Infrared Neural Stimulation Of The Cochlear Nucleus:
Towards A New Generation Of Auditory Brainstem Implants

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Abstract
Rohit Umesh Verma

Submission for Doctorate of Medicine

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Infrared Neural Stimulation: Towards a New Generation of Auditory Brainstem Implants

In an effort to improve the auditory brainstem implant, a prosthesis in which user outcomes are modest, infrared neural stimulation (INS) was applied to the cochlear nucleus in a rat animal model. Pulsed INS, delivered to the surface of the cochlear nucleus via an optical fibre, evoked auditory brainstem responses (ABR) and generated broad neural activation in the inferior Colliculus (IC). Varying the parameters of the laser stimulation revealed laser peak power to be the dominating parameter for both ABR and IC responses. Strongest responses were recorded when the fibre was placed at lateral positions on the cochlear nucleus, close to the temporal bone. After deafening by auditory nerve section, ABR and IC responses to INS disappeared, consistent with a reported "optophonic" effect, a laser-induced acoustic artifact. Thus, for deaf individuals who use the auditory brainstem implant, INS alone does not appear promising as a new approach.
Declaration

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Dedication
This thesis is dedicated to my parents, sisters, Dhiren, Sia & Anand.
To my wife Arianna, the most significant discovery I have made.

Acknowledgements
This project has had collaboration at its heart, therefore it is difficult to name all the individuals who have been so kind and supportive throughout the experience. Med-EL and Fondation Bertarelli, in addition to the Helene & Grant Wilson ABI Program at the Massachusetts Eye and Ear Infirmary, provided invaluable funding.

Thanks must commence with my chief supervisor Professor Colette McKay, for gifting me her expertise, talent, dedication and inspiration (provided at all hours of the day & night). I will seek to emulate these qualities in my own research & clinical career. My co-supervisors Mr Simon Lloyd and Professor Andrew King illuminated the clinical background for this project. Their drive and enthusiasm gave me the confidence to overcome logistical challenges I encountered along the way. In Boston, Dr Daniel Lee gave insight, effort and expertise. I am indebted to him for providing the lab space and resources for the project in addition to leading his team from the front. I am privileged to have worked with such a dynamic physician–scientist. Dr Chris Brown delivered motivation, leadership and mentorship, providing a spine to build the project around. Dr Ken Hancock and the Eaton Peabody Laboratory engineers Evan Foss and Ishmael Stefanov worked tirelessly to make all software and hardware in the lab work in the way I needed it to. I would also like to thank Professor Nirmal Kumar for challenging me to see the peak above the clouds.

My laboratory partners deserve more than just thanks. Ms Amelie Guex performed excellent work in signal processing and marshalled the project whenever focus drifted. Her appearance corresponded with an upturn in fortune for this project. Dr Nedim Durakovic was my conscience, sense of perspective and better self for 2 years. These splendid people showed me the value of cross-disciplinary research and how adverse circumstances can be the breeding ground for new opportunity. Finer friends and colleagues would be hard to find. I am indebted to my lab team for helping this thesis materialise. Thank you for giving me some wonderful memories from my time in Boston. Ymthwr.
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<td>Auditory stimulus evoked auditory brainstem response</td>
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<td>ABR</td>
<td>Auditory brainstem response</td>
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<td>AVCN</td>
<td>Anterior-ventral cochlear nucleus</td>
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<td>CI</td>
<td>Cochlear implant</td>
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Chapter 1: Introduction

The Auditory Brainstem Implant was first developed by the House Ear Institute in 1979 as a neuroprosthetic or the rehabilitation of hearing in adults with Neurofibromatosis type 2 (Hitselberger et al., 1984).

Chapter 2 describes the development of the ABI, in addition to its current use and previous strategies that have been employed to improve the device, including the penetrating ABI. One of the possible explanations of the poorer audiological performance of ABI users compared to CI users is the notion that electrical stimulation is poorly focused with spread of the current. Direct stimulation of the cochlear nucleus with electrodes can lead to non-auditory side effects necessitating some electrodes to be turned off. In addition, whilst the cochlear implant electrode is able to access the tonotopic structure of the cochlea along its array, the tonotopic arrangement within the cochlear nucleus complex is much more complex and folds over on itself. Therefore, it is unlikely that tonotopic mapping using a surface electrode would be able to access the tonotopic gradient in a meaningful fashion.

INS could be a method of stimulating the cochlear nucleus. Infrared Neural Stimulation has been shown to generate focal, spatially specific stimulation to a variety of neural tissues. A comprehensive review of the literature discusses the use of Infrared Neural Stimulation including work in cochlea by Dr Richter’s group (Izzo et al., 2007a, Izzo et al., 2007b, Teudt et al., 2007a, Teudt et al., 2007b, Littlefield et al., 2010, Richter et al., 2011). A possible confounding factor resulting from a stress-relaxation wave developing at the tip of the optical fibre when the laser is fired is described. This ‘optophonic artifact’ is potentially audible in rats and drove the design of experiments described in chapter 5 where the subjects, where acutely deafened (Teudt et al., 2011).
Chapter 3 describes the experimental set up for experiments designed to test the hypothesis that INS could be applied to the cochlear nucleus and result in activation of the central auditory pathways. The responses were to be recorded in the form of auditory brainstem responses and inferior colliculus responses. An animal model was chosen (Sprague Dawley Rat) and the surgical techniques refined to allow a stable physiological preparation to allow high quality data to be recorded. The surgical steps are described in detail. Refinement of these techniques contributed significantly to increasing the survival of the subject. Anatomical pitfalls are also described, in addition to resuscitative and airway preservation techniques.

Chapter 4 details the results in animals with normal hearing. Building on the limited preliminary work in this field (Lee, 2009), the effect of varying the laser stimulation parameters was investigated. It was identified that varying laser peak power had a significant effect on both ABRs and inferior colliculus response. In fact, the laser peak power drove the response even when other parameters were varied. Wavelength and pulse width had no effect on ABR recordings or IC recordings. Increasing the pulse rate did have an effect on P2 latency, but no other effect on the extracted ABR parameters. Only peak power had an effect on IC responses. All IC responses to INS were broad, with poor spatial tonotopic specificity. These IC responses would be consistent with a broadband click.

The surface of the cochlear nucleus was subdivided into a 4x3 virtual grid of 12 squares. The tip of the optical fiber delivering the infrared energy was moved to each of these locations and a set of experiments with fixed laser parameters was undertaken, recording ABR and inferior colliculus responses. Responses were strongest at locations closest to the temporal bone. This raised the possibility that responses to INS in hearing animals could be the result of artifact. An optophonic artifact was described after these experiments had
been commenced. This paper described a mechanism by which rapid heating of air at the
tip of the laser could result in an acoustic artifact.

In Chapter 5, the optophonic artifact for the laser was measured. Varying the laser
stimulation parameters showed that an acoustic artifact was generated as a broadband click.
The artifact was sensitive to laser peak power only, with no effect of varying pulse width
or wavelength. This suggested that an artifact was in fact driving the responses seen in
hearing animals. Experiments were then conducted to investigate the effect of acute
deafening on the responses to INS. The same experiments (parametric studies and location
studies) from chapter 4 were repeated in deafened subjects and responses recorded as ABR
or IC recordings. Acute deafening removed any response to INS. Responses recorded were
the same as background noise or the noise level when the laser was switched to peak
power of 0%. We conclude that the responses to INS in hearing animals were driven
entirely by the optophonic artifact.

Chapter 6 describes how the ABI might be improved. Understanding the cellular
generators of the ABR and the cyto-architecture of the cochlear nucleus may assist in
devising novel ABI devices. INS appears to work in other neural tissues, specifically in the
cochlea in deafened subjects and in genetically deaf cats (Matic et al., 2013). Whilst other
groups are reporting small responses to INS, or even inhibitory responses (Bec et al., 2012,
Duke et al., 2013, Cayce et al., 2014), the work of Dr Richter’s team stands out as the only
group to provide evidence that INS be used in a clinically significant fashion.

Whilst INS is unlikely to be useable as a stand-alone means of stimulating the cochlear
nucleus, it is possible that it may still have a role when used in combination with electrical
stimulation. Further work is required in this field to benefit ABI candidates and help
restore their auditory experience.
Chapter 2: Literature review

2.1 Implantable Hearing Prosthesis

2.1.1 The Auditory Brainstem Implant

Otologists, audiologists, auditory neuroscientists and engineers have developed hearing prostheses to improve the hearing capabilities of those with hearing loss. For those suffering with profound or severe sensorineural hearing loss, it is with implantable prostheses that most success has been made.

Implantable devices may bypass the hypo functional or non-functional hair cells of a severe to profoundly deafened cochlea, or bypass an absent or damaged auditory nerve, stimulating the peripheral or central auditory system respectively. The most commonly implanted auditory implant is the Cochlear Implant (CI).

With over 100000 devices placed worldwide, the CI represents the most successful of the implantable hearing prostheses and has been used successfully in both adults and children. The electrode array of the CI is inserted into the scala tympani of the cochlea and works by directly stimulating the adjacent spiral ganglion cells (first order neurons) in order to provide hearing. Since their first introduction in the 1960’s (Simmons, 1966) there has been a steady improvement in hearing outcomes as refinements were made in the technology and processing strategies use to encode speech. Cochlear implants are associated with high levels of patient satisfaction and provide meaningful sound and speech perception (Bond et al., 2009).

Aiming to build upon the success of the CI, attention focused on methods to restore the hearing of a different group of patients for whom a cochlear implantation is not an option.
The CI requires the presence of a cochlea and functional auditory nerve to conduct action potentials to the brain. However, there are circumstances when either the nerve is absent, due to surgery, pathological destruction, congenital absence or the cochlea is not suitable to accommodate a CI due to dysplasia or ossification. For these patients, vibro-tactile aids, lip reading and sign language were the only communication aids available (Brackmann et al., 1993). Therefore, attempts were made to create a device to rehabilitate the hearing of this group of patients for whom a CI was not feasible.

The Auditory Brainstem Implant (ABI) is a technological advance aimed at helping this specific cohort. The ABI bypasses the cochlea and auditory nerve to directly stimulate the cochlear nucleus, the first relay station in the brain for all ascending sound information originating in the ear. The ABI was first developed at the House Ear Institute in 1979 (Hitselberger et al., 1984) and has been implanted in over 500 patients worldwide (McCreery, 2008). The current device, approved by the FDA in 2000, can be conceptualized as a modified cochlear implant. It consists of an externally worn device that includes the microphone, speech processor, and battery pack, and a surgically implanted receiver-stimulator that delivers encoded information to the electrode array. Current ABI systems use a multichannel array with exposed platinum-iridium electrode contacts that are placed onto the surface of the auditory brainstem. Electrical current is delivered from the electrode paddle to the second order auditory neurons of the cochlear nucleus. This stimulation generates activity on the central auditory pathway and can manifest as perceived sound by the user. It is possible to record activity in the auditory brainstem by means of the Auditory Brainstem Responses (ABR). Clinicians and scientists commonly use this recording technique, analogous to an electroencephalogram, to assess the auditory system and it plays a significant role in hearing screening, especially for younger infants unable to participate in behaviour-based hearing tests (Ptok, 2011). It is important to note,
that whilst the ABR can identify activity in the brainstem, the quality and perception of hearing is not necessarily reflected in the ABR waveforms (Werner et al., 1994).

