition (as low as 17% for 2 kHz). The reason for the discrepancy between the two conditions is unclear but needs to be explored further.

One possibility that we explore in our first empirical study is the potential adverse effects of polarity on the offset potential. Shifts in wave V latency for low frequency ABRs, elicited with rarefaction and condensation onset-phase stimuli, can result in partial cancellation of wave V when the responses are combined (Schwartz et al., 1990, Orlando and Folsom, 1995, Hall, 2007).

It is difficult to say whether alternating polarity of stimuli as reported in Van Campen et al., (1997) could explain the relative scarcity of responses in the no ringing condition – such a study has never been undertaken in the offset potential. However, if the offset response behaves in a similar way to the onset response, then alternating polarity reduces amplitude, thereby reducing the signal to noise ratio and as a result some of the responses may be simply lost in the noise.

Subsequent studies have proposed three mechanisms which could explain the generation of the far-field offset response. One possibility is that the offset response reflects the synchronous termination of stimulus evoked neural activity. A second possibility is that it reflects a neural response to the offset transient. An alternative view is that the offset response is a reflection of synchronous post-stimulus resumption of spontaneous discharges. These are illustrated schematically below in Figure 7.1. Similar mechanisms have also been proposed at the level of the auditory cortex (Scholl et al., 2010).

Figure 7-1 Schematic showing the three hypotheses that have been proposed to underlie the neural response contributing to the far-field recorded offset response.

One possibility is that the offset response reflects the synchronous termination of stimulus evoked neural activity (A). A second possibility is that it reflects a neural response to the offset transient (B). A third possibility is that it reflects synchronous post-stimulus resumption of spontaneous discharges (C).

Clearly, more normal descriptive research is needed on the relationship of stimulus parameters, such as intensity, duration (rise/fall time, plateau), presentation rate, the type of stimulus (noise versus tone burst) and response acquisition parameters to offset ABRs.

Here, we present our offset-ABR data recorded in a normal hearing group of adults. We report a series of experiments, employing a wider range of frequencies and rise-fall times. This work replicates and extends previous studies published in Van Campen et al., (1997).

7.2.3 Methodology

7.2.3.1 Subjects

Ten normal hearing young adults (6 females, 18-30 years, mean age of 22.1 years) with no known predisposing factors for hearing loss participated in the study. Study participants were initially recruited through convenience sampling, and subsequently through snowball sampling. Ethical Committee approval was obtained for

the study with informed consent obtained from each subject. Participants were recompensed for their time at all times of measurement.

All subjects had pure tone audiometric thresholds of 15 dB HL across 0.25-8.0 kHz. Tympanometry confirmed normal middle ear status in each of the participants. Because of the length of testing and the number of stimuli involved, electrophysiological testing was performed across 3 sessions (typically 1-2 weeks apart) depending upon the availability of each participant. Otoscopy and tympanometry were performed at the beginning of each session.

7.2.3.2 Stimuli, recording method and procedures

All subjects had standard baseline audiological assessment as previously described in Chapter 3. The presence of hearing loss as assessed by abnormal audiometric and impedance audiometry tests was a criterion of exclusion from the study. During each session, participants were instructed to relax on a soft comfortable chair in a sound-treated room and encouraged to sleep (if possible). A click evoked ABR was undertaken in each participant at the initial electrophysiological session to establish a normal baseline measure. These ABRs were recorded in response to clicks (100 μs in duration) of alternating polarity presented at a rate of 11.1/s at an intensity of 80 dB nHL using TDH-49 headphones.

Onset and offset ABR was recorded in response to tone burst stimuli (duration 10 msec) at 0.25, 0.5, 1, 2, and 4 kHz with varying rise and fall times (from 0.5 to 5 msec). Table 7.4 summarises the number of conditions and stimuli employed. Because the stimulus rate was slower (to avoid overlapping stimuli) the length of testing was increased and it was necessary to undertake recording over several sessions.

Stimuli were presented monaurally through EAR-3A insert earphones using a Biologic NavPro auditory evoked potential measurement system. Tonal stimuli were presented at rate of 6.3/s using alternating polarity with an intensity level of 67 dB nHL. The presentation order of the different tonal stimuli (n=25) was randomised using a modified Latin Square Matrix. Only the right ear of each participant was tested.

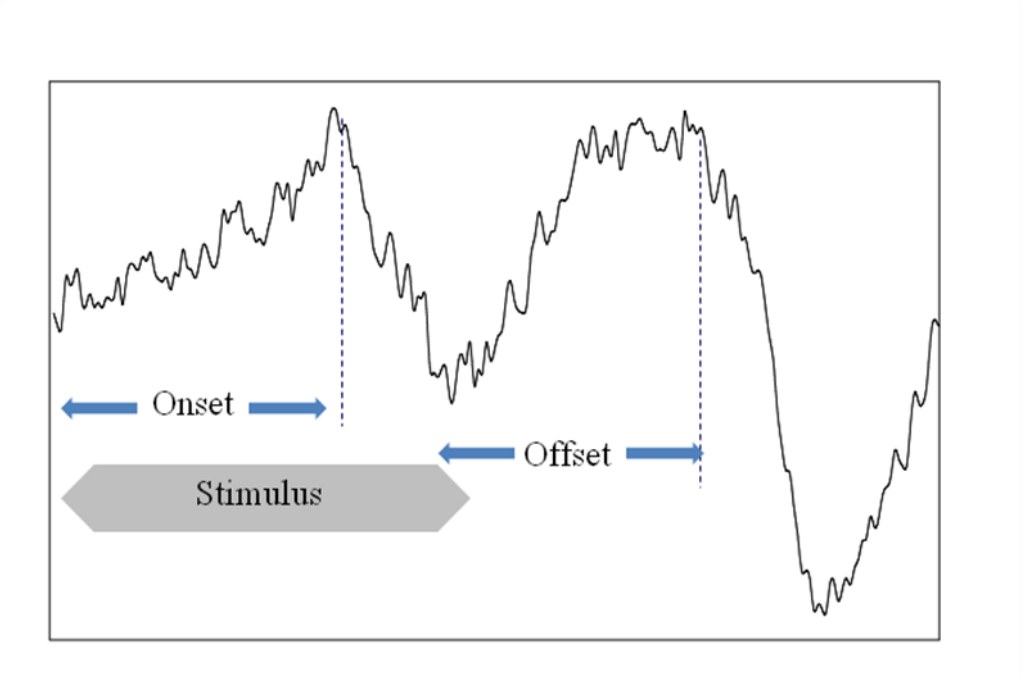
In order to replicate the Van Campen et al., (1997) study as closely as possible, a horizontal montage was adopted. The rationale for this selection in Van Campen et al., (1997) was based on optimising peak waveform identification. Electrode impedances were maintained below 5000 Ω and inter-electrode impedances were balanced to within 2000 Ω in order to maximise rejection of common mode signals. The EEG inputs were amplified by 100,000 and band-pass filtered from 30 to 3000 Hz (12 dB per octave roll-off) using a NavPro AEP system (Biologic, UK). Each waveform response represents an average of 1000 stimulus presentations over a 25-ms analysis window. All responses were replicated – in the event of a questionable response a third response was also obtained.

7.2.3.3 Data analysis

Onset and offset ABRs were analysed offline using the visual detection method previously described in Van Campen et al., (1997). For the purposes of present study, we adopted the same definitions of onset latency and offset latency. Thus, onset latency was defined as the time between the start of the rising ramp of the tone burst stimulus and the onset of wave V. Offset latency was calculated as the time between the start of the falling ramp of the tone burst and the offset V peak. Amplitude for each of these measures was measured based on a peak-to-trough calculation as shown in Figure 7.2.

A schematic of the method used to calculated onset and offset latency and amplitude is shown in Figure 7.2.

Figure 7-2 Illustration of the method used to calculate onset and offset latency as recommended in Van Campen et al. (1997)



7.2.3.4 Statistical analysis

The following descriptive statistics were presented for continuous variables: number of values, mean, standard deviation (SD), median, minimum, and maximum. We were unable to combine the onset and offset ABR data in any meaningful statistical analysis because of the small number of participants which was complicated by the varying number of absent waveforms across a number of conditions, so a descriptive analysis of ABR measures was undertaken instead.

7.2.4 Results

7.2.4.1 Descriptive summary of the onset-offset ABR

All participants (n=10) had peak and interpeak latencies that fell within clinical normative data (± 2SD) for click evoked ABR. Normative ABR data is shown in Appendix 2, section 9.2.6. Because of the clear responses obtained across these conventional ABRs, we did not seek to routinely record the onset ABR to short tonal burst stimuli as previous studies have suggested that the click-evoked ABR, when measured at suprathreshold levels, is generated in the apical region of the cochlea (2-4 kHz) (Hall, 2007).

The number of onset and offset ABR responses that were recorded varied widely across stimuli (n=25). The number of missing data was related to stimulus characteristics including frequency and rise-fall time. Generally, the onset was recorded in all participants across all frequencies when the rise time was fast (i.e. 0.5 ms). The exception for this was at 1000 Hz which was present in 8/10 participants. Interestingly, the onset response when recorded with 1000 Hz was highly variable and showed the poorest morphology for the onset response (8/10 absent cases) when recorded using a rise-fall time of 1ms. Furthermore the onset response was more reliable when recorded with high frequency stimuli compared to low frequency stimuli.

We have summarised the data and also presented the results published in Van Campen et al., (1997) for comparison in Table 7.4. Although we can only compare our data with Van Campen et al., (1997) at two frequencies (500 and 2000 Hz), our onset data that we present here is generally better than that previously reported as evidenced by the increased number of missing data at these frequencies.

The offset ABR had a similar morphology to the onset ABR and consisted mostly of wave V. The offset was reliably recorded in most participants for long duration, low frequency stimuli particularly when the ‘fall’ time was fast (i.e. 0.5ms). The offset response varied widely at higher frequencies with missing data ranging from

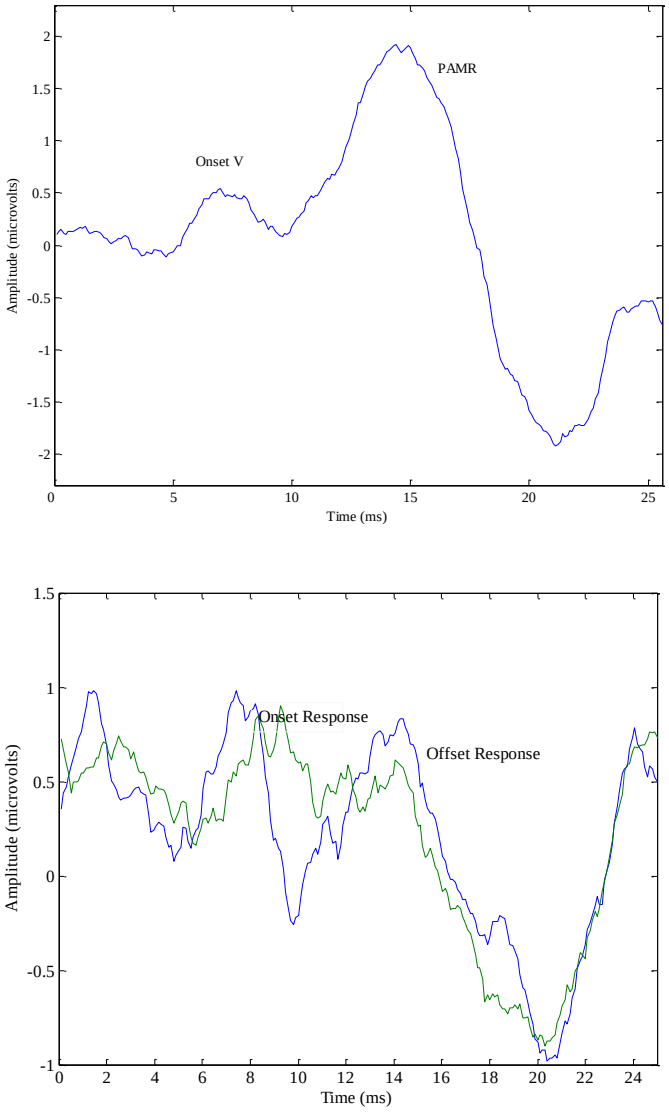
10-80% (Table 7.4). In several cases, the presence of the offset response was obscured by a large vertex positive component which appeared at the end of the analysis time (Table 7.4). A representative waveform is shown to illustrate this point in Figure 7.3

Table 7-4 Stimuli employed and the number of recordable onset-offset waveform responses in 10 normal hearing subjects. Data from a previous study (Van Campen et al., 1997) is shown here for comparison.

Frequency	Rise-Plateau-Fall (ms)	ABR (onset)		ABR (offset)		
		Present study (%)	Van Campen et al. (%)	Present study (%)		Van Campen et al. (%)
					Artifact (%)	
250 Hz	0.5-9.0-0.5	100	-	100		-
	1.0-8.0-1.0	100	-	90		-
	2.5-5.0-2.5	50	-	30		-
	5.0-0.0-5.0	10	-	30		-
	5.0-4.5-0.5	70	-	100		-
500 Hz	0.5-9.0-0.5	100	87	100		60
	1.0-8.0-1.0	70	100	90		72
	2.5-5.0-2.5	90	70	90		62
	5.0-0.0-5.0	70	97	70		70
	5.0-4.5-0.5	90	67	100		55
1000 Hz	0.5-9.0-0.5	80		80	10	
	1.0-8.0-1.0	20		0	30	
	2.5-5.0-2.5	60		60	10	
	5.0-0.0-5.0	70		70	30	
	5.0-4.5-0.5	80		80	20	
2000 Hz	0.5-9.0-0.5	100	97	70	20	60
	1.0-8.0-1.0	100	67	60	10	40
	2.5-5.0-2.5	80	85	40	20	17
	5.0-0.0-5.0	70	70	60	10	90
	5.0-4.5-0.5	90	87	80	10	85
4000 Hz	0.5-9.0-0.5	100		50	20	
	1.0-8.0-1.0	90		40	40	
	2.5-5.0-2.5	90		20	30	
	5.0-0.0-5.0	100		70	30	
	5.0-4.5-0.5	100		90	10	

This interference of offset identification may correspond to post-auricular muscle activity (PAM) as suggested in Perez-Abalo et al., (1988) or may reflect interference from some other mid-latency response (MLR) activity which has also been reported when rise and fall times are very brief (Hall, 2007). This finding may explain why the offset response is so variable across previous studies (Elfner and Barnes, 1983, Perez-Abalo et al., 1988, Van Campen et al., 1997).