2.1.2 Who Uses the ABI?

Many of the patients who are candidate for an ABI have been diagnosed with Neurofibromatosis Type 2 (NF2). This autosomal-dominant inherited tumour predisposition syndrome, results from mutations on chromosome 22. Affected individuals develop vestibular schwannomas (VS) characteristically affecting both sides, leading to hearing loss and eventual deafness (Evans et al., 2011). VS can disrupt the cochlear nerve, which shares the internal auditory meatus with its sibling the vestibular nerve, both directly and indirectly.

2.1.3 Management of VS

Growth of the VS can threaten the brainstem itself, with significant gross neurological impairment or even death if compression of this structure is allowed to continue unabated. In those patients where the rate of growth of the VS is increasing, the tumor is becoming symptomatic or threatens nearby structures, excision or size reduction is indicated. Some centers favour the use of stereotactic radio-surgery, a form of radiotherapy, especially for smaller tumors (Sarmiento et al., 2013). However, for some of the larger tumors, a surgical approach is often indicated.

The surgical management of VS typically involves accessing the cerebello-pontine angle in one of three ways; via a trans-labyrinthine approach, via a retro-sigmoid approach, or through a middle-fossa craniotomy. One of the cardinal risks to the patient is the loss of hearing on the operated side. This risk is the result of the close relationship between the vestibular nerve and cochlear nerve as they pass through the internal auditory canal. In some cases the nerve has already been compromised by compression rendering it non functional. In other cases, despite the presence of residual hearing on that side, the cochlear
nerve is so intimately associated with the vestibular schwannoma that it is impossible to undertake total resection of the tumor without sacrificing the cochlear nerve.

In a small number of cases surgical removal of the VS does not necessitate sacrifice of the cochlear nerve. In some of these patients, it has been possible to fit cochlear implants to rehabilitate hearing on the operated side (Piccirillo et al., 2008, Lustig et al., 2006, Vincenti et al., 2008). These patients usually have small, slow growing tumors, which optimises the probability of cochlear nerve preservation.

The majority of patients with a VS requiring surgical removal do not have tumors amenable to cochlear nerve preservation, and removal of the VS will lead to sectioning of the cochlear nerve, regardless of whether there was any functional hearing capability on that side. Patients are often faced with a tragic decision; to suffer total deafness on one side or premature death.

For the non-NF2 patient with a sporadic, unilateral VS, hearing in the contra-lateral side should be unaffected by the surgery and a hearing rehabilitation can focus on protecting the hearing in the ‘healthy’ ear.

The case for the NF2 patient is less optimistic as bilateral VS are pathognomic features of the disease (Evans, 2009). As a consequence, the patient with NF2 is likely to eventually lose all auditory sensation, either as a direct result of the disease process itself, or through the surgical resection of the tumors.

For these patients, whose hearing loss from the disease is almost guaranteed, surgery may save their lives, but renders the world silent to them. The ABI has provided these patients with an opportunity to gain some auditory sensation and for some users, is associated with high satisfaction, regardless of the functional outcome (Lenarz et al., 2002). However, there is no international consensus as to which patients should receive these devices.
2.1.4 Indications for ABI insertion

Currently, the United States Food & Drug Administration (FDA) regards the only clinical indication for an ABI to be fitted is in a patient over 12 years of age suffering with NF2. However, there is an FDA approved trial is progress in the US for non-NF2 candidates, and a second trial involving ABI in paediatric patients.

In Europe, broader cohorts of patients have been able to benefit from the ABI. These include children under 12 and those with cochlear nerve abnormalities, or cochlea abnormalities (Colletti, 2007, Colletti, 2006, Colletti et al., 2005). A consensus statement issued in 2011 by the leading ABI centers across Europe further defined appropriate criteria for ABI candidacy and included children less than 3 years old (Sennaroglu et al., 2011).

The willingness of some centres to adopt new indications for ABI candidates, especially non-NF2 patients, reflects the differences in outcomes between NF2 and non NF2 ABI users.

2.2 Functional Anatomy of the Human Cochlear Nucleus

Before considering the ABI device itself, let us focus upon the intended therapeutic target, the Cochlear Nucleus, which is the gateway to the central auditory pathway. This collection of neurons is located on the dorsal-lateral aspect of the brainstem and spans the junction of the pons and medulla.

2.2.1 The Auditory Pathway

The Cochlear Nucleus (CN) receives inputs from the cochlear branch of the vestibulocochlear (VIIth) cranial nerve, which stems from the spiral ganglion cells of the cochlea, and has outputs to the higher auditory centres. The structure itself can be sub-
divided anatomically into the dorsal cochlear nucleus (DCN) and the ventral cochlear nucleus (VCN). The VCN can also be subdivided into the antero-ventral (AVCN) and postero-ventral (PVCN) portions. Projections from the CN head further upstream along the auditory pathway. Fibres head to both the ipsilateral and contralateral inferior colliculi (IC) via the lateral leminisci. The path from the leminisci to the IC is monosynaptic. Whilst the VCN projects only to the IC, the DCN receives efferent innervations from higher auditory relay centres including the auditory cortex, inferior colliculus and superior olivary complex. These retrograde innervations play roles in middle ear function such as protective reflexes that help preserve hearing when exposed to potentially damaging noise levels (Mukerji et al., 2010). From the IC, the auditory pathway continues to the medial geniculate body and then onto the auditory cortex.

2.2.2 Macro-Anatomy of the Cochlear Nucleus

The macro-anatomy of the cochlear nucleus is well described in humans. Abe et al (Abe and Rhoton, 2006) have discussed the relation of the human cochlear nucleus to its surrounding structures after preparing formalin fixed brainstems and analysing the microstructures. The DCN lies on the floor of the lateral recess of the fourth ventricle on the posterior surface of the inferior cerebellar peduncle. The ventral cochlear nucleus is positioned on the lateral surface of the inferior cerebellar peduncle where it straddles the anterior edge of the foramen of Luschka at the junction of the lateral recess and cerebello-pontine angle. The VCN produces less of a noticeable prominence on the surface of the brainstem than on the dorsal nucleus. The VCN may be better accessed through a retro-sigmoid approach.

Whilst some controversy remains as to the optimal target for the ABI and how best to access the cochlear nuclei, the Abe et al (2006) paper has 2 key messages. Firstly, the Cochlear nuclei, especially the Ventral aspects would easily be susceptible to damage from surgery approaching, or tumors in, the cerebello-pontine angle.
Secondly, the proximity of the cochlear nuclei to other key neuro-anatomical structures is highlighted. The glossopharyngeal nerve, facial nerve, accessory nerves, inferior cerebellar peduncle and flocculus are all within a short distance of the cochlear nuclei. Therefore sub-optimal placement of the ABI electrode array, due to poor technique or technical difficulties arising from the disease process, could conceivably lead to stimulation of these adjacent structures through current spread. These anatomical findings are consistent with report of non-auditory side effects (Shannon et al., 1997a) reported by ABI users (Otto et al., 1998, Brackmann et al., 1993). The CN offers a unique set of challenges as a therapeutic target for an auditory implant. Comparisons of its anatomy with the cochlea could perhaps partially explain the difference in hearing outcomes achieved by users of ABI compared to the CI.

2.2.3 Comparing the Architecture of the CN and Cochlea; Implications for Electrode Array Design

The functional architecture of the cochlear nucleus does not possess an intuitively accessible tonotopic structural organization when compared to the cochlea. High frequency sounds maximally stimulate the base of the cochlea, whilst low frequency sounds cause maximal activation of the hair cells at the cochlea’s apex. It is therefore possible to effectively predict what sort of pitch will be perceived by directly stimulating points at different lengths along the cochlea. It is upon this functional anatomy that the multi-channel cochlear implant has based its success, by inserting an electrode array into the scala tympani via the round window. The electrode array lies adjacent to the basilar membrane and functions by directly stimulating the spiral ganglia cells through a series of electrodes positioned along the length of the electrode array. Although it is not currently possible for an electrode array to traverse the entire length of the cochlea and thus stimulate across the entire hearing spectrum, functional outcomes are almost universally positive (Lassaletta et al., 2006, Klop et al., 2008, Bond et al., 2009).
The anatomy of the cochlea provided an ideal platform for development of prosthesis. The cochlea is easily accessible with minimal morbidity caused by surgery: damage to the cochlear is usually the result of cellular degeneration rather than any physical disruption to the organ and there are few non-auditory structures in the vicinity of the implantation sites that could be subject to stimulation due to current spread. These structural features, which seemingly lend the cochlea to being an ideal candidate for the development of an implantable auditory prosthesis, contrast sharply with the anatomy and functional architecture faced by those developing the ABI.

2.2.4 Cochlear Nucleus Micro-Anatomy

2.2.4.1 Tonotopic Organisation

Characterization of the tonotopic organization of the auditory system is summarized by Clopton et al (Clopton et al., 1974). The tonotopic organization within the AVCN and PVCN exists in a low to high pitch association from dorsal to ventral. In the DCN, the arrangement is slightly altered, with progression from high to low being along a dorsal-medial to ventero-lateral axis. Lesion studies of the cochlea and auditory nerve appear to identify the presence of iso-frequency laminae within the CN.

An appreciation of the nature of tonotopic arrangement within the CN is essential in understanding the difficulties this presents to neuroscientists, surgeons, audiologists and engineers in developing an ABI that works effectively to enhance the user’s listening experience. The close proximity of surrounding structures coupled with the inaccessibility of the iso-frequency laminae deep within the structure combine to hamper efforts at hearing rehabilitation in this cohort. In addition, the CN may also play a significant role in
neural signal processing, reflected in the arrangement of different cell types with specialized function that exist within the CN.

2.2.4.2 Cochlear Nucleus Micro-Anatomy: Role in Signal Processing

The anatomy of the cochlear nucleus also reveals its role in sound processing. Much of the basic temporal and spectral components of the sound are identified and processed through parallel streams within the CN. Functionally and morphologically distinct nerve populations undertake processing. McCreery D.B. (2008) provided a description of the differing cell types that exist within the mammalian cochlear nucleus. The branching pattern of the cochlear nerve maintains inputs to these parallel processing pathways within the CN. However, in pathological situations when a cochlear nerve is either absent or has severely compromised function, the inputs to these pathways are compromised and their processing properties may be disabled. An ABI, functioning by stimulation of the surface of the CN, may be unable to access or appropriately activate these processing channels. This may lead to a poor quality, unprocessed signal being sent higher up the auditory pathway, resulting in some of the perceptual hearing problems identified by psychoacoustic testing.

The complexity of sound processing increases from the rostral pole of the AVCN, through to the caudal pole of the PVCN and eventually through into the DCN. The VCN contains spherical cells rostrally, a central region containing a mixture of multipolar cells, globular bushy cells and spherical cells. The caudal region contains octopus cells.

These differing cell types have differing outputs, passing to either the superior olivary nuclei, or lateral leminisci, and differing functions in signal processing.
The Type 1 multipolar cells are narrowly tuned to specific frequencies of tone bursts, responding best to acoustic signals that are amplitude modulated. This behaviour suggests that they are specialized to extract envelope information from an acoustic signal. It is worth noting that in comparisons of NF2 and non-NF2 users, modulation detection thresholds (MDT) of the NF2 users were worse than those of non-NF2 subjects. Although the relatively poor performance of the NF2 patients cannot be entirely attributed to their worse MDT, it is worth noting that this is a possible psychoacoustic discriminator between non-NF2 and NF2 patients (Colletti and Shannon, 2005a), though the r-value of 0.59 suggests extreme caution must be used with this conclusion.

Multipolar cells’ sensitivity to amplitude modulation is possibly enhanced by numerous inhibitory inputs from GABA-ergic and glycine-ergic neurons in the VCN. Almost all of these inhibitory neurons are located in the small cell cap, a micro-structure that is especially prominent in humans (Moore et al., 1996). Type 1 multipolar cells have been strongly implicated in speech perception specifically by a lesion study (Egan et al., 1996). In that case report, the patient developed profound bilateral deafness after a pontine haemorrhage, disrupting the ventral acoustic stria pathway that carries outputs to the contralateral IC, but conserving the ipsilateral projections from the DCN. Only type 1 multipolar cells project directly to the IC whilst the large bushy cells and large globular cells that share the same output also have substantial ipsilateral projections. The patient was able to perceive pure tones, but had suffered a total loss of speech perception. This case report may help to identify an anatomical basis for the MDT differences identified by Colletti et al (2005). It is possible that Type 1 multipolar cells are particularly affected by the NF2 pathology, or that the loss of innervation to these cells caused by tumour growth or removal of the cochlear nerve may lead to a loss of sound envelope information and subsequent difficulties in auditory perception experienced by most users of the ABI.
The DCN contains many types of neurons, but only 2 types of output neuron, the more numerous fusiform cells and giant cells. As previously discussed, the primary target of the fusiform cells is the contralateral IC, where their tonotopic organization overlaps with the multipolar cells of the ventral nucleus. The DCN is a relatively large structure in humans, yet it has a relatively smaller output pathway when compared to the VCN. This difference may suggest that the human DCN has the primary function of intrinsic signal processing.

In summary, an examination of the functional anatomy of the cochlear nucleus reveals an architecturally complex structure with a tonotopic arrangement that is not fully accessible from the surface. In addition, it is clear that signal processing that occurs in the CN is unlikely to be facilitated if only selected parts of the CN surface are activated through the application of exogenous stimulation. The multiple parallel processing pathways are fed by the cochlear nerve and any pathological disruption to this essential structure is likely to alter the performance of the CN as both a relay centre and initial processing hub.

In spite of the challenges, the desire to offer patients without a functional vestibulocochlear nerve a rehabilitation device has fuelled efforts to create a viable device. The ABI is the culmination of these efforts.

**2.3 The Basis of Electrical Stimulation**

In order to function as a stimulation device, the ABI must be able to induce action potentials in a functionally relevant population of neurons. To this end, the ABI stimulates the 2nd order neurons of the auditory pathway, using an array of electrodes in contact with the surface of the cochlear nucleus.

Testing of the perceptual thresholds for these surface electrodes reveals a large degree of variability across the patient populations. Colletti and Shannon (2005) reported that the
average thresholds for activation were higher in NF2 patients than in the non-NF2 users. In addition, many of the NF2 patients were unable to pitch-rank their electrodes, although there did not appear to be significant correlation between the ability to pitch-rank and performance in speech perception tests.

2.3.1 Development of the ABI

Despite the obvious difficulty in trying to provide meaningful sound perception by stimulating the surface of the cochlear nucleus, attempts at developing prosthesis to stimulate the central nervous system have a long history. In 1982, the House Institute published their findings of generating hearing by stimulating the CN with electricity (Edgerton et al., 1982). This initial work was developed into a rudimentary device consisting of a single bipolar electrode made of platinum contact plates, attached to a 3M/House cochlear implant processor. Initial results appeared to be encouraging with the subject reporting improved sound recognition without visual cues. The group reported that these results were ‘comparable to Cochlear Implant users’ which demonstrates perhaps not the efficacy of the ABI, but highlights the improvement in CI users’ outcomes since 1985 (Bond et al., 2010).

2.3.2 Current ABI Devices.

The present range of ABI devices that are commercially available share a similar structure. They typically consist of an implanted electrode array that is in contact with the CN and receiver/stimulator that is surgically placed behind the ear within the temporal bone. An ear-level microphone converts sound into electrical code via a body worn external speech processor. The information from the processor is passed across the skin via electromagnetic induction.

The Nucleus 24 ABI is the only ABI device licensed by the FDA. The device has the standard array of components as described above and uses the Spectral Peak (SPEAK)
strategy for speech processing, although it can also use the Advanced Combination Encoder (ACE) strategy. The electrode array consists of 21 platinum disk electrodes mounted onto a silicone and Polyethylene terephthalate mesh measuring 3x 8.5mm. Each of the electrodes measures 0.7mm in diameter. In addition, there are 2 return or reference electrodes: a plate electrode on the lateral surface of the receiver/stimulator and a ball electrode that is commonly placed beneath the temporalis muscle. These ground electrodes allow different stimulation modes to be employed (e.g. monopolar, bipolar or common ground).

The MED-EL ABI has similar components to the Nucleus system. The electrode array in this device consists of 12 contact electrodes embedded in a soft silicone matrix measuring 5.5 mm x 3.0 mm. A major advantage of this device is the presence of an advanced surgical placement system that allows the surgeon to assess a potential implant site on the CN prior to implanting the device.

### 2.3.4 The Penetrating Auditory Brainstem Implant

Criticisms of the current generation of ABI devices include their inability to take full advantage of the tonotopic arrangement of the CN. Attempts to overcome this deficit have included the development of a penetrating auditory brainstem implant (PABI). This device was developed with several electrodes piercing the surface of the CN in order to access populations of neurons that were previously beyond the influence of surface stimulation. Unfortunately, clinical trials of this device did not yield the improved outcomes that were hoped for and in fact led to some significant side effects, causing the development of this device to be halted (Otto et al., 2008).

Although the PABI did not become a commercially available device, it did start to focus attention on alternative ways of stimulating the auditory system, and its development by
the same team that developed the original ABI device was tacit acknowledgement that the then-current technology was not without inherent problems. Significant changes in strategy would be needed in order to optimize stimulation of the central auditory system to meet the functional needs of patients.

### 2.3.5 The Auditory Midbrain Implant

The Auditory Midbrain implant (AMI) is a further attempt at stimulating the central auditory pathways. Taking advantage of the tonotopic gradient contained within the central nucleus of the inferior colliculus (ICCN), the AMI consists of a single-shank multisite array designed according to the dimensions of the human IC with the goal of stimulating the different iso-frequency laminae contained within the ICCN (Lim et al., 2009). The inferior colliculus is unlikely to be directly involved in NF2 disease, nor is it likely to suffer iatrogenic damage during surgical removal of vestibular schwannoma. However, by stimulating the inferior colliculus directly, there may still be ongoing perceptual difficulties, similar to those reported by ABI users, as the processing function of the CN would be entirely bypassed by an AMI device.

Trials of the AMI are ongoing, but this device represents a novel attempt to overcome some of the inherent problems of stimulating the CN using conventional electronic means.

### 2.4 Outcomes For ABI Users

Outcomes for the users of Auditory Brainstem Implants vary widely. The general consensus is that in NF2 patients hearing outcomes do not match those achieved by CI users (Schwartz et al., 2008). Furthermore, the outcomes for individuals operated on by the same group also appear to be highly variable.
2.4.1 Complications from ABI Insertion Surgery: Colletti’s cohort

Colletti et al (2010) described the complications they had experienced in their patient group, the largest series published to date. This analysis of 114 ABI's inserted into 83 adults and 31 children gives an insight into some of the short term, intermediate and long-term effects of this type of surgery. Within this cohort, 36 patients were NF2, whilst 78 were non-tumour candidates (Colletti et al., 2010). The major specific complications included lower cranial nerve lesions and facial nerve palsy. Minor complications included balance disorders, headaches and non-auditory side effects. This final group incorporated experiences of facial twitching, ipsilateral body tingle, dizziness and headaches.

Analysis of the complication rates revealed differences in the side-effect profiles between Non-NF2 patients and NF2 patients. Major complications were experienced by the non-NF2 group in approximately 6% of cases, with almost identical rates in both children and adults. In NF2 patients, the rate of major complications was recorded at 33%. In terms of minor complications, these were observed as either intra-operative or immediately postoperative. These features were generally transient in nature, but were present in 17% of non-NF2 patients compared to 58% of NF2 patients.

The difference in complication rates does suggest that surgery is more complex in the NF2 individuals. The major complications for NF2 patients included 3 deaths: however they were not the direct result of the surgery itself, although they may reflect the systemic upset caused by the disease and resultant loss of physiological reserve.

The non-auditory side effect rate also differed significantly between NF2 and non-NF2 patients. One or more non-auditory side effects were reported in 32% of non-NF2 adults and 23% of children. In comparison, 73% of NF2 patients reported such side effects. In the
case of children, the complications were also inferred from data gained from EABRs that had waves present after 4.5ms, indicating a myogenic response.

The Colletti series provided comparison of ABI insertion with other procedures that access the cerebello-pontine angle. A meta analysis performed by this group suggested that placement of an ABI in a non NF2 patient confers no greater risk of major or minor complications when compared to removal of a VS alone.

Therefore they suggested that the rate of complications in ABI placement in the NF2 patient is higher as a direct consequence of the disease process. ABI surgery in the non-NF2 patient carries similar rates of both major and minor complications as micro-vascular decompression procedures, performed using similar techniques. When a comparison was made between the rates of complications experienced between cochlear implant insertion procedures and ABI placement, then there was no significant difference between the rates of complications. The authors acknowledged that the nature of potential complications involved in an ABI placement via retro-sigmoid craniotomy are generally agreed to be more serious than those associated with CI placement.