Figure 7-3 Top panel: Representative trace showing a large amplitude artifact that commonly obscured offset response with 4 kHz tone. Bottom panel: The figure shows two superimposed trials of the onset-offset ABR recorded using a 2000 Hz tone (rise-plateau-fall: 2.5-5.0-2.5) from the right ear of a normal hearing female.



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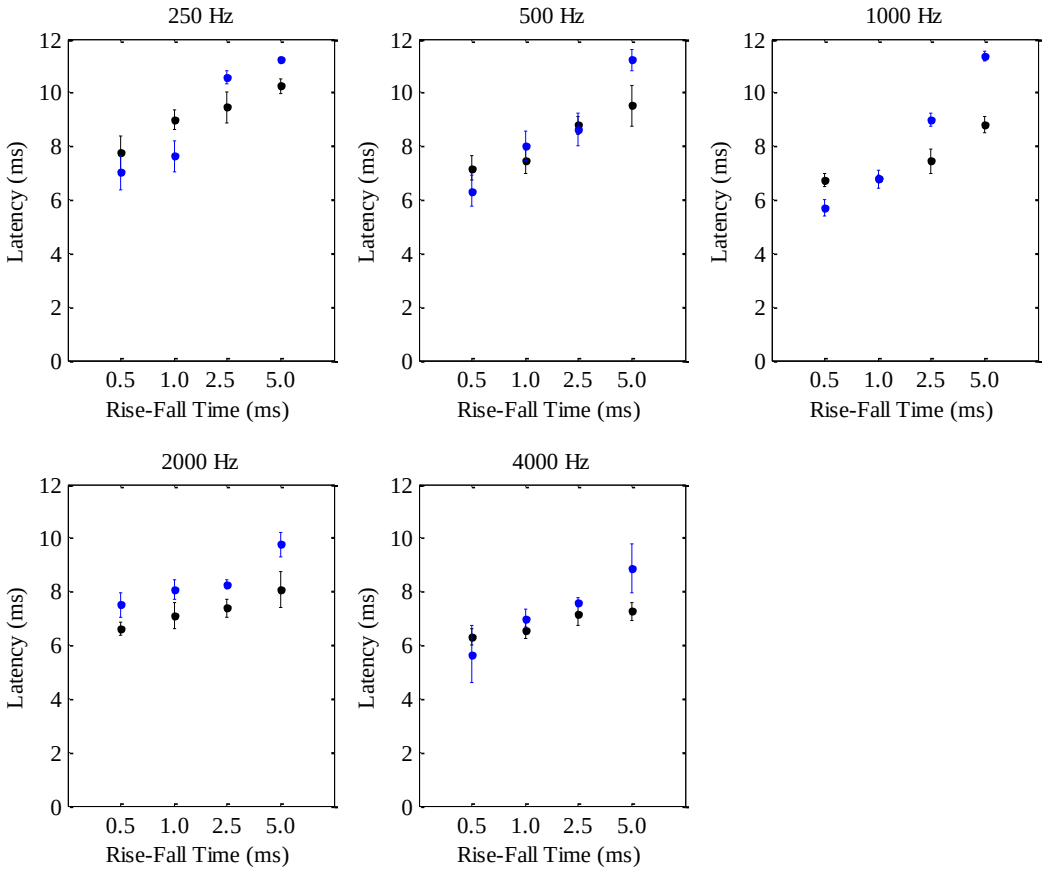
7.2.4.2 Effects of the rise-fall time on onset and offset ABR

Previous studies have argued that although the onset and offset ABR components may have similar morphology; they have very different functional responses. They have cited different responses to rise-fall time of different stimuli as evidence to support this claim (Perez-Abalo et al., 1988).

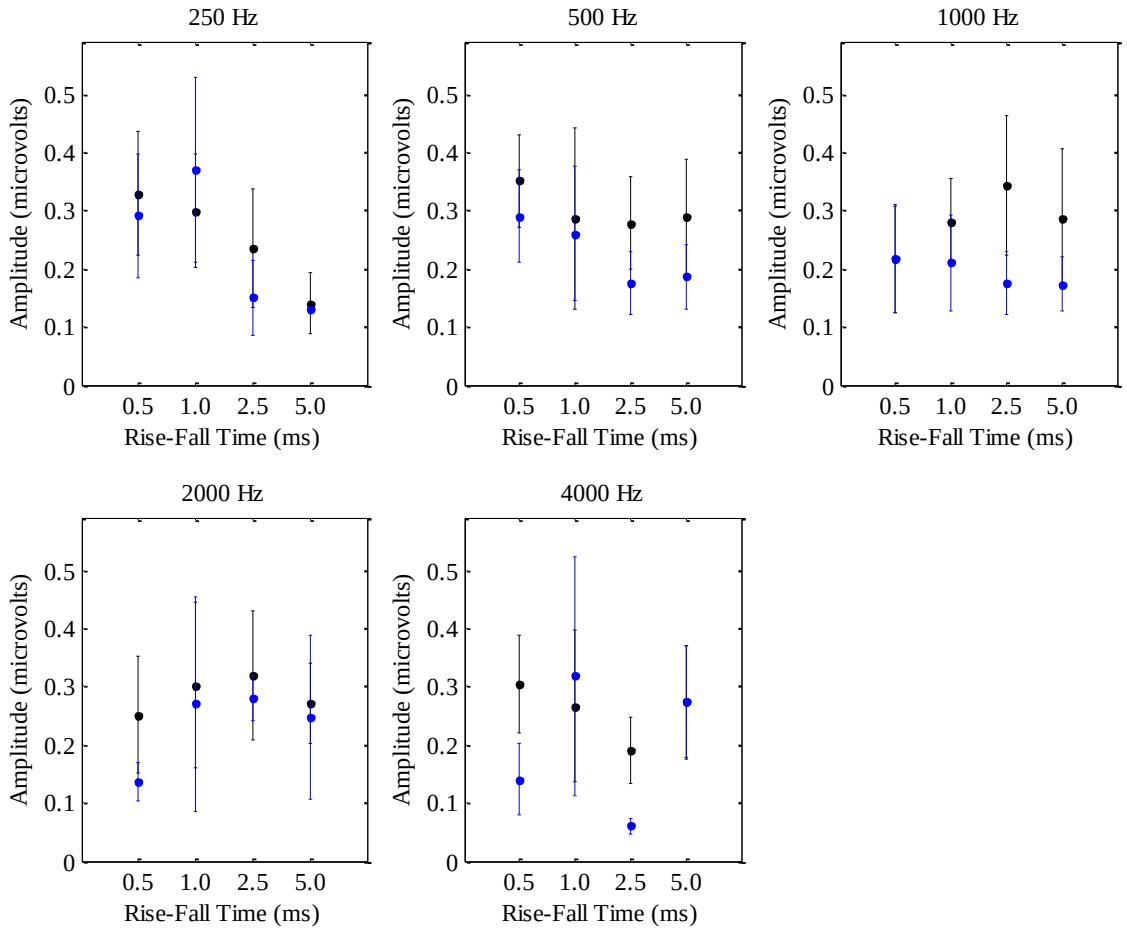
In order to examine this in more detail, we investigated the effect of four different rise-fall times (0.5, 1, 2.5 and 5 ms) for a wide range of frequencies (0.25, 0.5, 1, 2 and 4 kHz). We have plotted the data in a series of error bar plots for the latency of onset and offset responses in Figure 7.4 and corresponding amplitude data in Figure 7.5.

Figure 7-4 Error bar graph showing the mean onset (in black) and offset (in blue) wave V latency for frequencies plotted as a function of rise-fall times (ms)



We observed several trends in our data. First, the onset and offset ABR showed a marked trend of increasing latency with increasing rise-fall time across all frequencies. The slope of the latency rise-fall tie function was less steep for the onset ABR. This is in good agreement with data published by Van Campen et al. (1997). Second, an increase in frequency correlated with a decrease in latency for both components.

Figure 7-5 Error bar graph showing the mean onset (in black) and offset (in blue) wave V amplitude for frequencies plotted as a function of rise-fall times (ms)



Third, when we compared the amplitudes for both onset and offset components, we see that the onset amplitude is generally greater for low frequency stimuli and greater than the offset amplitudes recorded at the same frequency. This finding has been reported in previous studies (Brinkmann and Scherg, 1979). These earlier studies have reported that the amplitude of wave V offset are typically smaller by as much as 1/3rd to 1/5th (Brinkman and Scherg, 1979)

7.2.4.3 The effects of rise-time on the onset-offset ABR

We examined the onset-offset ABR for two different rise times (0.5 ms an 5.0 ms) while the fall time was held constant at 0.5ms. We compared the latency and amplitude of both components in Figures 7.6 and 7.7 respectively. In general the onset latency decreased with an increase in frequency. This finding was mirrored to a lesser extent in the offset response. Interestingly, the latency of the onset ABR was more sensitive to an increase in the rise time compared with the offset latency. This trend was observed across all frequencies but was more noticeable in the lower frequencies.

Figure 7-6 Error bar graph showing the mean onset (in black) and offset (in blue) wave V latency for frequencies (0.25, 0.5, 1, 2 and 4 kHz) plotted as a function of rise time.

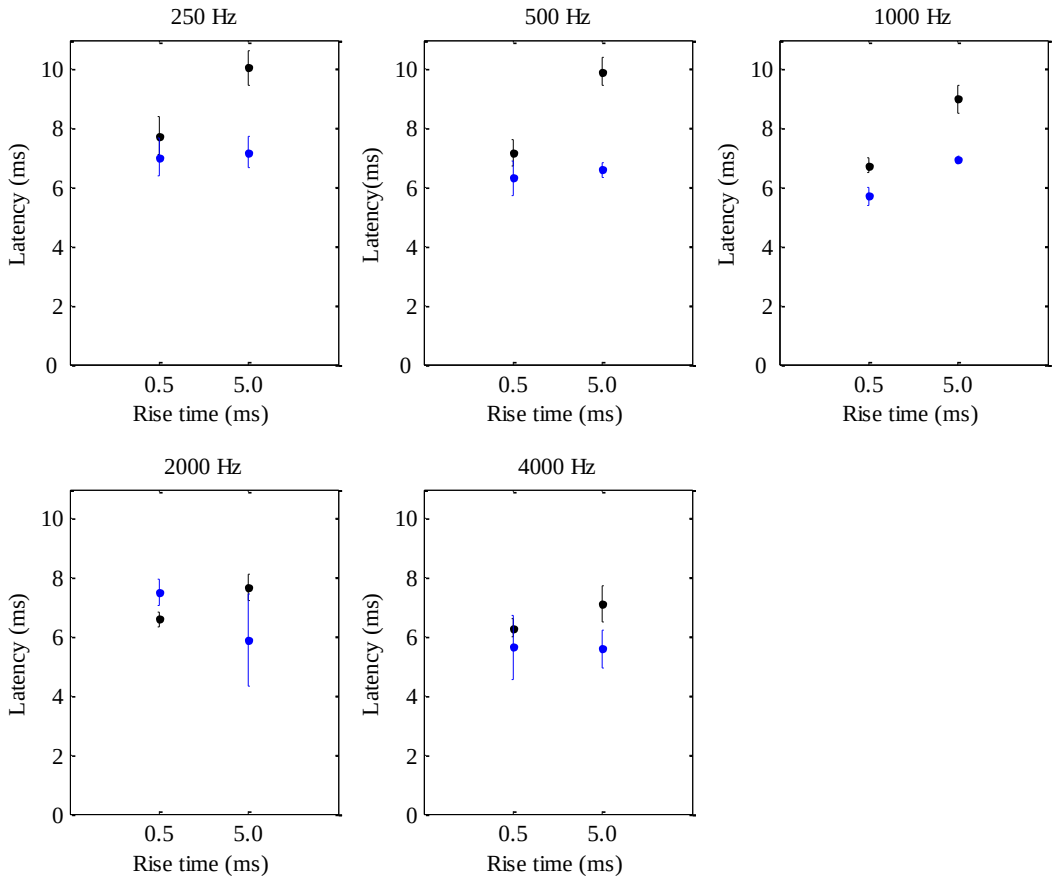
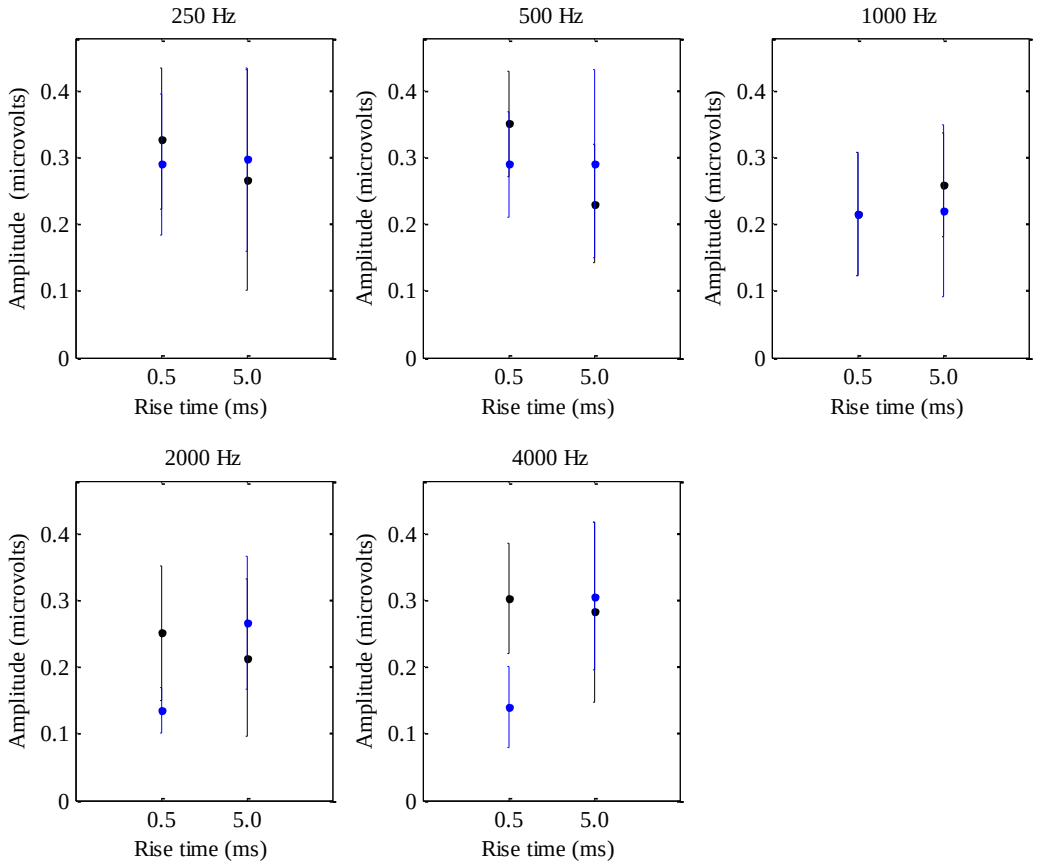


Figure 7-7 Error bar graph showing the mean onset (in black) and offset (in blue) wave V amplitude for frequencies (0.25, 0.5, 1, 2 and 4 kHz) plotted as a function of rise time.