### 2.4.2 Functional Outcomes After ABI Insertion: Colletti & Shannon (2010)

In terms of hearing outcomes, Colletti et al (2010) limited their description of hearing outcomes to the number of electrodes producing auditory sensation. The devices were activated at a mean of 32 days after insertion. Of the 21 available electrodes, a mean of 11.4 electrodes were available for use, providing some auditory sensation. Once electrodes producing mixed responses (auditory and non-auditory) were also deactivated, this left an average of 9.6 electrodes that were left active and available to use to stimulate the central auditory pathway.
A subset of the same cohort of patients previously had their auditory outcomes evaluated in 2006, when the total number of patients was 80, with 26 NF2 patients and 54 non-NF2 patients (Colletti, 2006). When initial behavioural testing was done at 1 year after insertion, open set speech recognition varied between 12-100%, with an average of 59%. The study then focused specifically on 39 consecutive adult patients operated on over a 5-year period (April 1999–April 2005). This cohort was subjected to a battery of psychoacoustic tests undertaken by Dr Bob Shannon from the House Ear group. Not all patients received the same ABI device, nor used the same sound processing strategies, which makes it more difficult to determine why certain patients had better outcomes than others. Devices used include the Nucleus 21 Cochlear ABI (Cochlear Ltd., Sydney, Australia), Nucleus 24 Cochlear ABI and Pulsar ABI (Med-El Innsbruck, Austria). In keeping with the complications reported in 2010, 92% reported non-auditory side effects, with 82% of those complaints being related to transient dizziness. Other reported side effects include throat sensations or limb symptoms secondary to activation of the spino-thalamic tract.

In order to assess the neurophysiologic properties of the implant’s electrodes upon activation, the threshold and maximal comfort levels for each functional electrode were assessed to ensure that the patient’s map was in an optimized configuration for testing. Tests included both open and closed set recognition of words and sentences. In general, devices were programmed with the SPEAK encoding strategy for the first 6 months of the study and then programmed with the ACE strategy after that time if the subject appeared to have reached a plateau in auditory performance. At 1-year post activation the patients underwent a series of behavioural tests to measure their auditory outcomes. These tests were

- Recognition of environmental sound or sound detection tests
- Closed-set vowel confusion test
• Closed-set consonant confusion tests
• Closed-Set word lip reading test (with and without sound mode)
• Open-set sentence lip reading tests (with and without sound)
• Speech tracking tests

This battery of tests was based upon an a standard set of tests, though they were translated into Italian in this circumstance.

In closed set word recognition testing, Non NF2 patients had results ranging from 40-100% with a mean of 84% and median of 80%. In the NF2 cohort, closed set word recognition results ranged from 5-41% with a mean of 25% and median result of 24%.

Shannon also investigated differences in the threshold to electrical stimulation in the NF2 and Non NF2 group. There was no significant difference in the average thresholds (12.6nC in Non NF2 and 8.6nC in NF2). This specific finding contradicts the findings from Coletti & Shannon’s 2005 paper (Colletti and Shannon, 2005b). Electrode interaction and by inference, spatial selectivity of stimulation was compared between the 2 cohorts. This was measured by measuring the electrode separation at which the interference (masking) decreased by 1dB from the maximum. Electrode selectivity did not significantly correlate with vowel or speech recognition. In fact some patients with excellent speech comprehension had poor selectivity and vice versa.

This study identified that there was a significant difference in the auditory outcomes between NF2 and non-NF2 patients. The best outcomes by far, demonstrating ‘excellent open-set speech recognition’, were in patients who had injury to the cochlear nerve for reasons other than a VS or VS removal. The paper highlighted a female patient who received an ABI on the opposite side to that of a failed CI. A month after activation she demonstrated sentence and word recognition on speech tracking at 29 words/min. At one-
year post activation, her rate had increased to 47 words/min. This patient routinely uses the telephone for normal conversation.

The authors hypothesised that a factor underlying the differences in average outcome between NF2 patients and non-NF2 patients is that tumor growth and removal may induce damage to a portion of the CN that is critical for speech recognition.

2.4.3 Results from Other Groups: Hanover & Verona

The Hanover group reported on 14 NF2 patients in 2001, with results described for 13 NF2 patients (Lenarz et al., 2001). These patients were implanted with either the Nucleus M22 ABI or the Clarion 1.2 device (Advanced Bionics Corp., Sylmar, CA, U.S.A). The results reported by the Hanover group were consistent with those previously described by the Verona group, with the non-auditory side effects mainly involving dizziness, and some reports of limb tingling or throat discomfort. These effects were generally induced at supra-threshold levels of stimulation. This cohort demonstrated auditory outcomes consistent with the results of the Verona group. There was some evidence of a deterioration of auditory outcome between 12 and 18 months in some patients. However there was acknowledgement that patients with an ABI may not receive full benefit from their devices for up to 2 years after implantation. The Hanover Group also discussed the influence of device type on auditory outcome with a suggestion that the Nucleus 24 device had an advantage over the Clarion device as it allowed multiple processing strategies to be used, with SPEAK being the preferred technique.

The results from the Hanover and Verona groups are consistent with findings from other groups who have reported from smaller cohorts. The consensus is that outcomes and non-auditory side effects are worse with NF2 patients as compared to non-NF2 patients (Behr
et al., 2007, Nevison et al., 2002, Otto et al., 2002). In the study by Behr et al, the group utilized a different type of ABI based upon a Med-el device with 12 channels, but using the Continuous Interleaved Sampling (CIS) high rate strategy. Using this device, one patient (Patient 12) had open set sentence recognition results of 91% and like Colletti’s patient T.L., had sufficient hearing to use a telephone for basic communication.

Otto et al (2002) discussed the relationship between the positioning of electrodes and their likelihood to cause non-auditory side affects. Of the 440 electrodes (in a total of 61 implants) 26% could not be used because of the non-auditory stimulation they caused. Analysis of the location of these electrodes identified these electrodes to most commonly be those located at the medial border of the CN.

What is evident from the above studies is that there is no clear difference in outcomes derived from varying the device, the surgical methods (trans-labyrinthine or retro-sigmoid) or the processing strategy. All groups reported similar broad conclusions.

The ABI is able to deliver a reasonable degree of hearing rehabilitation to non-NF2 users. However, the original target population, the NF2 cohort, has yet to receive the same average degree of benefit. Despite this limited improvement in auditory perception, ABI users with NF2 do report an improvement in their quality of life.

2.4.4 Differences In Outcomes Between NF2 and Non-NF2 Patients

The results from the Hanover and Verona group showing the ABI’s positive influence on hearing rehabilitation and consequent improvement in quality of life is supported by the Colletti series of patients, tested by Dr Shannon. However it is worth noting that that just one surgeon operated all the Colletti cohorts of patients. Whilst this condition is useful in ensuring a consistency of operating technique between patients, it is also possible that the
techniques employed by a single surgeon may not necessarily be optimal for NF2 patients. In reviewing the evidence from this data set, a caveat must be that it is impossible to rule out sub-optimal operative technique as a potential cause for the outcome differences.

Academic discussion seems to suggest that some groups are experiencing outcomes with NF2 patients that exceed the standards set by Behr et al (2007) and Colletti et al (2006). A more recent paper by Colletti et al suggests that the NF2 patients can achieve audiological outcomes that are equivalent to non-NF2 ABI users (Colletti et al., 2012). This latter paper details conclusions drawn by a cohort of ABI surgeons held in Munich in March 2012. The discussion focused on causes for variability between ABI candidates, with a consensus that surgical factors were the most likely cause of differing outcome. Specifically, this paper mentions the potential excito-toxic effects of bipolar diathermy for electro-cautery and mechanical or vascular damage caused to the brainstem during tumour removal. As further groups publish their outcomes, a re-evaluation of the outcomes in the non-NF2 and NF2 patients may be necessary.

2.5 Channel Interaction and Current Spread in the ABI

2.5.1 Channel Interaction

The concept of channel interaction is well recognized in cochlear implant studies. Channel interaction can occur when two or more electrodes fire simultaneously to produce direct current field summation. This resultant summation can cause focal depolarisation and subsequent neural activation in a region that may not have received direct stimulation from either electrode when activated on its own. This process will usually distort the response to stimulation and in a device such as the cochlear implant, it may lead to an alteration of sound perception. Alternatively, if a population of neurons exists in a location that is under the influence of 2 electrodes that are firing asynchronously, then that population of neurons
may be suppressed, over-stimulated or may even accommodate. This will lead to altered patterns of neural response that could affect sound perception.

If adjacent electrodes can be activated simultaneously, then a focused third point of stimulation can be created that is between the focal point of each electrode on its own. This 3rd point is known as a ‘virtual channel’. Current Steering is the process of delivering simultaneous stimulation to adjacent electrodes. By altering the proportion of current delivered to each of the pair of electrodes, the point of maximal stimulation between these electrodes can be changed or ‘steered’ thus altering the precise point of the ‘virtual channel’. In the cochlear implant, this phenomenon can be used to create ‘virtual channels’ (Choi and Hsu, 2009). The tonotopic arrangement of the spiral ganglia allows the creation of these virtual channels to generate an in-between pitch. When the 2 adjacent electrodes activate independently, they should only generate 2 distinct points of stimulation and hence 2 points of pitch should be perceived. The creation of the 3rd virtual channel, could in theory increase the number of distinct pitches that can be perceived, which in turn may improve the functional effectiveness of the CI (Wilson and Dorman, 2008). It is important to note however, that the virtual channel is not always perceived (Firszt et al., 2007). In addition, the normal sequential stimulation in CI’s will by definition generate virtual channels, as there are areas of stimulation overlap generated by the linear electrode array.

The principles of current steering and virtual channels could be a technique used in ABI to increase the number of distinct regions of the CN that an electrode array can stimulate (Bonham and Litvak, 2008).

Current spread reduces the spatial selectivity of electrical stimulation. Studies of channel interaction and speech perception have demonstrated that performance is compromised by electrical-field interactions (Stickney et al., 2006). In Stickney et al’s (2006) study,
interactions were evident at distances of less than 2mm. The designers of the ABI are faced with a dilemma regarding the number of electrodes to provide in an ABI. Increasing the number of electrodes may increase the chances of accessing the tonotopic architecture of the CN and result in a greater number of available channels. However, the size of the CN and close proximity of electrodes is likely to generate significant electrode interaction and subsequent smearing of perception as several populations of cell types within the CN may be simultaneously excited. Given the intrinsic processing that occurs in the CN, stimulating differing cell types in an uncoordinated fashion could degrade the quality of the signal being sent further up the auditory pathway.

In order to increase the spatial selectivity of electrical stimulation, different stimulation strategies have been employed. Electrodes can be set to function in a monopolar setting, which will generate the least spatial selectivity as the ground electrode can be located some distance from the stimulating electrode. In a bipolar configuration, electrodes are paired, with one acting as the anode and one as the cathode. In theory, current passes only between these 2 points, providing a leaner spatial profile and hence greater spatial selectivity (Shivdasani et al., 2008). The Nucleus 24M ABI device employs this strategy. Tripolar stimulation uses a configuration where multiple ground electrodes surround the stimulating electrode, which further sharpens the field potential leading to maximal spatial selectivity of stimulation. Multipolar techniques, where the entire electrode array is activated to limit current spread is another means of focusing electrical stimulation (van den Honert and Stypulkowski, 1987).

Kutcha et al (2004) investigated the relation between the number of functional electrodes and audiological outcome for ABI users, all of who were implanted with a Nucleus eight-electrode multichannel ABI (Cochlear Corp.) Of the cohort of 61 NF2 users, 6 had no functional electrodes. The perceptual tests included recognition of environmental sounds,
vowel and consonant recognition in addition to word and sentence recognition. In the tests that required spectral cues, at least 3 functional electrodes were required to provide satisfactory speech recognition. However when 6 or more electrodes were functional, performance became asymptotic. The authors suggested that at electrode numbers greater than this, there was no benefit conferred as the access to the tonotopic axis of the CN became the limiting factor (Kuchta et al., 2004). The conclusion drawn by this study was that the focus for development should be on optimizing placement of the electrodes. This recommendation is in contrast to the development of ABI devices with increasing numbers of electrodes.

2.5.2 Current Spread & Non-auditory Stimulation

Current spread may also play a role in the non-auditory side effects experience by some users. The anatomical location of the Cochlear Nucleus places it in close proximity to the cerebellar tonsils, which it straddles, in addition to other brainstem structures. Whilst placement of the ABI is performed under a surgical microscope, it is possible that some of the electrodes may not be optimally placed to provide auditory sensation and may in fact cause activation of non-auditory structures.

Common side effects include dizziness, throat and tongue sensations and limb pains. These symptoms could be the result of stimulation of the cerebellum, the spino-thalamic tract and lower cranial nerves, in particular the glossopharyngeal nerve. These side effects can be removed or lessened when the user has their map created. However, if the extra-auditory side effects cannot be mitigated, then the offending electrode may have to be switched off.

2.6 Improving the ABI: Summary

The ABI has had some success as a tool for hearing rehabilitation for patients with retro-cochlear pathology or cochlear pathology that renders cochlea implantation impossible.
However, hearing outcomes remain behind those achieved with Cochlear Implants. Though attempts have been made to improve outcomes using modified processing strategies, altering electrode array design and exploring ways of accessing the deeper architecture of the CN, a game-changing development remains elusive.

The complex anatomy of the CN may necessitate a paradigm shift in efforts to stimulate the structure in a way that gives meaningful hearing to a greater proportion of users. The CN has multiple cell types and contains numerous intrinsic processing pathways. In addition, the tonotopic arrangement within the CN is not fully accessible from the surface. The present electric methods of stimulation are therefore limited in their efficacy when faced with this arrangement. In order to stimulate the deeper sub-structures and access the processing pathways, surface electrodes will have to stimulate all the tissue between the point of contact and the desired target. This is likely to lead to a highly disordered signal being sent up the auditory pathway as large populations of neurons are stimulated. This strategy severely compromises the spatial specificity of the stimulation. If one considers just surface stimulation, the effect of current spread makes it difficult to focally activate discrete points of the CN. Therefore, the access to the tonotopically arranged tissue is inefficient, as electrical stimulation makes it difficult to generate multiple discrete points of stimulation.

A means of providing more focal and spatially selective stimulation could potentially allow a more effective ABI to be built. Infrared Neural Stimulation may provide an answer to some of these problems and constitute the innovation this area of neuroprosthetics requires.
2.7 Infrared Neural Stimulation

2.7.1 Non-Electrical Neural Stimulation

Electrical stimulation has been the means of choice for activating neural tissue for study since the 1800s. However, inherent problems with electrical stimulation are well documented and include the requirement of contact between electrode and subject material increasing the risk of damage to the structure being stimulated, the poor spatial specificity and the population response generated by recruitment of large numbers of axons (Harper, 2004, Wells et al., 2005b, Wininger et al., 2009).

In order to overcome these inherent problems, multiple modalities have been trialed for stimulating neural tissue. Activity can be elicited through the use of mechanical, magnetic and chemical methods (Wagner et al., 2004). The concept of using a pulse of energy from the electromagnetic spectrum is not novel. Initial studies demonstrated that central nervous fibers in rats could be stimulated by pulsed exposure to light in the Ultraviolet spectrum (Allegre et al., 1994). However the practical application of this technology was limited as the excitation threshold was very close to the photo-ablation threshold.

Further studies looked at the possibility of applying caged compounds that were released by photons. This is a viable method for selectively delivering chemicals capable of stimulating electrical activity to a cell. However, this method requires the caged compounds to be infused into the subject, making it less applicable for human use (Mayer and Heckel, 2006).

2.7.2 Infrared Neural Stimulation- Preliminary Experiments

In 2005 Wells et al published the first demonstration of pulsed infrared light being used to stimulate neural tissue (Wells et al., 2005b). Using a rat sciatic nerve model, they were
able to demonstrate compound nerve action potentials (CNAP) and compound muscle action potentials (CMAP) using a non-contact technique involving optical fibers delivering pulsed infrared light. Electrical stimulation was then applied to the same animals and the responses recorded. This short paper reported several key findings. Direct comparison of the CNAP and CMAP demonstrated that the wave pattern and timing of the electrophysiological responses were almost identical, suggesting that the action potential magnitude was identical for conditions when either INS or electrical stimulation was used. The authors also noted that whilst a stimulation artifact was evident with electrical stimulation, there was no evidence of a stimulation artifact in the optically stimulated preparation. Spatial selectivity of stimulation was crudely demonstrated by stimulating proximally along the sciatic nerve up to nerve branches, from which isolated muscle groups were recruited instead of whole muscle groups. Assessment of the damage caused to tissues caused by this infrared stimulus was assessed histologically 3-5 days after the experiment. Staining with Haematoxylin and Eosin did not show any thermal damage when the tissue was subjected to radiant energy levels 2.5 times higher than the threshold energy for stimulation.

Wells et al (2005) assessed the relationship between the wavelength of light used and the physiological response and characterized the relation between the magnitude of stimulation and amplitude of the CNAP(Wells et al., 2005a). As the incident energy was increased, larger CNAP and CMAP amplitudes were observed. Wells et al speculated that this response was a demonstration of a strength-response curve. In classical electrophysiology experiments, the amplitude of the CNAP increases with increasing magnitude of the stimulation (Geddes and Bourland, 1985). Whilst individual fibers demonstrate an all–or–nothing response to a stimulus, the CNAP amplitude reflects the population response to a stimulus. As the stimulation energy increase, the amplitude of the response increases until
the maximum number of axons within the nerve bundle is stimulated, resulting in a maximal voltage.

Wells et al (2005a) described efforts to determine the mechanism behind Infrared Neural Stimulation (INS). By applying Succinylcholine, a depolarizing neuromuscular blocker, to the muscles in preparation, the CMAP was diminished whilst the CNAP remained. This result appeared to confirm that the potentials generated by Infrared incident energy is generated within the nerve and propagated to the muscle using conventional synaptic transmission.

Wells et al (2007) also suggested that INS is spatially specific method of stimulating nerve tissue. Similar to the work discussed in Wells et al 2005 (Wells et al., 2005b), INS elicited CMAP when the fiber was located directly over the nerve fascicles innervating a specific muscle. As the fiber was moved onto an adjacent fascicle on the main sciatic nerve trunk, the CMAP disappeared. However, when electrical stimulus was applied in the same locations the CMAP was maintained in both test locations. This was likely to be due the current spread beyond the electrode resulting in stimulation of adjacent fascicles. This absence of stimulation of adjacent fascicles demonstrates that INS is capable of a greater degree of spatial specificity than electrical stimulation. In addition, it is possible to finely focus laser light, theoretically increasing the spatial specificity even further.

Wells et al (2005 a) and (2007 provided a proof of concept that INS could be used to provide non-contact, spatially specific stimulation without a stimulation artifact in the peripheral nervous system. A means of stimulating the peripheral nervous system had been identified. Further studies were required to see whether INS could be applied to the auditory system. Once again, the cochlea proved to be an ideal starting point for such experiments.
2.7.3 INS of the Peripheral Auditory System

Infrared neural stimulation of the auditory system has yielded promising results. Stimulation of the auditory nerve was achieved in 2006 by Izzo et al, who successfully recorded cochlear compound action potentials (CAP) from the round window (Izzo et al., 2006) by applying infrared light through the round window into the cochlea of gerbils. After opening up the bulla, the laser was inserted into the basal turn of the cochlea and oriented towards the modiolus. Experiments were performed in animals with normal hearing and acutely deafened subjects. Deafening was achieved by an intra-peritoneal injection of Kanamycin followed by an intravenous infusion of ethacrynic acid. The subjects then had their hearing levels tested pre and post deafening to confirm that a threshold shift had occurred. Results in the non-deafened and deafened subjects demonstrate CAP responses to INS. This stimulation was also non-contact and not associated with an electrophysiological stimulation artifact. Further studies by Littlefield et al confirmed these initial findings using a similar experimental model (Littlefield et al., 2010).

With the successful stimulation of the cochlea and a reliable method of recording auditory nerve stimulation, it has become possible to generate data that identifies how parametric variations can influence the response to INS. These parameters include pulse width, pulse rate and the wavelength of infrared light delivered (Izzo et al., 2007b). Izzo et al found that increasing the pulse width resulted in alteration in the morphology of the CAP waves recorded as initial negative and positive deflections were joined by a second positive deflection that increased in size as the pulse width increased. In addition, CAP amplitude varied as the wavelength of light applied was altered. In preparations where the radiant energy applied was kept constant whilst wavelength was altered, Izzo et al showed that the CAP amplitude increased as the wavelength decreased (from 1880nm to 1840nm). This relation can be explained by understanding that optical penetration depth is inversely
proportional to the wavelength of light applied and that the radiant energy decreases with depth. The optical penetration depth is subject to an absorption coefficient. These relations imply that the increase in CAP amplitude with decreasing pulse width will reach a natural plateau and this hypothesis was supported by the results of Izzo et al, who show that CAP amplitude does not significantly change between 1850nm and 1844nm. The CAP appeared to be stable and non-exhaustible as CAP amplitudes did not vary significantly after 6 hours of continuous stimulation.

The importance of establishing optimal parameters and stability over prolonged periods of time has considerable significance if INS is to become a future possible mode of stimulation for an optically based hearing implant. However, whilst the efficacy of INS appears to be validated by the published literature, spatial selectivity of stimulation is a key feature sought for a new stimulation modality.

2.7.4 INS: A More Spatially Selective Method of Stimulation?

Improved spatial selectivity of INS, when compared to electrical stimulation, can be demonstrated in the peripheral auditory pathways. By identifying the transiently expressed transcription factor cFOS in activated spiral ganglion cells using an immuno-histochemical staining method (Morgan and Curran, 1991), Izzo et al were able to compare the differing expression of cFOS when gerbil cochleae were exposed to auditory, optical and electrical stimuli (Izzo et al., 2007a). Increased levels of cFOS expression could be directly correlated to the beam path through the cochlea suggesting increased levels of metabolic activity in cells exposed to INS. In addition, altering the orientation of the beam, relative to the modiolus, resulted in differing, discrete populations of spiral ganglion cells expressing cFOS. The parameters for INS in this experiment had been used to evoke either CAP or ABR- so were at a ‘therapeutic’ level.
Initial investigations suggested that, in the cochlea, INS is spatially specific and that only tissue areas directly exposed to the light are activated. In contrast, when stimulation of the cochlea was undertaken in analogous preparations using biphasic electrical current pulses, the expression of cFOS spread into spiral ganglion cells in adjacent turns of the cochlear, far from where the electrode was actually positioned.