7.2.4.4 Does polarity have an effect on the recording of the onset-offset ABR?

Figure 7.8 shows an error bar graph plot of onset ABR latency measured against rise-fall time when the stimulus is measured for two conditions: rarefaction and condensation. Two trends emerge from the data, when examined, which we have already reported earlier. First, onset ABR latency for both condensation and rarefaction polarity show an increase in latency with increasing rise-fall time. Second, latency for onset ABR is greatest when recorded with low frequency stimuli. Our data suggests little, if any, difference between onset latency when measured with rarefaction or condensations; i.e. onset latency appears to be insensitive to changes in polarity.

Figure 7-8 Error bar graph showing the mean onset V latency responses in milliseconds for rarefaction (in black) and condensation (in blue) for frequencies (0.25, 0.5, 1, 2 and 4 kHz) plotted as a function of rise-fall time.

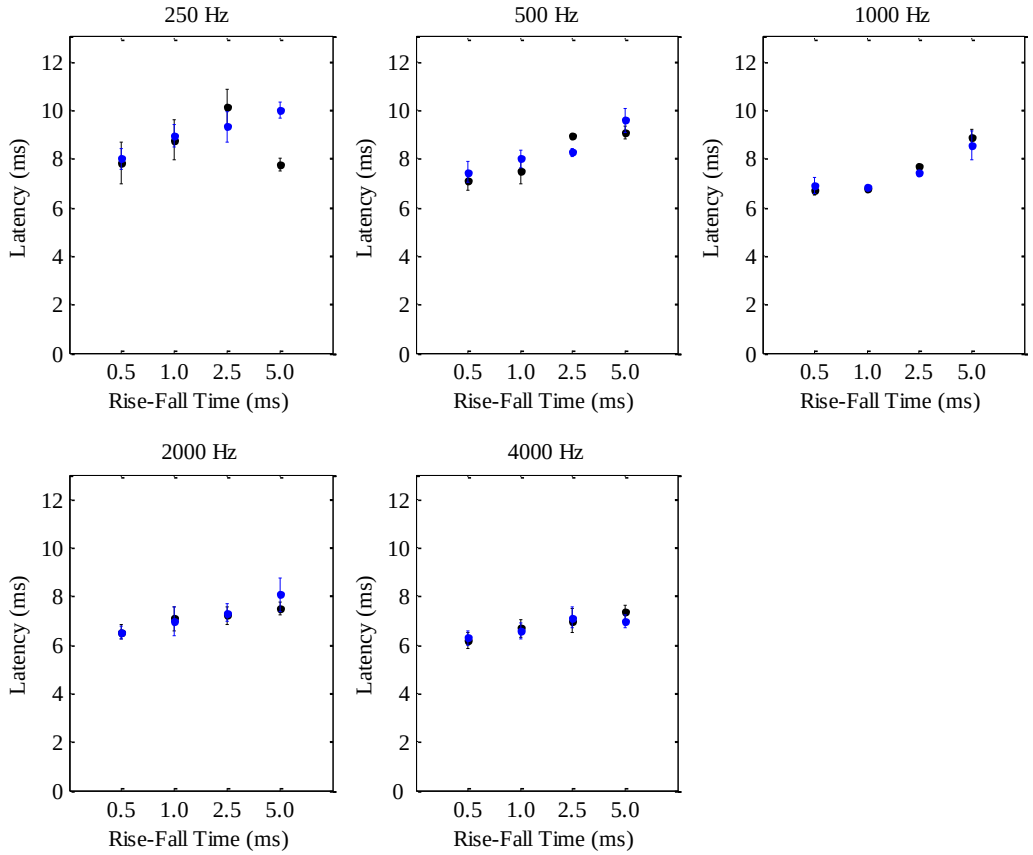
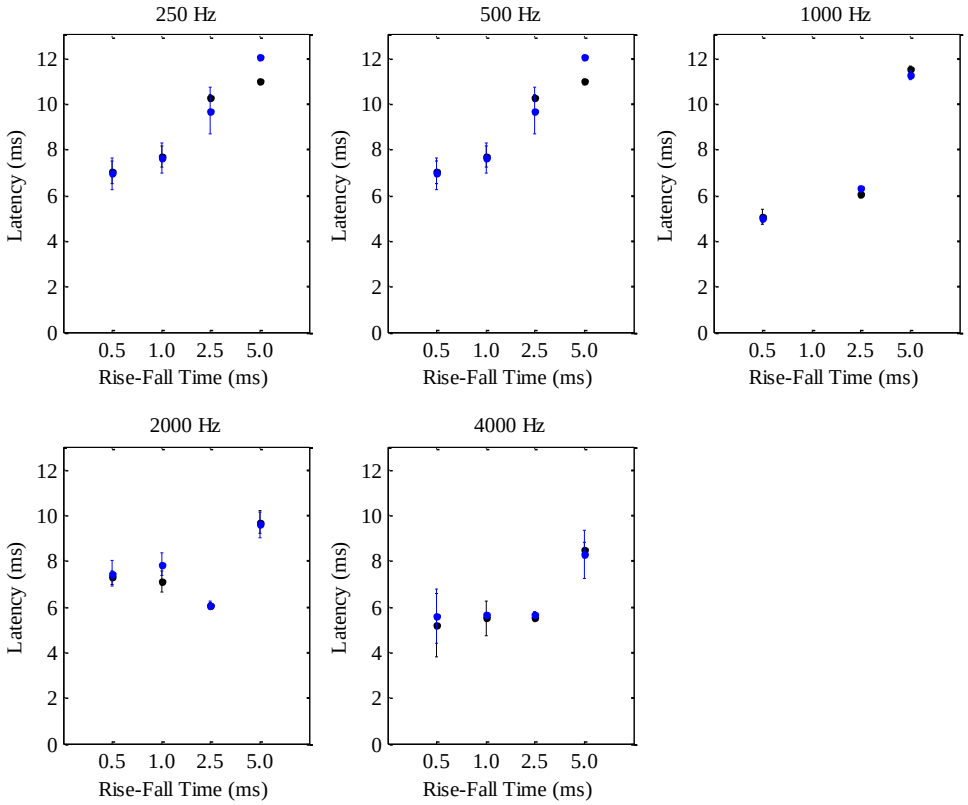


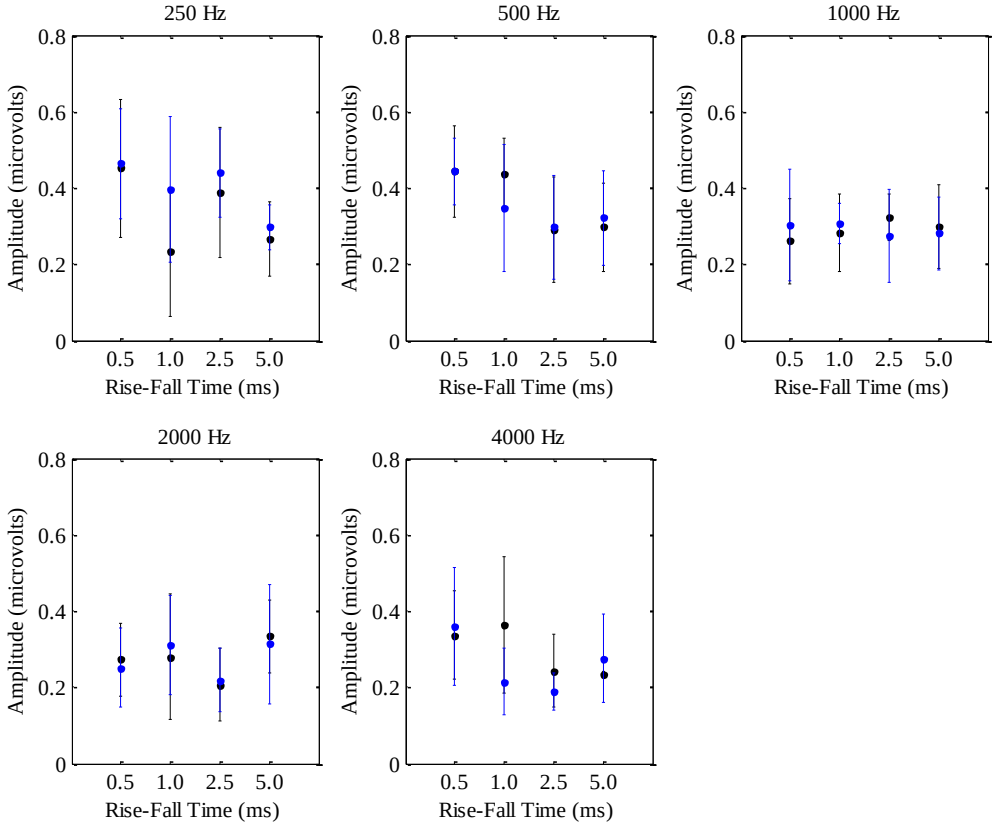
Figure 7.9 shows the corresponding data for the offset latency measures for both polarity measures. The same trends emerge for the offset data that were seen in Figure 7.8. Although we observe, a much steeper increase in offset latency with increasing rise-fall time. This is less obvious at higher frequencies.

Figure 7-9 Error bar graph showing the mean offset V latency responses in milliseconds for rarefaction (in black) and condensation (in blue) for frequencies (0.25, 0.5, 1, 2 and 4 kHz) plotted as a function of rise-fall time.
No offset ABR data is available when recorded with a 1kHz tone (rise-fall time of 1.0ms). This is due to poor morphology of the waveform and a general lack of wave reproducibility which we have attributed to PAMR.



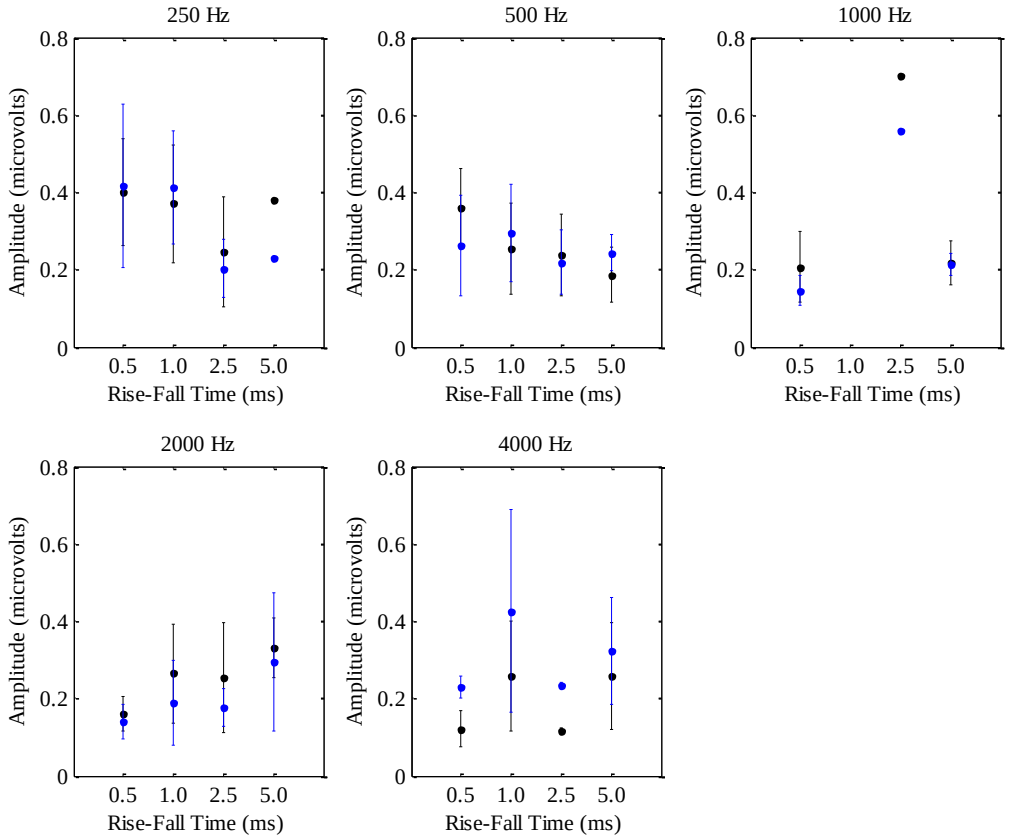
From the series of error bar plots shown in Figure 7.10, we can make several observations regarding the relationship between the onset ABR component and polarity. First, the ABR onset amplitude is greater for low frequency stimuli, particularly at 250 Hz, than when elicited with higher frequency stimuli. Second, onset ABR amplitude is sensitive to the rise-fall time. Amplitude is greater with faster rise fall times, although this is less obvious at in the onset response recorded with higher frequencies. Third, onset amplitude also appears to be influenced by polarity at low frequencies. In general, the onset amplitude is greater when recorded with condensation click stimuli compared with a rarefaction click. This is seen at 250 Hz and 500 Hz. However, we cannot see any evidence for this at higher frequencies.