Recordings taken from higher up in the central auditory pathway can also demonstrate spatial selectivity of stimulation. The ICCN has a tonotopic arrangement that runs in an orthogonal fashion to the dorso-lateral to ventro-medial axis.

The use of multi-channel recording electrodes to access this anatomical arrangement is well documented, as is the development of spatial tuning curves to demonstrate the spatial selectivity of electrical stimulation of the cochlear (Snyder et al., 2004, Snyder et al., 2008). Demonstration of the spatial selectivity of INS in the peripheral auditory system has been shown by recording responses to INS in the cochlea at the level of the IC (Richter et al., 2011). The experiments by Richter et al used a guinea pig model. The animal was prepared in a similar fashion to the gerbils used by Izzo et al (2006) in their experiments using INS. The animal was deafened after insertion of the recording electrode into the inferior colliculus by injecting a buffered neomycin solution directly into the scala tympani. Hearing thresholds pre-deafening were compared to those post deafening. Thresholds were raised by at least 40dB above baseline in all the animals used. A cochleostomy was performed that allowed a 200micron optical fibre to be inserted into the cochlea and rotated. This rotation allowed the orientation of the optical fiber to change in both the antero-posterior axis as well as the dorso-ventral axis. The results from Richter et al (2011) suggested that INS of the cochlea has a spatial selectivity that is comparable in the best instances to acoustically derived spatial tuning curves. The group demonstrated that when the optical fiber was aimed perpendicularly to the spiral ganglion, spatial tuning curves...
were narrower than when the beam was fired in a more tangential fashion. This result re-emphasized the spatial selectivity of INS. This selectivity could enable a cochlear implant to have an increased number of channels as each optode along the length of the implant would stimulate a smaller population of spiral ganglion cells than their electrode counterparts. However, long-term safety and efficacy studies have not been published at this time.

The spatial selectivity of INS is of particular relevance to its use as a stimulation paradigm for an optically driven ABI. Spatial selectivity increases the number of discrete points on a tissue that can be stimulated. This increased resolution could limit extra-auditory stimulation. In addition, the fine area of stimulation could result in an ABI array with a greater number of channels. In addition, Light, unlike electricity, can be focused using lenses, which may also increase the highly focused nature of INS.

### 2.7.5 INS of the Central Nervous System

Research on the use of INS in the central nervous system has been limited, with little published data available. One available study shows that INS of the rat somatosensory cortex produced an inhibitory effect on neuronal firing (Cayce et al., 2011). This inhibition was focal, with no other changes to the patterns of firing noted in non-stimulated areas during the laser firing. The authors commented that this inhibitory effect, opposite to the excitatory effect observed in other uses of INS, might be due to the spatial integration of inhibitory neurons in the cortex as compared to peripheral neural structures. Studies undertaken by (Cayce et al., 2011) suggest that pulse width, radiant energy exposure and pulse rate affect the response to INS, as was similarly demonstrated in the peripheral auditory system (Izzo et al., 2007b, Littlefield et al., 2010). More recently, Duke et al (2013) published results demonstrating that INS can cause transient inhibition of electrically initiated axonal activation in a rat sciatic nerve preparation. This result
supported the initial findings of Cayce et al who also suggested that locally applied INS could lead to inhibition. However, new data from Cayce indicates that infrared light applied to the CNS may cause a stimulatory response (Cayce et al., 2014). Using single unit recording techniques, this group evoked local neural responses in the primary visual cortex of primates. INS applied to retinal and vestibular neurons also appears to give a stimulatory response, but required patch clamping techniques to record the very small responses they generated (Bec et al., 2012).

2.7.6 INS of the Central Auditory Pathway

All the published literature to date using INS in the auditory system focuses on its use in the peripheral nervous system. There is currently no published literature regarding the use of INS to stimulate the central auditory system, although preliminary results presented in abstract form suggest that it is possible to generate ABRs if the cochlear nucleus is subjected to INS (Lee, 2009).

2.7.7 Mechanism of Infrared Neural Stimulation

There is no definitive consensus on the precise mechanism of INS. There was some initial thought that the mechanism may be due to a heating effect, activating trans-membrane channels (Wells et al., 2007a). More recently, Shapiro et al described a potential mechanism for INS that involves the transient development of capacitance currents (Shapiro et al., 2012). The initial experiments were undertaken in oocytes, but further experiments in synthetic lipid bilayers, free of membrane proteins, demonstrated the same capacitive currents. Shapiro et al’s paper leads to an interesting conclusion, that all cells with a lipid bilayer possess the innate ability to respond to INS. However, this paper does not give any further information as to why some tissues are more readily stimulated i.e. peripheral nervous tissue than other tissue i.e. central nervous tissues.
2.7.8 INS and Optophonic artifact

Whilst INS is not known to produce any electrophysiological stimulus artifact, one group has identified and investigated a possible acoustic artifact associated with INS (Teudt et al., 2011). Experiments undertaken by this group identified that when pulsed infrared laser light was directed at a tissue, a small volume in front of the laser tip was rapidly heated, resulting in a rapid expansion of this volume and a stress-relaxation wave was created. The experiments focused on identifying whether this stress-relaxation wave was audible.

In-vitro experiments in a soundproof booth were done where a microphone was placed in a perpendicular fashion 2mm from a 200-micron optical fibre. Laser pulses of up to 100 µs generated an acoustic event, with wavelength of 1850 nm. Increasing the radiant energy of the laser pulses, by increasing the laser peak power, generated greater peak pressure levels up to 62 dB SPL. As the microphone was moved further from the tip of the optical fibre, the pressure levels diminished.

These experiments were conducted in air. When they were repeated in conditions when the microphone was surrounded by nitrogen only, there was no audible acoustic artifact. This suggested that the laser energy was absorbed by oxygen, carbon dioxide or water in the air with conversion of radiation into heat generating the pressure wave. The absorption characteristics of infrared light suggests that water is the most likely candidate (Hale and Querry, 1973).

Teudt et al also performed in vivo experiments using adult Sprague–Dawley rats. The bulla was opened to access the cochlea and the fibre placed in different positions around the cochlea whilst infrared laser light pulses were fired. Responses were recorded in the form of compound action potentials generated by placing a silver electrode at the bony rim of the round window. When the tip of the laser was close to the cochlea (<2mm), responses were recorded. In addition, applying white noise masking diminished the responses when
the laser was applied to the middle turn of the cochlea. Teudt et al’s result demonstrated that the acoustic artifact generated by the stress-relaxation wave was within the rat’s audible range. The optophonic artifact is not a stimulation artifact as such, but a true response caused by an additional stimulus. The optophonic artifact was a potential confounding factor as it produces an audible stimulus that must be controlled for in order to assess whether INS applied to the CN resulted in responses mediated by the infrared radiation acting upon neural tissue, or were responses indirectly generated via the audible stress relaxation wave. Efforts to control for the optophonic artifact are detailed in chapter 5.

### 2.8 Auditory Brainstem Implants: An Optical Future?

The experiments described in this thesis were devised to ascertain whether INS applied to the cochlear nucleus could stimulate responses in the central auditory pathway. ABR’s were chosen as the measure of activity as the ABR is used intra-operatively to assess the correct positioning of the ABI, it seemed appropriate to test whether INS could generate this clinically applicable measure of auditory pathway activity (Nevison, 2006).

Experiments were also performed to identify the effect of altering the stimulation parameters such as peak power, wavelength of light, pulse width and pulse rate upon an INS evoked ABR. Previous research has shown that, when INS was applied to the peripheral auditory system, there was an effect of wavelength and pulse rate. In addition, radiant energy has also been shown to have an effect (Izzo et al., 2006, Izzo et al., 2007a, Izzo et al., 2007b). The effect of wavelength is particularly interesting, as varying the wavelength will alter the depth of penetration of the infrared radiation through the tissue. Lower wavelengths penetrate the tissue more deeply (Hale and Querry, 1973). The effect of applying INS at different surface sites on the dorsal cochlear nucleus was also tested to
ascertain where there was an optimal placement location for stimulation. Experiments was also undertaken to assess responses in the inferior colliculus when INS was applied to the CN. This set of IC experiments used a multi-channel electrode placed within the central nucleus of the inferior colliculus (ICCN) to develop spatial tuning curves. The IC experiments were mirrored the parametric studies and location studies described above

In order to control for the possible effects of the optophonic artifact described in 2.7.8, the experiments were repeated in acutely deafened animals.

The ABI represents the only hearing rehabilitative option for many adult patients, and is increasingly an option for pre-lingually deaf children unsuitable for cochlear implants. Attempts to improve the ABI with a novel stimulation paradigm may offer a new generation of neuroprosthetics with improved auditory outcomes.
Chapter 3: General Methods & Experimental Set-Up

3.1 Introduction

The aim of the following experiments was to investigate whether infrared neural stimulation (INS) could activate the central auditory system when applied to the cochlear nucleus. Specifically, we exposed the dorsal cochlear nucleus (DCN) of male Sprague Dawley rats and applied INS by means of a 400-micron diameter optical fibre to the surface. We evaluated the responses by taking 2 measurements of activity of the auditory pathway. In the first set of experiments we recorded auditory brainstem responses (ABR) using subcutaneously placed silver iridium electrodes. In the second set of experiments we placed a multichannel electrode into the central nucleus of the inferior colliculus (ICCN) contralateral to the exposed DCN and recorded responses from iso-frequency laminae that exist within this structure.

Each set of experiments consisted of further subsets of tests. We investigated the effect of altering different stimulation parameters. These parameters included laser peak power, pulse width, wavelength of the infrared light applied and pulse rate. We also tested the effect of applying INS to different locations across the surface of the DCN by dividing the surface of the DCN into sub-sites.

After these experiments were performed in animals with normal hearing, further testing was undertaken on subjects who were acutely deafened. In these animals the auditory nerve was sectioned as it exited the internal acoustic meatus and deafening was confirmed by testing the auditory brainstem response to acoustic stimulation, confirming elevated thresholds (above 70dbSPL). In addition the structural integrity of the DCN was confirmed by successfully eliciting an electrically evoked auditory brainstem response generated by placing a silver ball electrode onto the surface of the DCN.
In order to obtain consistency, we attempted to perform as many subtypes of experiments on the same animal. However, due to the nature of the experiment and physiological/survival constraints it was not possible to obtain all data sets from the same animal in all cases. Therefore the results are based upon 3-8 animals per subtype of experiment. A total of 120 rats were used during this study. This figure includes subjects that were use to study the anatomy of the rodent auditory system in order to optimize surgical approaches to the auditory brainstem and the inferior colliculus. In addition, a number of subjects died intraoperatively or during the experiment as a result of hypovolaemia, anaesthetic overdose or respiratory complications.