Figure 7-10 Mean onset amplitude for rarefaction (black) and condensation click recordings measured in microvolts



The amplitude data for the offset ABR is plotted for five frequencies (250, 500, 1000, 2000 and 4000 Hz) for different rise-fall times in Figure 7.10. The data for both condensation and rarefaction are shown. The published data concurs with the data we present in Figures 7.10 and 7.11 respectively.

Figure 7-11 Mean offset amplitude for rarefaction (black) and condensation (blue) click recordings measured in microvolts.
No offset ABR data is available when recorded with a 1kHz tone (rise-fall time of 1.0ms). This is due to poor morphology of the waveform and a general lack of wave reproducibility which we have attributed to PAMR.



Interestingly, when we examine the offset ABR data plotted in Figure 7.11, we can see that the choice of polarity does not appear to influence the amplitude of the offset response. Moreover, while the amplitude measures for low frequencies are generally larger compared with higher frequency stimuli, this relationship is less clear when compared to onset data shown in Figure 7.10.

7.2.5 Discussion

7.2.5.1 Comparison of our data with previous studies

Our research identified four main findings from the data. First, we showed that both components of the onset-offset ABR increased in latency with an increase in the rise-fall times, particularly the offset response, which had a more non-linear response to increased rise-fall times. This is consistent with previously reported studies for the onset response (Fausti et al., 1991, Hall, 2007) and the offset response (Kodera et al., 1977b, Perez-Abalo et al., 1988, Van Campen et al., 1997).

Second, we showed that both of the onset and offset ABR components had shorter peak latencies when recorded with progressively higher frequencies. Thus peak latencies were longest at 250 Hz compared with 4000 Hz. Once again our results are in good agreement with published reports in animal and human studies (Kodera et al., 1977b, Henry, 1985a, Perez-Abalo et al., 1988, Van Campen et al., 1997). This well-documented finding has been explained by the delay in the travelling wave such that low frequency responses are generated apically and higher frequencies have been attributed to more basal regions.

Van Campen et al., (1997) maintained that this data provides evidence for an apical generator site for the offset ABR component. They argued that:

"the higher frequency stimuli offset wave V latencies were significantly longer than onset latencies across stimulus conditions. These results suggest that the high frequency stimuli did not optimally stimulate the cochlear partition responsible for initiating offset ABR" (p44)

Perez-Abalo et al., (1988) also argue that the offset ABR comes from a narrow, apical region of the basilar membrane. They demonstrated that the offset ABR had smaller decreases in amplitude and smaller changes in latency than the onset counterpart particularly when the high-pass masking filter was lowered.

Third, our data showed that amplitude was smaller for the offset ABR compared with its onset counterpart. Interestingly, both components had greater amplitudes when recorded with low frequency stimuli compared to higher frequency stimuli. When offset responses are reported they are usually described as having smaller amplitudes than the onset responses (Antonelli and Grandori, 1984). This differs from the data presented in Perez-Abalo et al., (1988) who reported that all of the offset responses were of a similar size or greater amplitude compared with the onset response.

Finally, we showed that the onset ABR was more sensitive to rise time than the offset ABR. This trend was observed across all frequencies but was more noticeable in the lower frequencies. We could see no difference in the amplitude for either component for the two rise time conditions. This result is not surprising when we consider that only the earliest portion of the waveform is important in eliciting a response for the onset, thus the transient nature of the ABR favours short rise times and enhanced neural synchronicity (Hall, 2007). Our data did not show any evidence to suggest that the offset ABR is dependent on rise time. Collectively this supports the idea that these components are generated by different mechanisms.

Our data here differs significantly from Van Campen et al., (1997). They reported that the offset ABR had an 'enhanced' response when measured with a longer rise time (5ms) relative to the shorter 0.5 ms rise time. The discrepancy between our studies is not clear. Previous studies investigating the effect of rise and fall times on the offset ABR postulated that the offset ABR was more sensitive to rise-fall time than onset ABR due to subsequent changes in the amplitude of acoustic ringing. Alternatively, this may reflect a difference between our recording parameters, for example, different gating parameters used to generate our stimuli. Unfortunately they did not publish their stimulus waveforms so we cannot judge the degree of ringing in each condition and therefore we cannot draw any further conclusions.

7.2.5.2 Polarity

The published data examining the relationship between polarity (or stimulus phase) and the ABR is conflicted. There is no consensus on the effects of polarity on the ABR. Some studies report very little effect of polarity (Hall, 2007); others report that polarity has a significant effect on the ABR (Gorga and Thornton, 1989). Moreover, most of these studies have concentrated on the potential influence of polarity on peak latency, with less attention given to the influence on wave amplitude (Hall, 2007).

Here we present the first data from the onset-offset ABR, examining the possible effect of polarity on the latency and amplitude of both components. We hypothesised that this may provide a partial explanation for the poor response reported in previous studies (Van Campen et al., 1997). The use of an alternating polarity click can disrupt the phase locking properties of afferent auditory fibres; cancel low frequency phase locked components, while summing the input from fibres tuned to high frequencies. This can produce responses that are out of phase, which can lead to a poor wave morphology or complete absence of the waveform, and in turn this can lead to misinterpretation of abnormal brain stem function (Schwartz et al., 1990, Orlando and Folsom, 1995)

Our data showed that the onset ABR is sensitive to polarity but only when recorded with lower frequency stimuli. Amplitude was greater when recorded with a condensation click compared with the rarefaction click stimulus. This was particularly clear at lower frequencies but not obvious at higher frequencies. This result is not surprising especially as Gorga and Thornton (1989) maintain that low frequency stimuli are separated in time as compared to higher frequency stimuli whose positive and negative half-cycles occur closer in time. This would mean that stimulus polarity effects are more prominent at low frequencies because of the dependence upon phase-locking properties of the cochlear and auditory nerve (Gorga and Thornton, 1989).

However, we observed no difference in latency onset to either rarefaction or condensation. This differs from empirical data published in Orlando and Folsom (1995) that examined the ABR for morphological differences using both polarities and found large latency differences between rarefaction and condensation stimuli for low frequency stimuli. They argued that this was related to the phase sensitive neural responses within the cochlea and auditory nerve (Orlando and Folsom, 1995). However, the interpretation of polarity (or stimulus phase) is complicated by several factors including the acoustics of the external auditory meatus, stimulus gating parameters and stimulus intensity to name but a few (for a review see Hall, 2007). The differences between our data and previously published results may just be a reflection of different parameters or due to our smaller sample.

Interestingly, the offset ABR was relatively unaffected by polarity. Indeed, we could find no evidence to support a difference in offset ABR amplitude or latency for either condition. This may provide evidence for different functional properties of the offset ABR compared with the onset response or may simply reflect a difference in the neural generators (i.e apical versus basal cochlear mechanics). Although our sample is small, we must conclude that polarity does not affect the offset ABR and cannot be used to explain the poor responses (i.e. the number of absent measurements that have previously been described). A partial explanation for the poorer offset response may lie in the choice of electrode montage and the overlapping MLR components. There are a number of possible approaches that could be used to try and eliminate the possible contributions of myogenic and neurogenic sources such as selecting a different electrode montage (to avoid any contribution from the mastoid) or amending the stimulus rate. These should be tested in future studies.

7.3 Experiment 2: The offset ABR in a clinical population

7.3.1 Introduction

The remarkable ability of humans to reliably identify and categorise phonemes with high fidelity – despite considerable natural variation across speakers, and distortions brought by noisy and reverberant environments – has been the subject of intense study for decades (Anderson and Kraus, Anderson et al., Holt and Lotto, Cacace and McFarland, 1998, Griffiths, 1999, Kraus, 1999, Allen et al., 2000, Bellis et al., 2000, Emanuel, 2002, Fuente and McPherson, 2006, Banai et al., 2007, Hackett, 2011).

Scientific studies of these phenomena are also strongly driven by our need to understand what can cause dysfunction in these critical processes; i.e. states in which the *brain is unable to accurately represent or process speech* as seen, for example, in hearing loss, auditory aging or neurological disease.

One potentially important and related area of research that has received little attention is the encoding of sound offsets, particularly in the auditory brainstem. Studies of the offset ABR have been strictly limited to normal hearing adults (Palmer, 1987, Van Campen et al., 1997). Study of the offset ABR has never been undertaken in any clinical disorders although a number of studies have suggested it may have clinical relevance (Van Campen et al., 1997).

More recently, study of the rat auditory cortex has provided convincing evidence supporting the existence of two pathways for auditory processing. This study clearly showed that one set of synapses was strongly activated by the onset of tones but a different group of synapses responded strongly at the offsets, or the sudden ending of the tones. No overlap between the two responding sets of synapses was seen, i.e. the end of one tone did not affect the response to a new one (Scholl et al., 2010).

Since the anatomical pathways controlling the two responses are thought to be different, at least in some respects, it is not unreasonable then to expect that lesions and disease processes may differentially affect these functional pathways. Therefore to the clinician it may be just as useful to test the offset response as it is to test the onset response. Furthermore, knowing how the brain responds to, and organises, sounds could lead to better treatment.

In this chapter, we present our preliminary findings from a small cohort of children with atypical saccadic eye movements. A key question that we attempt to address is '*whether the neural responses of the brainstem in children with oculomotor disease is sufficiently rich to support the discrimination of sound offset?*

7.3.2 Methodology

7.3.2.1 Subjects

The study group was composed of 12 children diagnosed with atypical eye movements. This group represents a smaller cohort of participants who had taken part in our previous studies (as presented in Chapters 4 – 6). Four children were diagnosed with neuronopathic GD (4 female, age range 7-19 years; mean age 10.5 yrs) and 8 were diagnosed with DES (4 males, age range 4- 15 years; mean age 8.75 years). Table 7.5 summarises the clinical other features and treatments of the study group.

An equal number of age and gender-matched children with normal hearing threshold levels (normal pure tone audiogram and tympanometry) with no known predisposing factors for hearing loss were used as controls. Ethical Committee approval was obtained for the study with informed consent was obtained from the parents of the younger children and from the older subjects themselves.

Table 7-5 Summary of clinical characteristics

Case	Age (yrs)	Sex	Diagnosis	Other neurological signs
1	8	F	GD3	Some difficulties with reading and writing but attends a mainstream school.
2	7	F	GD3	No concerns
3	8	F	GD3	No concerns
4	19	F	GD3	Learning difficulties
5	6	M	DES	Stroke in the cerebellum; Ataxic and very clumsy; Learning difficulties
6	11	M	DES	Dyslexia; LTM deficits; Dysarthric; Learning difficulties
7	4	F	DES	Learning difficulties
8	15	F	DES	Learning difficulties, Still unsteady on her feet; Attends a special needs school
9	8	M	DES	No concerns
10	8	M	DES	Learning difficulties, Still unsteady on his feet; Attends a special needs school
11	6	F	DES	Learning difficulties but attends a mainstream school
12	12	F	DES	Learning difficulties

Abbreviations: DES – dancing eye syndrome; F- female, GD3 – type 3 Gaucher disease; LM – long term memory deficits; M- male, SIF – saccade initiation failure.

7.3.2.2 Recording methods

The onset and offset ABR was recorded as previously described in section 7.2.3. Tone burst of 0.5 kHz and 2 kHz , with a rise time of 5 ms and a fall time of 0.5ms (total duration of 10 ms) were used to obtain the offset ABR. These stimuli were selected as our previous studies (and those reported by Van Campen et al. 1997) had shown that the offset response was more prominent when recorded with a longer rise time and shorter fall time.

7.3.2.3 Data Analysis

The analysis of each peak component (onset and offset) was undertaken using a visual inspection method as previously described in section 7.2.3. Peak waveform latency and amplitude data for onset and offset were calculated. The following descriptive statistics were presented for quantitative data: number of values, mean and

standard deviation (SD). Because of the small sample size, we have confined ourselves to providing a descriptive analysis of our pilot data only.

7.3.3 Results

7.3.3.1 Comparison of the onset-offset ABR in normal hearing adult and children

To the best of our knowledge, the offset ABR has not been measured in younger participants. Because of the possibility that age may be influence the ABR, we compared the onset–offset ABR from two different age groups. All of the participants had normal hearing.

The onset ABR was identifiable in 9/10 older participants for 500 and 2000 Hz. The offset ABR was present in all participants for 500 Hz but only present in 8/10 participants at 2000 Hz. The onset ABR was less reliable in the younger group, and present in 8/10 cases when recorded with both 500 Hz and 2000 Hz. The offset response was present in 9/10 participants for both 500 Hz and 2000 Hz.

When we compare our data with that reported in Van Campen et al., (1997) we see that our data is similar for both components (87% onset and 85% offset when measured with 2000 Hz. However, our onset and offset data at 500 Hz for both younger and older subjects was better than previously reported by Van Campen et al., (1997) (Table 7.4).

Table 7.6 shows the mean and SD for ABR latency and amplitude in adults and young participants with normal hearing. We could see no evidence from our data to support a difference in the latency or amplitude for the onset or offset ABR component when measured at 500 Hz or 2000 Hz.

Table 7-6 Latency and amplitude data for the onset and offset response to 500 Hz and 2000 Hz for normal 'older' hearing adults and 'younger' children

Group	Stimuli	Onset response				Offset response			
	(kHz)	Latency (ms)	SD	Amplitude (μV)	SD	Latency (ms)	SD	Amplitude (μV)	SD
Older	0.5	9.93	0.47	0.23	0.09	6.60	0.25	0.29	0.14
	2.0	7.66	0.46	0.21	0.12	5.87	1.57	0.27	0.10
Younger	0.5	8.89	0.84	0.35	0.24	6.78	1.10	0.26	0.12
	2.0	7.14	0.79	0.28	0.18	6.89	2.45	0.48	0.14

7.3.3.2 The onset-offset ABR in neuronopathic GD

Of the four children who participated in this study, we were only able to successfully complete recording in three cases (1, 3 and 4). We were unable to reliably record the onset-offset ABR in case 2 (one of the younger participants) due to poor co-operation. Their excessive movement during the testing resulted in the need to repeat as well as increasing the number of artifacts. Both of these factors increased the total length of testing and as a result the testing had to be stopped.

We were unable to identify any onset or offset waveform component for any of these participants for either 500 Hz or 2000 Hz. Figure 7.12 shows a representative figure illustrating the response to click stimulus in recorded in the right ear and the onset-offset response recorded to 500 Hz.

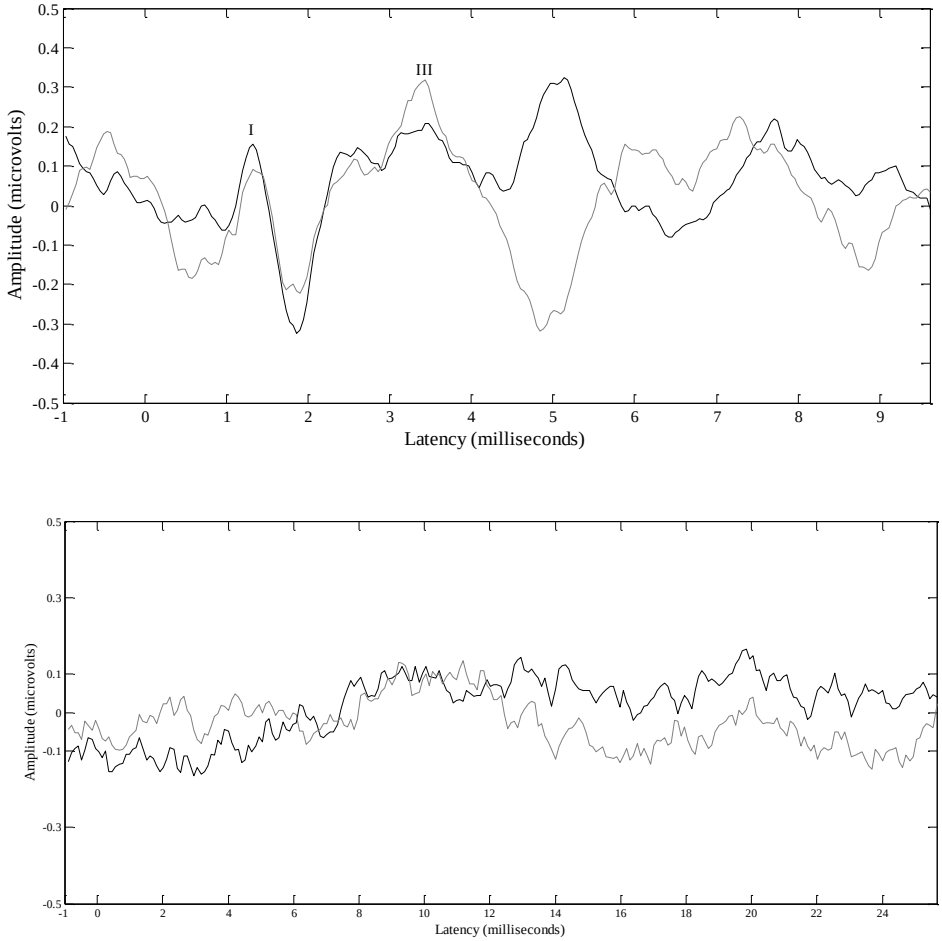
Only wave I and III are identifiable on the click ABR recording. There are no clear responses for the onset or the offset ABR (Figure 7.12 bottom panel). This was a typical response for all of the GD participants across the 500 Hz and 2000 Hz.

7.3.3.3 The onset-offset ABR in DES

In this study, we were able to successfully measure the onset and offset response in 7/8 DES cases. We could not complete testing in case 7 because of poor co-operation. In the remaining 7 cases, both components – the onset and offset – were clearly discernible. The mean latency and amplitude data for individual DES cases is shown

below in Tables 7.7 and 7.8 respectively.

Figure 7-12 Representative onset and offset ABR recordings from one child diagnosed with nGD (case 1).
The top panel shows a traditional onset-ABR recording using a click stimulus, only waves I and III are clearly reproducible in the traces. The bottom panel shows two superimposed traces of the onset-offset ABR recorded with a 500 Hz stimulus (right ear data only shown).



The mean onset latency response at 500 Hz was 8.16 ± 0.55 ms (n=7) in children diagnosed with DES compared with a mean onset latency of 8.89 ± 0.84 ms in normal hearing children (n=9) (Table 7-7). The mean onset amplitude at 500 Hz was 0.41 ± 0.20 µV (n=7) in children diagnosed with DES compared with a mean onset amplitude of 0.35 ± 0.24 µV in normal hearing children (n=9). There were no observable differences in onset latency or amplitude between the groups at 500 Hz.

The mean onset latency response at 2000 Hz was 7.42 ± 0.57 ms (n=7) in children diagnosed with DES compared with a mean onset latency of 7.14 ± 0.79 ms in

normal hearing children (n=9) (Table 7.7). The mean onset amplitude at 2000 Hz was 0.53 ± 0.16 μV (n=7) in children diagnosed with DES compared with a mean onset amplitude of 0.28 ± 0.18 μV in normal hearing children (n=9). There were no observable differences in onset latency or amplitude between the groups at 2000 Hz.

Table 7-7 Latency and amplitude data for the onset and offset response to 500 Hz for individual DES subjects participants. Control data is shown for n =9 cases for onset and n=8 for offset response. SD shown in brackets.

Case	Onset response		Offset response	
	Latency (ms)	Amplitude (μV)	Latency (ms)	Amplitude (μV)
5	9.2	0.29	7.83	0.31
6	8.99	0.18	9.60	0.49
7	NT	NT	NT	NT
8	8.16	0.81	8.03	0.34
9	9.72	0.41	7.41	0.50
10	9.41	0.48	7.58	0.40
11	8.68	0.32	7.18	0.25
12	8.47	0.35	7.11	0.40
Controls	8.89 (0.84)	0.35 (0.24)	6.78 (1.10)	0.26 (0.12)

When we examine the latency data we see that children with DES and the younger, normal hearing controls both demonstrate longer latencies for onset and offset components when measured with 500 Hz compared to 2000 Hz (Tables 7.7 and 7.8). The finding of a frequency-latency dependent relationship for both the onset and offset response is similar to that seen in our first empirical study in this chapter and concurs with previous published data (Perez-Abalo et al., 1988, Van Campen et al., 1997).

The mean offset latency response at 500 Hz was 7.82 ± 0.85 ms (n=7) in children diagnosed with DES compared with a mean onset latency of 6.78 ± 1.10 ms in normal hearing children (n=8) (Table 7.7). The mean offset latency response at 2000 Hz was 7.50 ± 0.64 ms (n=7) in children diagnosed with DES compared with a mean onset latency of 6.89 ± 2.45 ms in normal hearing children (n=8) (Table 7.8). There were no observable differences in onset or offset latencies between the groups at 500 Hz.

Table 7-8 Latency and amplitude data for the onset and offset response to 2000 Hz for individual DES participants. Control data is shown for n =9 cases for onset and n=8 for offset response. SD shown in brackets.

Case	Onset response		Offset response	
	Latency (ms)	Amplitude (μV)	Latency (ms)	Amplitude (μV)
5	8.06	0.38	7.41	0.27
6	7.33	0.41	7.62	0.76
7	NT	NT	NT	NT
8	7.64	0.44	7.76	0.62
9	6.6	0.56	8.76	0.63
10	8.16	0.65	6.87	0.45
11	6.91	0.64	6.99	0.81
12	7.22	0.62	7.1	0.60
Controls	7.14 (0.79)	0.28 (0.18)	6.89 (2.45)	0.48 (0.14)

We observed a difference in the offset amplitude data between the two groups at 500 Hz (Table 7.7). The mean offset amplitude was 0.38 ± 0.09 μV (range 0.25 - 0.5 μV, n=7) in children diagnosed with DES compared with a mean offset amplitude of 0.26 ± 0.12 μV in normal hearing children (n=8) (Table 7.7).

There is no observable difference in the offset amplitude at 2000 Hz in DES (0.59 ± 0.18 μV; range 0.27 - 0.81 μV, n=7) compared with normal hearing children (0.48 ± 0.14 μV; range 0.27 - 0.81 μV, n=8) (Table 7.8).

7.3.4 Discussion

7.3.4.1 Comparison of the onset-offset ABR in adults and children

In this study, we investigated whether younger children with normal hearing would have a different onset-offset ABR profile compared to an older group of adults with normal hearing. Establishing the normal morphological characteristics of the onset-offset ABR in a younger group – and whether maturation has any overall effect on the waveform components or morphology – was an essential undertaking prior to any application of this technique in our clinical populations in our later study.

We found the onset ABR was a more reliable measure in the adult group especially when recorded with low frequency (500 Hz) stimulus as evidenced by the fewer number of missing data values. Furthermore, our onset and offset data at 500 Hz for both younger and older subjects was better than previously reported by van Campen et al., (1997). As stated earlier, this may be due to a technical factor (such as ringing) which enhances the offset response in our conditions. Such a response has been reported in some studies to result in an increase in amplitude and therefore making it easier to discern waveform components (Brinkmann and Scherg, 1979).

We could find no evidence of any difference in latency or amplitude between the two age groups for either the 500 Hz or 2000 Hz stimuli. Such a finding is not surprising as there is substantial evidence from click evoked ABR studies to support a fully myelinated brainstem and a 'mature' ABR response from the age of 18 months (Moore, 1987a, b, Sininger et al., 1999, Hall, 2007, Moore and Linthicum, 2007). However, the age difference between our two groups (mean of 22 years in the older group and 9 years in the younger group) was not vast and our sample was very small so our results may not give an accurate representation in a wider population. Clearly this issue should be re-examined in a much wider age range including neonates and much older adults (>65 years) as evidence from the click-evoked ABR would indicate a difference in latency (Hall, 2007). While these results need to be validated in future studies, pilot studies examining test-retest reliability and the effect of different electrode montages, particularly in order to minimise PAMR would also be useful additions in this area of research.

7.3.4.2 The onset-offset ABR response in nGD

In this study, we present for the first time the onset-offset ABR in 3 children diagnosed with nGD. We have previously shown in Chapters 4 and 5 that the click evoked ABR in nGD is abnormal, despite normal peripheral hearing. Of the 6 patients who participated in this study, we were only able to successfully complete our recording in 3 cases. The morphology of the waveforms in each of these 3 cases was grossly abnormal – *we were unable to identify any onset or offset waveforms* – to either the 500 Hz or 2000 Hz stimuli.

There are three possible explanations for our data. Firstly, the onset-offset ABR is not a wholly reliable measure. We were able to record the onset and offset ABR in 8/10 children in the first part of our study. It is plausible then that this is an unreliable measure of auditory processing and that the cases did have a response but we failed to capture or measure it using our current method. Secondly, our stimulus may have been 'too challenging' for the auditory brainstem in these three cases. The stimulus used in this study had a very long rise time (5ms) which is a less than optimal for evoking the conventional 'onset' ABR. Previous studies have shown that the onset ABR is highly dependent upon the time-locking transient properties of the stimuli. In a disorder – characterised by poor neural synchrony – any stimulus that is 'suboptimal' would present a challenge portion not ideal for neural synchrony and eliciting an 'onset' response. Finally, an alternative explanation is that children with nGD don't have recordable onset-offset responses because of the neurological damage within the auditory system. The slower rise time of the tonal stimulus that we used in our study, compared to the click we previously used could potentially enhance the effects of poor neural synchrony in this group and provide some explanation for the absent responses in these patients.

The origin of the offset ABR is still highly debated in the scientific community. Because the conventional ABR is time-locked to stimulus onset, it presumably

represents synchronized single unit activity of onset neurons (Moller, 1985). It follows then that the offset ABR could reflect single unit activity of offset neurons or a synchronized decrease in neural activity. Other studies have concluded that the offset ABR could reflect a synchronised 'second onset' response to stimulus cessation. Unfortunately we are unable to draw any conclusions in this regard based on our data. It may be of interest to examine the onset-offset ABR in other neurological disorders that present with dysynchronous ABRs such as those seen in auditory neuropathy.

The distinct lack of any 'onset' response is an important finding and warrants future investigation. Onset responses have been shown to have a wide range of functional significance including an important in temporal discrimination, music perception and loudness perception (Allen et al., 2000, Phillips and Hall, 2002, Phillips et al., 2002a, Phillips et al., 2002b, Banai et al., 2005, Abrams et al., 2006, Hornickel et al., 2009a, Kraus and Chandrasekaran, 2010). Furthermore, accurate encoding of auditory transients is particularly important in speech perception, because both the amplitude onset slope and the relative timing of the onsets of energy in different frequency bands can be important cues for phonetic identity (Kraus et al., 2000, Phillips et al., 2002a). Future studies in children with nGD, or indeed any patient with 'paradoxical' hearing should include a wider range of behavioural and psychoacoustic measures of temporal function. Indeed there is emerging evidence of temporal asynchrony and perceptual timing deficits in many of these cases (Starr et al., 1996, Kraus et al., 2000, Starr et al., 2001). Such studies provide a model for studying the role of synchrony in auditory perception and could lead to more effective hearing therapies and devices.