### Hearing animals

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### Deafened Animals

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<td>Location Studies</td>
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**Figure 3.1:** Summary of experiments types
3.2 Anaesthesia & Preparation of Subject

All procedures were conducted in accordance with guidelines of the National Institutes of Health and were approved by the Animal Care and Use Committee of the Massachusetts Eye and Ear Infirmary and Harvard Medical School. All experiments were conducted within a sound-attenuating chamber, with a soundproofed penetration hole to allow passage of wires and the optical fibre into the chamber from the control station located outside the chamber.

3.2.1 Subject Preparation & Positioning

Male Sprague-Dawley rats (410-600g) were used as our experimental subjects. Anesthesia was undertaken using ketamine (800 mg/kg I.P.) and xylazine (8 mg/kg I.P.). Atropine (0.4 mg/kg) was given to minimize respiratory secretions. Dexamethasone (0.8 mg/kg) was administered at induction to minimize the effect of brain swelling during surgery. Booster injections of ketamine (30mg/kg I.P.) and/or xylazine (3 mg/kg I.P.) were given approximately each hour or as required after paw pinch assessment or other signs of agitation of the subject. The head, ventral neck and dorsal neck were shaved using electric

**Figure 3.2:** Representation of the experimental set up
clippers to facilitate insertion of electrodes to record ABR.

In addition, a 3cm x 3cm midline patch of skin over the lumbar vertebrae was shaved to allow a ground electrode to be inserted. At this stage, a sound source was placed in the left ear. After acoustic calibration, acoustic ABRs were derived from presentation of click stimuli to the subject. Normal hearing thresholds for the subject were between 20-30 dB SPL. If the hearing thresholds were not within this range, the animal would be allowed to wake up from anesthesia and removed from the study. This step of recording auditory ABRs also helped to identify any problems with excessive electrical noise in the set up and ensured that all the equipment required for recording ABR responses was in working order before any non-reversible steps had been performed on the subject.

Once we were satisfied with the acoustic ABR levels and experimental set up, tracheotomy was performed to allow suction clearance of secretions from the respiratory tract during the experiment. Intra-peritoneal and sub-cutaneous injections of saline were administered to the rat to maintain normo-volaemia.

### 3.2.2 Exposure of the Cochlear Nucleus

![Figure 3.3: Rat cranium showing the approximate positions of the craniectomies performed to access the CN (blue) and the IC (red). (George Paxinos and Charles Watson: The Rat Brain in Stereotaxic Coordinates, Academic Press, New York, 1998, p. 11).](image-url)
The rat was held in a prone position in a stereotactic head holder and animal platform (David Kopf Instruments, Tujunga, CA) and placed on a warming pad. The animal platform was placed on a vibration isolation table. Lidocaine and Adrenaline (7mg/kg) was injected sub-dermally as a local anaesthetic agent before a 4cm midline incision was made over the occiput extending caudally. The musculature overlying the posterior cranium was split at the midline raphé and nuchal muscles detached from their cranial attachments to give clear exposure of the occiput of the subject’s skull up to the level of the foramen magnum. A self-retaining retractor was placed to maintain the exposure during the remainder of the surgery. The periosteum was removed using a periosteal elevator and tenotomy scissors.

A posterior craniectomy measuring approximately 10mm x 8mm was created using a craft drill and cutting burr to expose the dura and underlying left cerebellar hemisphere (Fig 1). The dura was opened with a cruciform incision and bleeding controlled with thermo-coagulation. The left cerebellar lobe was gently aspirated using a fine ‘Belluci’ sucker to reveal the fourth ventricle in the midline and the cochlear nucleus at the lateral margins of the exposed brainstem. Care was taken to avoid damage to the cerebral vasculature at the lateral margins of the craniectomy. Residual pieces of tissue were swept away from the surface of the exposed CN in an atraumatic fashion using fine dental points moistened with 0.9% saline and held with fine forceps.

The exposed musculature was covered with saline soaked gauze during the procedure to prevent similar drying and to decrease the volume of insensible fluid losses.

3.3.3 Exposure of the Inferior Colliculus recording electrode insertion site

An anterior craniotomy was performed over the right temporal-parietal suture, just rostral
to the tentorium to provide access for the stereotactic insertion of a multi channel electrode into the right ICCN (Fig 1). A small incision was made into the dura mater and the multichannel electrode array was inserted through the overlying occipital cortex into the ICCN using a 3-D micromanipulator (David Kopf Instruments Tujunga, CA) attached to the head-holder. The electrode was inserted into the ICCN on a dorsal to ventral trajectory. The site of insertion was determined from stereotactic co-ordinates with the bregma as the fixed anatomical reference point. The Point of insertion was approximately 18mm caudal from bregma and 8mm lateral to the sagittal suture.

Extreme caution was required in order to avoid puncturing or tearing the dura overlying the sagittal sinus and sigmoid sinus during insertion of the electrode. In the event of significant bleeding caused by iatrogenic puncture of the dural sinuses, gelatin sponge was applied to the bleeding point and left in situ for approximately 10 minutes. Fluid resuscitation with intra-peritoneal and / or subcutaneous 0.9% saline was provided to restore normovolaemia. Correct insertion of the electrode along the trajectory described above allowed the recording tip of the electrode to pass in an orthogonal orientation to the tonotopic axis of the ICCN. Correct placement of the electrode was confirmed by presenting acoustic tones ranging from 1 kHz to 32 kHz in increments of 2 steps per octave to the left ear. Acoustic stimuli were delivered via a custom acoustic assembly comprising two earphones to deliver sound and an electret condenser microphone (Knowles FG-23329-P07) coupled to a probe tube to measure sound pressure level. Successful placement of the electrode array was confirmed when neural responses could be generated across the full length of the array in response to these acoustic stimuli. Correct insertion required several insertion attempts in some instances. The surface of the cortex and the CN were kept moist with regular application of 0.9% saline and kept covered with saline moistened gauze.
3.3.4 Acute Deafening
In order to isolate the cochlear nucleus from the peripheral auditory system, acute deafening of the subject was required for some sets of experiments. In order to achieve this quickly and safely, a technique was developed to mechanically deafen the animal by dividing the VIIIth cranial nerve. This was achieved by gentle retraction of the brainstem using fine dental points. This provided a small amount of space between the lateral brainstem and the medial wall of the temporal bone. A fine mounted needle or sickle knife could then be passed lateral to the brainstem in order to divide the VIIIth nerve as it passed out of the internal auditory meatus.

Successful deafening was confirmed in a two-fold fashion. Firstly, attempts were made to generate acoustically driven ABR by presenting an acoustic stimuli to the left ear. If a threshold shift of greater than 60 dB SPL was recorded, then the animal was deemed to be successfully deafened. In order to ensure that the process of mechanical deafening had not damaged the structural integrity of the cochlear nucleus, bipolar electrical stimulation was applied to elicit an eABR (see section 3.6.2).

3.3.5 Termination of experiment
The experiment would come to an end either when the subject died, or when no further data was required or could be extracted. As per local animal care protocol, the subject was euthanised by intracardiac injection of Sodium pentobarbital euthanasia solution (390mg/ml), with dose adjusted by weight as per local protocol. Decapitation was undertaken after euthanasia.

3.4 Development of Surgical Technique
The surgical steps described above were developed in-house by the author with supervision from Dr Daniel Lee and Dr Chris Brown from Eaton Peabody Laboratory. Optimal exposure of the cochlear nucleus requires a dorsiflexion of the skull, which was
not possible with the head-holder that was initially available in the laboratory (model and manufacturer unknown). Review of literature highlighted the prevalent use of the Kopf stereotactic head-holder, which was subsequently purchased. This device allowed for a more secure head positioning, increasing access to the posterior cranium. The Kopf stereotactic head-holder provided better access to the tracheostomy intra-operatively and during the experiment. A common finding intra-operatively was that the tracheostomy tube would become blocked with respiratory secretions. These secretions could be cleared either by passing a fine suction tip directly into the tracheostomy tube, or by constructing wicks from tissue paper that were narrow enough to pass down the tracheostomy tube. Use of the Kopf stereotactic head-holder corresponded to a decrease in the mortality we observed from respiratory complications, a reduction in the time required for surgery and a performed. With increased experience it became possible to perform greater degrees of exposure of the dorsal cochlear nucleus. Because of the domed nature of the structure, which lies as a swelling over the bulk of the brainstem, damage to the superficial surface layer of the DCN was a significant risk during cerebellar aspiration. At times when the cerebellum could not be entirely removed from the surface of the DCN without risking significant harm a technique was developed that involved using fine dental points to gently push any residual cerebellar tissue off the DCN, causing minimal trauma. It was observed that the greater the degree of DCN preservation during exposure, the longer the DCN held its structural integrity and the longer the experiment could run for.

The inferior colliculus work provided the greatest technical challenge. There was no experience of the operative steps existing within the research group, consequentially the technique was developed by adapting it from Dr Claus-Peter Richter’s technique in Guinea pig. During a week-long visit to Dr Richter’s laboratory at Northwestern University, Chicago, Illinois, USA surgical and data analysis techniques were learnt. The specific difficulty in accessing the rat IC, when compared to guinea pig IC, is the close proximity
of the optimal electrode insertion site to the sagittal sinus and sigmoid sinus. The initial steps for access involved identifying a surface marking on the cranium for the medial and posterior limits of the craniectomy. The sagittal suture and the lamboid suture provided these surface markings and were roughly positioned over the sagittal and sigmoid sinus. Once the cranium was opened using a drill, the dura was exposed as fully as possible. The dura was then incised as described above and reflected to expose cerebrum. The electrode would bend and warp if forced to pass through dura. The location of the ICCN was identified using stereotactic co-ordinates taken from the bregma. A consistent pattern of blood vessels on the surface of the cerebrum was identified that seemed to identify a particularly reliable location for IC electrode insertion into the cerebrum and onwards into the ICCN. This point corresponded to a site where two vessels bifurcated. If the stereotactic co-ordinates did not give a good tonotopic map, the blood vessel method was used. Bleeding from the sinus was initially considered a terminal event. With time and practice it became possible to reduce and control bleeding using the techniques described above. Fluid resuscitation was also critical in the event of bleeding. IM or IP injections of 0.9% sterile saline were given as a 2ml bolus doses.