7.3.4.3 The onset-offset ABR response in DES

In this study, we were able to successfully measure the onset and offset response in 7/8 DES cases. Both components – the onset and offset – were clearly discernible in the DES group. Our latency data was comparable to the normal hearing age-matched controls, with longer latencies observed for both onset and offset components when elicited with low frequency stimuli (500 Hz) and shorter latencies seen in response to 2000 Hz. The data supports previously reported studies (Van Campen et al., 1997).

We could find no evidence to support any differences in onset amplitude data for children with DES compared to normal hearing children. However, our data showed a difference in the offset amplitude data between the two groups at 500 Hz which was significantly greater in children diagnosed with DES. There was no difference in amplitude at 2000 Hz. The novel finding – that the offset response is greater in DES, particularly at low frequencies – is interesting and may provide a partial explanation for the processing difficulties that children report in DES (Table 6.7, Figures 6.4 – 6.6).

Like the other responses seen in DES this 'over-representation' of low frequency information at stimulus offset may be a reflection of the neural 'hyper-excitability' seen in other aspect of the disease. Our finding suggests that there is an abnormally high level of sound induced neuronal activity occurring within auditory brainstem pathways, which could be due to abnormal amplification of sound evoked neural signals, particularly in low frequencies.

It is difficult to account for the enhanced offset at 500 Hz in the DES group because the actual mechanism by which the offset response is generated is unknown. Offset responses could be generated within the cochlea (i.e.) a mechanical transient in the basilar membrane caused by the sound offset. Therefore our finding could suggest

dysfunction within the cochlear micromechanics, the result of which causes a cascade of disordered auditory information, upstream to higher cortical networks.

Alternatively, the sound offset could be generated by post-inhibitory rebound at some point along the auditory hierarchy. Therefore our data could imply that there is a distortion in the neural coding of the auditory input that may cause abnormal growth in loudness or failure of the central nervous system to habituate the startle response.

The cause of DES is generally assumed to be related to either antibodies or immune cells that are produced, most likely as part of a natural 'defense' response to the neuroblastoma cells. It is plausible then that in DES these antibodies also attack cells in key areas in the brain, including cells within the inner ear. These rogue cells could cause damage in the inner ear by releasing cytokines (after a delay), resulting in additional immune reactions. Alternatively, antibodies or rogue T-cells could cause accidental inner ear damage because the ear shares common antigens with a potentially harmful substance, virus, or bacteria that the body is fighting off.

Accurate encoding of sound offsets is important for perceptual grouping and auditory scene analysis and there is research that suggests that the auditory system may use off responses to 'reset' itself, at points of rapid amplitude change, in order to update the 'auditory scene'. Failure to 'reset' or accurately integrate fine-grained acoustic cues from the temporal envelope could manifest behaviourally as an abnormal perception of loudness (or decreased sound tolerance). This finding has been widely reported in other conditions associated hyperacusis including patients diagnosed with Autism, Spina bifida and Williams syndrome, although we are unaware of any study that has investigated the 'offset' ABR in these cases (Khalfa et al., 2004).

This finding has important implications for understanding speech, particularly when the background environment is challenging (e.g. speech in noise). Speech is

composed of complex sound waves and is dependent on rapid spectral and temporal changes, which are separated by discrete gaps. The enhanced over-representation of low frequency information could mask other important spectral cues, potentially compromising speech perception in sub-optimal environments. Evidence supporting this finding was recently published by Elsabbagh and colleagues (2011). They examined whether hyperacusis interfered with speech processing in children and adults diagnosed with WS. They found a significant relationship between the effect of hyperacusis and speech discrimination performance (i.e. speech discrimination was worse in cases with more severe hyperacusis. They attributed this to auditory and non-auditory factors including altered auditory attention and context dependent cues (Elsabbagh et al., 2011) Clearly this is an area which requires further study but our novel finding that suggests that the speech offset should also be more closely examined in these studies.

7.3.5 Conclusions

In this chapter we have presented the preliminary results from the onset-offset ABR using simple tonal stimuli. The origin of the onset-offset ABR is controversial and research into this potential application has been severely limited. In our first study we have shown that with careful recording, an offset potential with a unique set of characteristics but similar in morphology to the onset ABR can be recorded. This response is highly variable and more sensitive to some changes in the stimulus envelope, than its onset counterpart. We have also shown in this study that it is unlikely that the use of alternating polarity can explain the discrepancies in identifying the offset ABR. It is more likely that the absence of the response is due to stimulus artefact obscuring the offset potential waveform.

Our second experiment examined the application of the onset-offset ABR in two clinical conditions with established brainstem oculomotor disorders. Previous studies of these two conditions have shown that the ABR when recorded with simple click is (a) absent or significantly dysmorphic in children with nGD and (b) that there is a slight prolongation in the children with DES. The onset-offset ABR was significantly different from age-matched controls in both groups. Firstly, we were unable to identify any clear waveform component (onset or offset) in the nGD group. This may reflect the recording parameters used to elicit the response but is more likely to be related to the poor neural synchronicity that is evident in the click ABR in this group. Our latency data from the DES population was comparable to the normal hearing age-matched controls but the amplitude data, particularly at 500 Hz was significantly greater in children diagnosed with DES. This data may reflect some of the difficulties that this group report with hyperacusis but further psychophysical testing to correlate with our findings are required.

Finally, our studies in clinical setting have identified a number of serious concerns with the use of the onset-offset ABR as it is currently measured. Without significant investment in basic and applied research studies, any clinical application of the onset-offset ABR at this stage would be extremely premature although our data offer some very interesting insights that should encourage future work in this area.

Chapter 8
General Discussion and Conclusions

8.1 Overview

Firstly, let us look back to the original question that motivated our thesis. We asked whether *children diagnosed with GD and DES with documented saccadic eye movement abnormalities, originating in the brainstem, also have auditory problems.* Our research was motivated anecdotal patient reports of auditory complaints; previous (incomplete) literature and the proximity of the structures. We hypothesized that since the neural substrates controlling the oculomotor and auditory systems are anatomically co-located within the brainstem then it would be reasonable to expect that auditory pathways would also be affected in these two conditions. Moreover, we argued that if there is brainstem disease in neighbouring anatomical areas – such as the brainstem oculomotor system – then we ought to anticipate some auditory abnormality.

Our thesis concentrated on two clinical disorders: GD and DES. The hallmark of both of these disorders is their abnormal eye movements: slow saccades seen in children with neuronopathic GD and opsoclonus as seen in children diagnosed with Dancing Eye Syndrome. We selected these conditions as saccadic models of slow saccades and high frequency oscillations (opsoclonus) had shown evidence to support that the *pons* – a neural substrate common to both in the oculomotor and auditory brainstem systems – is damaged in both of these disorders.

Our second research question was concerned with the utility of auditory electrophysiological measures in the study of eye movement disorders, such as GD and DES. Here we asked *if the application of the ABR could be used to complement current eye movement studies in GD and DES?* We argued that the ABR was a potentially useful instrument to examine this issue. We maintained that there were several advantages in applying this technique including an extensive literature supporting its ability to measure the integrity of brainstem pathways and the ability for this non-

invasive measure to translate successfully from the lab to clinic. We also presented literature, albeit limited, suggesting that children with established eye movement disorders also have abnormal ABRs.

Returning to the hypothesis posed at the beginning of this study, it is now possible to state that *there is ample evidence that the use of audiological assessment in children with GD and DES would contribute to clinical practice*. Moreover, we believe we have presented evidence to support the utility of the ABR alongside eye movement studies in a variety of roles.

In this final chapter, we begin by presenting a summary of our main findings and conclusions. We then proceed to outline what we consider are our core contributions and discuss their significance and their relevant implications. Finally, we discuss the limitations of the thesis and suggest possible directions for future work.

8.2 Findings and conclusions

8.2.1 General introduction and background literature

Our initial chapters were concerned with 'setting the scene' – providing a reasoned argument for our research questions – highlighting the importance of such a study in Neuroscience. These studies we argued were of growing import when set against the emerging evidence for a 'dynamic' brainstem: a system that demonstrated plasticity and was able to learn.

We began to develop this argument further in Chapter 2 by outlining the saccadic eye movement and auditory brainstem systems. Here we clearly showed the close anatomical relationship in the brainstem. We argued that brainstem lesions do not respect sensory boundaries and in order to understand the functional implications of any disease, that may affect the brainstem, study *across* sensory modalities was required.

Therefore, we argue that a more powerful approach is to combine multimodal techniques such that the resultant data measures augment one another.

We followed this discussion with a brief overview of eye movement techniques and audiological techniques that are currently used to examine the brainstem. We had previously argued that these techniques are readily available in most Audiology clinics – although the routine investigation of eye movement disorders is considered by many as beyond the scope of clinical practice.

Since our thesis was concerned with establishing the central importance of the auditory brainstem response (ABR), we asked whether there is any documented evidence of auditory abnormalities in other aetiologies associated with slow saccadic function or opsoclonus. We explored the scientific literature to see whether both signs and symptoms have been reported in conditions. Our findings from the scientific literature suggested that, in general, auditory deficits – as indexed by the ABR – were commonplace in a number of aetiologies that give rise to slow saccades or opsoclonus. However, there was limited multimodal study examining both systems. We believed this provided sufficient evidence to warrant further study.

8.2.2 Chapter 4: Audiological profile of Gaucher Disease

In this chapter, we introduced our first clinical population – Gaucher disease. A general assumption made in the clinical literature was that the presence of abnormal eye movements – characterized by slow saccades and saccade initiation failure – was a universal sign, and therefore diagnostic of the neurological subtype. However, we could find no study that categorically supported this assertion. Indeed, we found several studies that argued that abnormal eye movements were also present in patients with

non-neuronopathic phenotypes. Clearly before we could commence any auditory study – we needed to establish the credibility of these claims.

We undertook a systematic literature review study to examine whether there was any evidence to support the theory that GD1 and GD3 are *not* distinct phenotypes on the basis of eye movement studies. Our findings showed that eye movement abnormalities in our GD *were pathognomonic of neuronopathic disease*, at least in children.

We began the first of our empirical studies in GD. Here we systematically investigated the audiological function in 25 children with enzymatically diagnosed GD using a series of tests that measured the integrity of the auditory system. We found that combined auditory and oculomotor studies can be used to better delineate the underlying neurological deficits in nGD. This finding provided our first evidence supporting our original hypothesis that *children with GD have auditory problems* and it suggested the possibility of a new role for the ABR and its modification by brainstem oculomotor pathology.

One of the more significant findings to emerge from this first study was that GD1 and GD3 are really distinct phenotypes on both measures. There was no indication of gradations on either auditory or eye movement tests between the two groups, consistent with the notion of two distinct phenotypes –neuronopathic and non-neuronopathic. The results of this research provide sufficient evidence to warrant the inclusion of audiological testing as part of the standard assessment of newly diagnosed GD patients.

8.2.3 Chapter 5: The use of audiological assessments in the longitudinal monitoring of GD

We began this chapter, by asking whether the ABR could have a wider application either as a longitudinal outcome measures for use in clinical trials or value as a neurological marker for monitoring disease burden in the brainstem pathways. The cost of not developing quantitative measures was extreme: the urgent need to accurately diagnose and monitor treatments in these rare diseases – as early as possible – was a recurring theme throughout the literature.

We presented two experimental studies. Our first study was concerned with examining whether the ABR could be used to reliably measure the disease burden in the neuronopathic subtypes of GD. Here we reported data of serial ABR recordings in type 2 and type 3 GD.

We drew two conclusions from the first part of this Chapter. First, our data clearly showed that the ABR could be used to provide objective evidence of the time course of neurological involvement (progression or remission) in patients diagnosed with nGD who were undergoing high dose ERT. Second, we showed, for the first time, evidence that high-dose ERT does not prevent the deterioration of ABRs in neuronopathic GD. Here we provided empirical evidence to support our second research question: *if the application of the auditory brainstem response could be used to complement eye movement studies?* As a result, we proposed that patients should undergo these tests prior to commencing treatment to establish a baseline for future measures.

Our second study examined the merit of using the ABR as an outcome measure in clinical trials. Only three small case studies had been described in which eye movement and auditory assessments were used as outcome measures. All of these studies indicated that substrate restriction therapy (Miglustat) could improve

neurological manifestations in GD3. Here we presented our data on use of the ABR as a secondary outcome measure in randomized control drug trial of Miglustat in children diagnosed with GD3. We could find no evidence to support any improvement in ABR waveform components or morphology over a two year interval. Moreover, we found that children who had previously had normal peripheral hearing developed hearing loss over this short time frame. Our novel finding raised an interesting question – is hearing loss is a previously unidentified feature of GD? Such data lent further support to our argument for the use of audiological assessment in children diagnosed with GD.

8.2.4 Chapter 6: Audiological profile of Dancing Eye Syndrome

We began this chapter by introducing our second clinical study group: Dancing Eye Syndrome, a rare disorder characterized by remarkable ('chaotic' or 'dancing') saccadic eye movements. Anecdotal reports painted a picture of extremely distressed children when exposed to loud or sudden sounds – reminiscent of hyperacusis. Here, we asked whether the hyper-excitability expressed in the eye movement system, as opsoclonus, was mirrored in the auditory system, i.e. *do children with DES have hyperacusis?* Our parental questionnaire provided preliminary evidence to suggest that many children with DES did have an abnormal sensitivity to ordinary environmental sounds.

Our second study took up the challenge of establishing whether the auditory brainstem was impaired in DES. We highlighted the major gap in our clinical knowledge – the inability to accurately identify the site of lesion – in DES. Eye movement studies had implicated the brainstem and the cerebellum as possible candidates. Here we presented for the first time a systematic audiological evaluation of DES. The most obvious finding to emerge from this study was that children with DES

had evidence of longer-term brainstem disease, even in the absence of any overt eye movement abnormalities. This major finding implicated the auditory brainstem in DES.

Taken together, these results suggest that *children with DES do have auditory problems*, including an abnormal sensitivity to ordinary environmental sounds. Furthermore, *these deficits can be reliably measured by the ABR*.

8.2.5 Chapter 7: The 'offset ABR' in saccadic eye movement disorders

In our final experimental chapter we presented pilot data for the onset-offset ABR. The origin of the onset-offset ABR is controversial and research into this potential application has been severely limited. We argued that application of the offset ABR merited further investigation in light of new data from animal models. In our first study we clearly showed that an offset potential with similar morphology to an onset potential can be recorded. This response was highly variable. We showed in this study that it is unlikely that the use of alternating polarity can explain the discrepancies alone in identifying the offset ABR. It is more likely that the absence of the response is due to stimulus artifact obscuring the offset potential waveform.

Our second experiment examined the application of the onset-offset ABR in two clinical conditions with established brainstem oculomotor disorders. Previous studies of these two conditions have shown that the ABR when recorded with simple click is (a) absent or significantly dysmorphic in children with nGD and (b) that there is a slight prolongation in the children with DES. The onset-offset ABR was significantly different from age-matched controls in both groups. Firstly, we were unable to identify any clear waveform component (onset or offset) in the nGD group. This may reflect the recording parameters used to elicit the response but is more likely to be related to the poor neural synchronicity that is evident in the click ABR in this group. Our latency data from the

DES population was comparable to the normal hearing age-matched controls but the amplitude data, particularly at 500 Hz was significantly greater in children diagnosed with DES. This data may reflect some of the difficulties that this group report with hyperacusis but further psychophysical testing to correlate with our findings is required.

8.3 Research issues examined

The experiments reported in this thesis present many pieces of an evolving puzzle. Moreover the contributions of this thesis also make it clear how little of the entire territory has been investigated to date. We now consider the original contributions of this thesis concentrating on those research issues we have explored and relating our findings back to those questions that motivated our thesis.

8.3.1 Children with GD and DES have audiological abnormalities

Our ABR investigations, using onset and to a much lesser extent offset stimuli, have clearly identified a diverse number of ABR abnormalities in two conditions associated with atypical SEM. These findings have not previously been documented. The findings from our thesis make several contributions to the current literature which we summarized earlier in Chapter 2.

First, we clarified the usefulness of the ABR as a neurological tool in nGD and DES. For example, we presented literature in Chapter 4 to show that the utility of the ABR as a clinical measure of neurological dysfunction in GD1 and GD3 was extremely confused. Earlier studies in this area had compared the ABR findings in GD and while some degree of similarity has been identified, several inconsistencies across studies were also reported. Further examination of these studies revealed marked methodological heterogeneity and incomplete reporting that limited the interpretation of

earlier research in this area. Our study as presented in this thesis was the first to present quantitative data and not just a descriptive analysis of ABR waveforms.

Second, our findings add substantially to our understanding of the pathophysiological mechanisms in both of these conditions. The auditory abnormalities that we have identified in children with nGD have shown that not only is the ABR a sensitive subclinical measure of neurological deterioration in these groups but that the peripheral hearing mechanism may also be involved in the latter stages of the disease (see 8.3.2 for further discussion). Our data, for the first time, have also clearly implicated the auditory brainstem as a site of lesion in DES, even after the oculomotor abnormalities resolved.

In Chapter 4, we also presented a model linking eye movement and audiology findings. We argued that the unusual pattern of audiological and oculomotor abnormalities identified in our nGD patients suggested an excitotoxic mechanism which predisposed nerve cells to glucocerebroside toxicity. In Chapter 6, we discussed the link between the eye movement abnormalities in DES and the presence of hyperacusis. Our study identified hyperacusis as an undiagnosed concern *in at least 43%* of children with DES. We argued that this finding could be consistent with hypothesis of serotonin disturbance in these patients. We showed that this finding is consistent with eye movement studies and other biochemical studies in DES. The developments of these theories – although speculative – provide strong support for multimodal study that we advocated earlier in Chapter 1.

Third, the this thesis provides additional evidence that auditory tests, such as the ABR, in addition to eye movement studies, can improve the phenotype/genotype discordancy that has been previously described in this disease. This data has important clinical implications in terms of diagnosis and treatment. Major advances have been

made in developing treatments for GD and other associated LSD. Given the prohibitive costs of many of these treatments, there is an impetus on the early distinction between different phenotypes (non-neuronopathic and neuronopathic disease) to allow the appropriate treatment to commence as soon as possible.

Taken together, these findings clearly suggest a role for ABR measurement in children with atypical SEM. This research will serve as a basis for future studies. Given the exciting developments in learning and plasticity studies of auditory brainstem pathways there is a very real possibility for amelioration of some of these deficits and auditory rehabilitation in these clinical conditions.

8.3.2 Systematic application of audiological tests provides important clues in understanding the pathophysiology in GD

Our data presented in Chapters 4 and 5 underscored the importance of systematically testing the auditory system. By combining standardised audiological tests with other tests of overlapping afferent and efferent pathways, including the acoustic reflex threshold test, the medial olivocochlear suppression test (MOCS) and the ABR, we clearly showed that the auditory system is affected in neuronopathic GD.

Our multimodal test strategy suggested that the disease affected multiple auditory brainstem structures including the cochlear nuclei and the olivary nuclei. We argued that a midline lesion near the genu could affect both fascicles to give a bilateral lesion and also affect the horizontal saccade centres in the PPRF. Although a number of these abnormalities could be accounted for by a solitary lesion located in the dorsomedial brainstem (in and around the saccadic eye movement centres), our auditory data suggested that it is more likely that there are additional lesions, possibly affecting inner hair cell function.

Our longitudinal studies, reported in Chapter 5 also showed that the audiological abnormalities often mirrored the severity of the phenotype (i.e.) the ABR was more impaired in GD2, than GD3. The pathophysiology of these abnormalities is speculative but suggests that multiple factors could be involved including segmental demyelination and axonal and neuronal loss. Treatment with ERT failed to halt the progression of the neurological manifestations and treatment with Miglustat showed no significant improvement in ABR abnormalities.

Serial auditory studies also revealed that abnormalities within the peripheral auditory system. These appear to emerge later in the disease course, suggesting subclinical cochlear dysfunction in some cases, and more severe inner ear damage (resulting in hearing loss), in others. Because of new and more aggressive treatments, which have extended the life expectancy in these rare orphan diseases, we could start to see the emergence of other auditory manifestations previously not reported (or observed) prior to the introduction of these treatments.

The site of lesion(s) leading to the auditory abnormalities in neuronopathic GD is uncertain. The neuropathological features of the GD3 phenotype are poorly defined. Post-mortem data are limited to a single case report in which the only morphological abnormality was infiltration of the Virchow-Robin spaces with lipid-laden macrophages. A direct neuronal insult was not found. On the basis of this evidence, we must conclude that toxic or metabolic factors extrinsic to the neuron play an important, and yet, undefined role in the pathogenesis of GD.

Based on this new knowledge – *gained from auditory and eye movement studies* – we posited that the unusual pattern of audiological and oculomotor abnormalities was consistent with an excitotoxic mechanism. The vulnerability of these neural pathways in GD reflects neurotoxicity – *induced directly and/or indirectly* – by elevated cellular cerebroside that potentiates excitotoxicity from excitatory amino acids, possibly

glutamate receptors, which is further exacerbated by high average neuronal firing rates. Such excitotoxic damage may be amenable to direct therapeutic intervention and warrants further study.

8.3.3 The application of the ABR as an outcome measure in clinical trials

Objective measurement of neurological dysfunction remains a major challenge when testing new drugs, especially in young children. The development of suitable neurological measures for monitoring the brainstem dysfunction has not kept pace with the new drug therapies that are emerging for the treatment of metabolic diseases.

We presented audiological data that showed the utility of the ABR as a longitudinal measure and as an outcome measure in a two year randomized clinical trial. Our data clearly showed that audiological investigations could be a sensitive longitudinal marker of disease burden. To the best of our knowledge, we presented the first data of the use of serial objective audiological testing to monitor the neurological progression in acute neuronopathic Gaucher disease (GD2). As far as we are aware this was the first objective evidence for progressive neurological involvement, despite ERT, that has included measurements pre-and post enzyme treatment**.** Our data added to an emerging literature which showed that ERT does not halt neurological progression in this severe form of the disease. Our application of the ABR in children with GD3 who were receiving high-dose ERT also showed evidence for the deterioration of the ABR.

The 24-month trial of Miglustat represented the first randomised, controlled study of a drug treatment in patients with GD3. We did not find any significant differences on the ABR assessed over 24 months. Indeed, our ABR data showed remarkable variability in the degree of abnormality in the ABR. Such variability needs to be taken into account when designing clinical trials and it is possible that this time

frame was too short to capture any meaningful data regarding the efficacy of the drug. Studies regarding treatment efficacy in DES are currently underway – these larger-scale randomised controlled trials could provide more definitive evidence about the utility of the ABR in this disorder.

Our findings add to an emerging literature on outcome measures, particularly the controversial use of eye movements and auditory tests in clinical trials. The ABR certainly meets some of the criteria of an 'ideal' biomarker. For example, it has been argued that a biomarker should be 'simple, rapid and inexpensive' – and the ABR easily fulfils these specifications. However, further study regarding ABR specificity and sensitivity are needed before the methods used in this thesis may be applied to other clinical trials. Large randomised controlled trials could provide more definitive evidence.

8.3.4 The ABR has a wider research and possible clinical role than previously indicated.

The data presented within this thesis both confirms previous findings and contributes additional evidence that an atypical 'offset' ABR response to stimulus cessation may account for some of the persistent auditory difficulties that are reported in some children with atypical saccadic eye movements. Whilst the study reported in Chapter 7 did not confirm the origin of the neural generators of the offset ABR, it did partially substantiate the potential usefulness of this technique. However, our studies also identified a number of concerns with the use of the onset-offset ABR as it is currently measured. Without significant investment in basic and applied research studies, any clinical application of the onset-offset ABR at this stage would be extremely premature although our data offers some very interesting insights that should encourage future work in this area.

8.4 Summary of Research Limitations

Finally, a number of important limitations need to be considered. Although we have attempted to address them as they arose, throughout the thesis, a number of caveats need to be noted. These can be divided into four main areas including: choice of population and sample size; limitations associated with poor source localisation of the ABR; lack of correlation to any behavioural measure and, finally, a number of technical and methodological issues.

First, the data applies only to children diagnosed with neuronopathic GD and DES. Furthermore, because of the small sample size – *a reflection of the rarity of these conditions* – we urge that caution must be applied when interpreting the data. These findings might not be directly transferable to other eye movement disorders presenting with slow saccades or opsoclonus. Moreover, we were unable to control for variables such as treatments or age effects. It is not clear from the studies presented here whether this is a significant source of variation on the ABR.

Second, because the exact generator sites of the ABR are not known, the pathophysiology of abnormalities seen in both conditions is speculative. The ABR is a far-field electrophysiological measure and their generators are complex and dependent upon a number of factors that are still not completely understood. Furthermore, although exquisitely sensitive to brainstem dysfunction, ABRs do not detect higher level (cortical) processing abnormalities. As such, we are unable to draw any conclusions about what effect (if any) functional deficits at these levels could have or how they may impair the information processing capacity of one, several or all of the subserving brain regions. This relationship has yet to be fully explored in healthy as

well as in clinical populations and to our knowledge, there have been no studies documenting this in children with saccadic eye movement disorders.

One of the main weaknesses of the studies presented here was the paucity of behavioural (or psychophysical) measures of auditory processing or other psychometric tests. What is the significance of an abnormal ABR on day to day listening experiences in these children? What is the potential usefulness of the ABR in this context? Given what we know about the neurological damage seen in these two conditions, it is not surprising that ABRs may be disordered in such individuals. However, it is apparent from some results that the ABR was normal. It is also unclear whether there are other conditions which could produce the same ABR 'signature'. Finally, the ABR's prognostic usefulness and functional significance is also unclear. Future studies should consider working at different levels of analysis, including at the symptom (or phenomenological level) and at the neurophysiological level of analysis. Such studies are urgently required not only so that we can monitor the neurological problems of children with brainstem disease but also so that we can make meaningful rehabilitative interventions.

Finally, we need to mention a number of methodological issues. One source of weakness in the studies presented here, which could have affected measurement, was the dependence of visual detection for interpretation of the ABR parameters – although this is a much wider issue affecting interpretation of all electrophysiological measures. Another limitation is the lack of validity for the questionnaire used in Chapter 6. At a practical level, our findings from Chapter 7 underscore the urgent need to significantly redevelop the onset-offset ABR technique before it is implemented clinically or used in research studies in a younger and potentially less co-operative population. Indeed, if all the patients in these studies were clinically confirmed as having auditory processing

deficits and yet only some of them had abnormal ABR then the onset-offset ABR seems to be a less reliable test than a clinical history and other findings.

Another example of a technical issue we faced when recording the onset-offset ABR was that we were unable to record from one child simply because of the length of testing involved – approximately 25 minutes per frequency – for one ear only. This is a significant barrier when considering the merits of incorporating this test into any test battery. In addition to examining the optimal recording methods, future studies should also investigate what the minimum number of stimulus presentations are required in order to evoke an onset-offset ABR. If it is significantly less than the 1000 presentations (per trace) used in our study, then the application of this procedure would certainly be more attractive to researchers and clinicians investigating the role of offset responses in auditory processing.

8.5 Potential Areas for future research

Audiological abnormalities indexed by the ABR are common in aetiologies associated with atypical SEM and our thesis has identified a number of questions in need of further investigation. Clearly, the limitations we outlined earlier in the previous sections must be dealt with. For example, correlating the ABR with other cognitive assessments and psychometric tests would certainly be beneficial. However, in this next section we outline some of the work that could be undertaken to extend the work contained here.

8.5.1 Extending the systematic measurement of the ABR to a wider group of conditions diagnosed with brainstem oculomotor disease

A natural extension of our study would be application of the auditory techniques and methods adopted in this thesis to a wider group of brainstem oculomotor disorders, particularly those presenting with slow saccades and opsoclonus. Future research should therefore concentrate on the systematic investigation of auditory processing in these disorders.

Another interesting application of our findings would be study of the ABR in neonates that are diagnosed with congenital opsoclonus; this could be achieved by using the national neonatal hearing screening programme. There is some data to support the presence of opsoclonus at birth. Opsoclonus has been reported in as many 3% of healthy infants – a statistic that rivals the detection of permanent childhood hearing impairment. In the majority of cases, opsoclonus recovers spontaneously (typically within 6 months), indicating some maturational component. An interesting question is whether the ABR would also show similar abnormalities? Or whether these infants develop auditory processing deficits at a later stage in their development?

8.5.2 The encoding of complex 'speech' stimuli in children with atypical eye movements

Our earlier experiments have only considered the neural representation of simple stimuli. We have not yet considered the significance of more complex or 'naturalistic' sound and how this might be encoded in children with atypical eye movements. Recently, a series of studies have been undertaken measuring the ABR recorded using a synthetic speech syllable, /da/. The aim of these studies has been to develop a more 'ecologically valid' test of brainstem integrity. Researchers have found that the 'speech-ABR' is able to detect subclinical abnormalities in the brainstem that are not evident

when using simple stimuli. These studies have been reported in normal hearing children with language and learning deficits and in other clinical populations such as autism.

These studies could provide insight into the accuracy with which the brain stem nuclei synchronously respond to acoustic stimulation and the degree to which the response reproduces the input. A key question we suggest should be tackled in future work is whether neural responses of the brainstem, in children with atypical SEM, are sufficiently rich to support detailed phonetic discrimination.

8.5.3 Development of other tests of brainstem function including the application of 'sensory gating' studies in children with atypical eye movements

The sensory gating hypothesis asserts that auditory processing anomalies are due to deficits in 'gating' or filtering of external sensory input by the CNS. Sensory gating experiments have shown that when two events happen within a short time frame (300 ms) of each other, the response to the second event is reduced ('gated' out) to allow priority processing of the first event. If the responsiveness to the second event is not reduced, a deficit in sensory gating may be assumed, and 'flooding' with irrelevant information may result. Two primary methods are used to assess gating: acoustic startle inhibition and P50 suppression.

Both of these measures involve overlapping auditory and oculomotor pathways. It is plausible that these pathways will also demonstrate some impairment. Measures of sensorimotor gating are assumed to reflect CNS gating mechanisms necessary for normal perception. Further gating deficits have been reported in a wide number of conditions which share very few clinical characteristics. However they are linked by a reduced ability to inhibit or gate sensory, motor, or cognitive information. Further work needs to be done to establish whether the gating mechanisms in brainstem oculomotor disorders are dysfunctional.

Appendices

9.1 Appendix 1: Systematic review methodology

We undertook a systematic review of the available literature, using the electronic database PUBMED. The search strategy was conducted using medical subject headings ('MeSH terms') and free text words and included sub-searches of the title, original title and abstract. This initial search was further refined using a combination of key words with the addition of Boolean operator terms[49]. The inclusion and exclusion criteria, that we applied to the original search is shown below. A total of 157 abstracts were returned.

Inclusion and exclusion criteria applied to the literature search

Inclusion criteria	Exclusion criteria
Studies that investigated the use of the core index test: the auditory brainstem response	Studies investigating other evoked potentials
English language	Non English publications
Published between 1970-2010	Conference abstracts
	Animal studies
	Not an original research article (ie) review articles, systematic review articles, editorials or theoretical papers

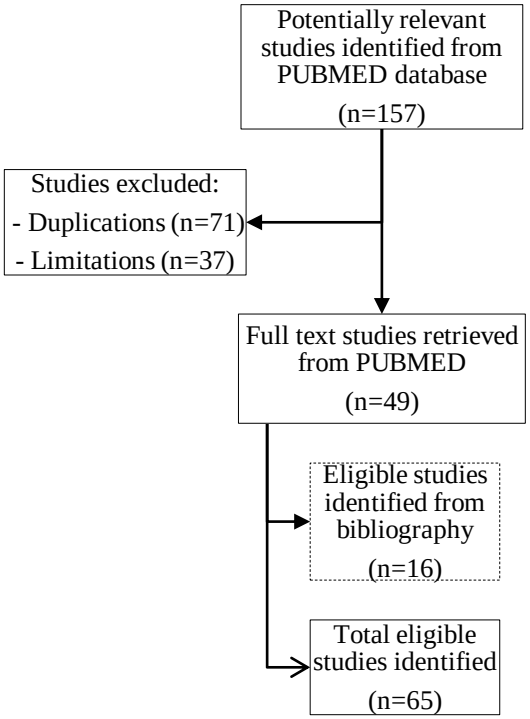
The title and abstract of each study included was used to determine its relevance to this review. Full publications were retrieved for studies that seemed relevant, and for those for which relevance was still unclear. Of the original 157 citations, only 49 studies were considered relevant. Bibliographies of selected studies were also searched by hand in order to identify any further relevant studies not detected by the electronic search.

Sixteen additional studies were identified from hand searching the bibliographies of those studies initially selected. This resulted in a final total of 65 studies (Miller et al., 1973, King, 1975, Sanders and Lake, 1976, Tripp et al., 1977, Cogan et al., 1981, Stowens et al., 1982, Winkelman et al., 1983, Erikson and Wahlberg, 1985, Erikson, 1986, Grafe et al., 1988, Gross-Tsur et al., 1989, Conradi et al., 1991, Sidransky et al., 1992, Uyama et al., 1992, Brady et al., 1993, Patterson et al.,

[49] Boolean operator terms ("AND" "OR"): were combined key words for the study population (Gaucher, non-neuronopathic, neuronopathic, type-1, type-2, type-3) and the eye movement abnormality (eye, gaze, looping, ocular, saccade, saccadic, supranuclear).

1993, Abrahamov et al., 1995, Chabas et al., 1995, Harris et al., 1996, Tsai et al., 1996, Schiffmann et al., 1997, Harris et al., 1999, Bohlega et al., 2000, Garbutt and Harris, 2000, Altarescu et al., 2001a, Garbutt et al., 2001, Sinclair et al., 2001, Tayebi et al., 2001, Accardo et al., 2005a, Accardo et al., 2005b, Pensiero et al., 2005, Alfonso et al., 2007, Capablo et al., 2008, Accardo et al., 2010, Alonso-Canovas et al., 2010).

A flow chart summarising the study selection process.



Data relating to the study population (genotype, phenotype, age, and gender), treatments, eye movement status (and measurement), and key findings were extracted.

9.2 Appendix 2: Normative data

9.2.1 Pure tone audiometry (PTA)

Audiometric reference thresholds published in BSA (2004)

Audiogram	Terminology	Description
Type	Sensorineural hearing loss	Average air-bone gap of less than 15 dB for 0.5, 1 and 2 kHz
	Conductive hearing loss	Normal BC thresholds and average air-bone gap of 15 dB or more for 0.5, 1, and 2 kHz
	Mixed hearing loss	BC threshold greater than 20 dB HL in combination with averaged air-bone gap 15 dB or more for 0.5, 1, and 2 kHz
Degree	Normal	Thresholds were better than 20 dB HL in both ears
	Mild	Thresholds were measured between 20-40 dB HL in both ears
	Moderate	Thresholds were measured between 40170 dB HL in both ears
	Severe	Thresholds were measured between 71-90 dB HL in both ears
	Profound	Thresholds were > 90 dB HL in both ears

9.2.2 Tympanometry

Normative data for tympanometry published in BSA (1992)

Jerger Pattern	Characteristics
A	Clear peak that occurs at 0 daPa ± 50 daPa with a base-peak compliance difference in the range of 0.3-1.6 ml
A_s	Clear peak that occurs at 0 daPa ± 50 daPa with a base-peak compliance difference in the range of < 0.3 ml
A_d	Clear peak that occurs at 0 daPa ± 50 daPa with a base-peak compliance difference in the range of > 1.6 ml
B	No clear peak (flat)
C	Clear peak that occurs at > 100 daPa, with a base-peak compliance difference in the range of 0.3-1.6 ml

9.2.3 Acoustic reflex thresholds

Reflex thresholds was judged subjectively by shape and looking for growth, although a reduction in compliance of 0.02ml or more was usually observed (BSA, 1992). In our study, we used the normative data published in Cohen and Prasher (1988). The ART was as 1) elevated for responses > 100 dB HL or 2) abnormal if they exceeded 105 dB HL at two or more frequencies or if the inter-aural threshold difference exceeded 10 dB on at least two frequencies (Cohen and Prasher, 1988). Acoustic reflexes at 4kHz were not considered as they are frequently absent.

ART patterns interpreted as indicating brainstem lesions were *vertical* (abnormal ART by stimulation of one ear only), *horizontal* (ART abnormal by contralateral stimulation of both ears), *inverted L* (combined vertical and horizontal) and *'full house'* (all ipsilateral and contralateral reflexes abnormal) (Cohen and Prasher, 1988).

9.2.4 Transient evoked otoacoustic emissions

The table shown below presents the mean and std. error data for TEAOE .

Ear	Variable	Mean	Std Err.	95% CI	
				Lower	Upper,
Right ear	**Resp**	16.528	0.6555	15.208	17.848
	Repro	88.326	1.8992	83.688	85.399
	Stim	84.543	0.4247	62.666	65.439
	Stab	89.717	1.9871	85.715	93.720
Left ear	**Resp**	14.515	0.8659	12.771	16.259
	Repro	88.326	1.8992	77.256	89.788
	Stim	84.609	0.3723	83.859	85.359
	Stab	91.761	1.1939	89.356	94.165

Abbreviations: Resp – Response (dB SPL), Repro – Reproducibility (%), Stim – Stimulus level (dB), Stab – Stability of the response. Data from 46 normal hearing children aged 2-20 years, 20 females.

9.2.5 Medial olivocochlear suppression test

The table shown below presents the mean and std. error data for MOCS test (n=30, 15 males).

Ear	Variable	Mean	Std Err.	95% CI	
				Lower	Upper,
Right ear	**Resp (q)**	14.921	1.3873	12.007	17.836
	Repro (q)	91.105	2.3987	86.066	96.145
	Stim (q)	64.053	0.6599	62.666	65.439
	Stab (q)	89.105	3.1175	82.556	95.655
	Resp (n)	12.505	1.2738	9.8290	15.181
	Repro (n)	86.895	2.7273	81.165	92.625
	Stim (n)	64.211	0.7116	62.716	65.705
	Stab (n)	89.1053	3.1748	82.557	95.654
	Difference	***2.416***	***0.2998***	***1.786***	***3.046***
Left ear	**Resp (q)**	13.826	1.19575	11.311	16.342
	Repro (q)	91.474	1.7578	87.781	95.167
	Stim (q)	63.895	0.6393	62.552	65.238
	Stab (q)	92.053	2.4465	86.913	97.192
	Resp (n)	11.674	1.1912	9.171	14.176
	Repro (n)	85.737	3.1473	79.125	92.349
	Stim (n)	63.895	0.6160	62.600	65.189
	Stab (n)	92.053	2.4465	86.913	97.192
	Difference	***2.268***	***0.2644***	***1.713***	***2.824***

Abbreviations: (n) – measurement recorded in the presence of contralateral noise, (q) - measurement recorded in quiet (i.e. in the absence of any contralateral noise) Resp – Response (dB SPL), Repro – Reproducibility (%), Stim – Stimulus level (dB), Stab – Stability of the response.

9.2.6 Click-evoked ABR test

The table below shows the normative latency range for peak waves I, III and V and inter-peak intervals (mean ± 2 SD, n=56).

Waves (ms)	I	III	V	I-III	III-V	I-V
	1.47 ± 0.22	3.61 ± 0.29	5.39± 0.46	2.13 ± 0.23	1.78 ± 0.37	3.92 ± 0.40

The table shown below summarises age-related normative ABR latency data recorded at 75 dB. Data shown in brackets is 2 SD range. Data from Hall (2007)

	Waves (ms)				IPL (ms)
Age	**I**	**II**	**III**	**V**	**I-V**
< 3months	1.87 (1.43-2.31)	3.11 (2.39-3.83)	4.66 (4.22-5.10)	6.77 (6.23-7.31)	4.90 (4.22-5.58)
3 -4 months	1.77 (1.45-2.09)	2.87 (2.51-3.23)	4.31 (3.93-4.69)	6.31 (5.81-6.81)	4.54 (4.12-4.96)
5-7 months	1.80 (1.56-2.03)	2.84 (2.50-3.18)	4.25 (3.87-4.63)	6.10 (5.76-6.60)	4.38 (4.04-4.72)
8-11 months	1.69 (1.43-1.95)	2.77 (2.37-3.17)	4.10 (3.76-4.44)	5.98 (5.52-6.44)	4.29 (3.93-4.67)
> 1 year	1.80 (1.42-2.18)	2.79 (2.33-3.25)	3.99 (3.53-4.45)	5.79 (5.19-6.39)	3.99 (3.63-4.35)
> 2 years	1.68 (1.42-1.94)	2.69 (2.35-3.03)	3.86 (3.46-4.26)	5.68 (4.88-6.48)	3.96 (3.68-4.24)

9.3 Appendix 3: Questionnaire and Listening profiles

9.3.1 Case history questionnaire

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9.3.2 Modified listening profile

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9.4 Appendix 4: Hyperacusis questionnaire

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