The techniques evolved over several months as technical skills improved and the need for quicker and less traumatic surgery became apparent. By the end of this study only 35 minutes was required from placing the anaesthetized subject into the stereotactic head-holder to first direct stimulation of the CN. The surgical time for IC exposure was approximately 10-15 minutes, reduced from 60 minutes at the outset. Correct placement of the multichannel electrode into the ICCN in an orientation orthogonal to the iso-frequency laminae could take between 10-90 minutes, including the time to test the position using acoustic stimuli as described below. In the final months of testing, the IC electrode could be placed within 3 attempts, allowing for testing of the acoustic map between insertions. Repeated insertions increased the chance of haematoma formation and damaging the ICCN.
3.5 Measuring Responses to INS: ABR & IC responses

3.5.1 Auditory Brainstem Responses

In order to identify whether INS resulted in stimulation of the central auditory pathway, we identified two specific measures of activity to record. The auditory brainstem response (ABR) is an evoked potential extracted from activity in the brain. The response is measured as a series of waves, each of which reflects activity in a different part of the auditory brainstem.

Wave I – Peripheral portion of vestibulocochlear nerve
Wave II- Central portion of vestibulochochlear nerve
Wave III- Cochlear Nucleus
Wave IV- Superior Olive
Wave V- Inferior Colliculus.

ABRs can be evoked by auditory stimulus presented to the ear, resulting in an auditory evoked ABR (aABR). Alternatively, responses can be stimulated by applying electrical current to the surface of the cochlear nucleus to generate electrically evoked ABRs (eABRs). These eABRs are generated intra-operatively in order to guide the positioning the ABI electrode array upon the surface of the human cochlear nucleus (Nevison, 2006). In these experiments, the aim was to elicit ABR evoked by the application of INS. We termed these responses optically evoked ABR (oABR). Successful generation of an ABR gives evidence of activation of the auditory pathway, but as discussed in chapter 2, the ABR itself gives no information regarding the qualities or perception of sound, merely activation of the central auditory pathway itself.
3.5.2 Inferior Colliculus Recordings

Another means of measuring activation of the auditory pathway is to take recordings from structures within the central auditory pathway. As previously described, the ICCN contains iso-frequency laminae. An electrode passed orthogonal to the ICCN will be in contact with multiple iso-frequency laminae. Therefore, by inserting a multichannel electrode into the ICCN, it is possible to record which iso-frequency laminae were being activated by auditory stimuli or stimuli presented directly to the surface of the CN.

![Figure 3.4: Representation of multichannel electrode inserted into ICCN, straddling the iso-frequency laminae.]

By ‘tuning’ the electrode acoustically, as described below, it is possible to assess which frequencies INS is stimulating. This was done by comparing which of the individual channels of the IC electrode showed increased activity when the CN presented with infrared radiation and then comparing this stimulation pattern to the acoustic map to identify which of the iso-frequency laminae are activated and which pure tone these iso frequency laminae optimally responded to responded to.
3.5.3 Recording of the Auditory Brainstem Response

ABRs were measured via silver-iridium electrodes placed subcutaneously. The first electrode was placed behind the left ear with the second electrode placed over the vertex of the cranium in the midline. A reference electrode was placed in caudally in the midline of the back, roughly in line with the hind legs. In order to facilitate the insertion, these areas of skin were shaved pre-operatively after induction of anaesthesia.

Each ABR signal filtered with an analogue band-pass filter with cut-off frequencies of 30Hz and 3kHz, amplified by 60dB, 500 sweeps were averaged to remove most of the electrical, EMG and EEG noise. The signal was then and A/D converted with a sampling frequency of 25 kHz using an Ithaco Model 1201 (DL Instruments, Ithaca NY.) This signal was then converted to digital with 16-bits resolution at 25 kHz (PXI-6221, National Instruments, Austin TX).

3.5.4 Recording of the Inferior Colliculus Responses

The recordings from the contralateral IC are performed with a silicon-substrate thin-film 16-channels penetrating electrode (A1x16-5mm-100-177, NeuroNexus Technologies, Ann Arbor, MI) inserted along the tonotopic axis of this structure. Each recording channel had a surface of 177µm² and the centre-to-centre interval is 100µm. A global reference was used, which was the average of all the signals. They were then converted to digital with 16-bits resolution at 20 kHz, and the raw data are stored.

Once the electrode was inserted in the IC, it needed to acoustically tuned in order to confirm the placement and to identify the characteristic frequencies corresponding to each
channel. This was done with pure tones at specific frequencies to the left ear. The range of frequencies used is 1 to 32 kHz, with 2 frequencies per octave.

3.6 Technical specifications for INS and Electrical Stimulation

3.6.1 Optical and electrical stimulation parameters

Infrared pulses were generated using a diode laser (Capella R-1850, Lockheed-Martin Aculight Corp., Bothell, WA). The left CN was stimulated by surface contact with the tip of the optical 400µm fiber. Optical stimuli were infrared light pulses generated using the diode laser. The laser unit was kept outside the sound attenuated chamber and the optical fiber was led into the chamber via a penetration hole. The distal fiber was mounted to a three-axis micromanipulator (Narashige Corp., Japan), aimed, and advanced under direct visual guidance using a surgical binocular operating microscope (Carl Zeiss International). The surface of the CN was subdivided into 12 territories using a grid system with the caudal end of the 4th ventricle used as a fixed anatomical reference point.

The optical fiber was advanced to the desired position. The surface of the left cochlear nucleus was focally stimulated by placing the tip of the optical fiber (400µm diameter) such that it was just over the meniscus of fluid overlying the brainstem. The fibre itself was not in contact directly with neural tissue in order to minimize the risk of trauma to the cochlear nucleus. In experiments where the laser parameters were altered (Parametric Studies) the stimulation parameters were varied as detailed below:

*Wavelength 1849-1855nm*

*Pulse Rate 3-63 Hz*

*Pulse Width 0.05-0.4ms*

*Radiant Energy (0-70%) 0-711mJ/cm²*
For experiments where the surface site of stimulation was altered (location studies) the parameters of stimulation were fixed as follows:

*Wavelength 1849mm*

*Pulse rate 23 Hz*

*Pulse width 0.1 ms*

*Peak Power 70% (2844 mW/cm²)*

### 3.6.2 Electrical Stimulation Parameters

Electrical stimuli were 50-µs anodic current pulses generated by passing the output of a 16-bit D/A converter (PXI-6221, National Instruments, Austin TX) through an analog stimulus isolator (Model 2200, A-M Systems, Carlsborg, WA). Current pulses were delivered to the exposed CN using a parylene-insulated platinum-iridium twisted electrode (51mm length, impedance = 0.1-0.5 MΩ) (Microprobe Inc., Gaithersburg, MD). Bipolar stimuli were presented at 5 pulses per second, and varied in amplitude from 0 to 0.4 mA. Electrical stimulation was used exclusively to test the functioning of the cochlear nucleus after nerve sectioning.

### 3.7 Subdividing the Dorsal Cochlear Nucleus: Location Studies

The exposed surface of the CN, mainly comprised of the dorsal cochlear nucleus in our preparation, is roughly 16x12mm. In order to test how responses to optical stimulation vary across the surface of the CN, we devised a virtual grid system. This divided the surface of the CN into 12 zones, each of almost exactly the same size. The rostral-caudal axis was split into 3 rows, labeled A, B & C. The medial to lateral axis of the CN was divided into 4 columns (see figure 3.5). The reference point for this grid is the posterior end of the 4\textsuperscript{th} ventricle, close to the site C4. This site is always fully exposed during surgery. The site A1, the most rostral and lateral site was referred to as the “sweet spot”. The auditory nerve entered the cochlear nucleus complex deep to this site, into the ventral
cochlear nucleus sub-division. The parametric studies were undertaken at this particular site as previous work preformed by this research group had demonstrated that it consistently gave strong optically evoked ABR recordings.

Figure 3.5. Virtual grid used on the CN to have reliable stimulation sites for the location studies

Figure 3.6: Schema of the CN separation into 12 zones. A1 corresponds to the ‘sweet spot’.
3.8 Quality Assuring Recordings & Normalisation

In order to ensure that the quality of data we regularly performed a control run test where a reference set of parameters are used. This was generally done after approximately every 5-6 runs or whenever the fibre was deliberately moved. The purpose of this was to ensure the results were accurate and any alteration was not due to animal factors such as a lightening of the depth of anesthesia, or that the fibre had not moved a result of the animal twitching or moving. In addition, by having several runs at the same parameters throughout the experiment, it became possible to normalize the data so comparison between different rat subjects is possible. The parameters used for these normalizing runs for ABR experiments are detailed in Table 3.1. After every 5-6 runs, the normalizing, or reference, parameters were used to record a set of data. During the data analysis phase, the P2 and P4 extracted parameters were identified and used as a standard to normalize the subsequent 5-6 runs (until a new set of normalizing data was captured after a further 5-6 runs). The values for P2 amplitude and P2 latency from these runs was set as 1 and the P2 amplitudes and latencies of the subsequent data sets were divided by the raw P2 amplitude and P2 amplitudes respectively to give a ‘normalised’ result. This process was repeated for P4 Amplitude and P4 Latency, with the reference parameter P4 latency given the value of 2 and the P4 amplitude of 0.5

<table>
<thead>
<tr>
<th>Peak Power %</th>
<th>Pulse Width ms</th>
<th>Pulse Rate Hz</th>
<th>Wavelength nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>0.25</td>
<td>23</td>
<td>1849</td>
</tr>
</tbody>
</table>

Table 3.1: Laser parameters for normalizing data
3.9 Signal Processing and Data Analysis

3.9.1 Auditory Brainstem Responses

3.9.1.1 Initial Processing

Data analysis was performed with in-house programs written with the Matlab software (Mathworks, Natick, MA) and in collaboration with Ms Amelie Guex. The ABR signal analysis was critical to detect the waveforms and to extract meaningful parameters from the signal. The goal was to remove the noise (mainly electrical, EEG and EMG noise) as much as possible in order to increase the Signal-to-Noise Ratio (SNR). The first step was to remove the noise is averaging, a technique widely used in evoked potential analysis (Shannon et al., 1997b). The assumption is that the signal does not change among trials, so it will add up whereas the noise is stimulus-independent, so it will be cancelled out by the averaging. 500 sweeps are averaged for each run, which has been shown in previous work to be sufficient to remove most of the noise (Izzo et al., 2007b). To further enhance the SNR, filtering can be used. During data acquisition, analog filtering had to be used over a very broad bandwidth to avoid waveform distortion (30Hz to 3kHz). (Laukli and Mair, 1981) Digital, phase-invariant filters were preferred because they avoid phase distortion. The cutoff frequencies have to be optimized in order to maximize the SNR and minimize the signal amplitude distortion.

In order to achieve this, we analyzed the signal frequency spectrum after application of the Fast Fourier Transformation (FFT) on the signal, to detect the frequencies contributing to the actual signal and those contributing mainly to the noise.

The filtering was done with a forward and reverse order 5 Butterworth filter. The cutoff frequencies were determined with the FFT (Fast Fourier Transform) spectrum, in which the frequencies containing most of the signal were selected, over many trials. Different
cutoff frequencies were used for optically and electrically generated ABRs, due to the different properties of the signal. For the INS generated ABRs, the cutoff frequencies maximizing the SNR and minimizing the signal distortion were 100Hz and 1.4kHz. For the electrically generated ABRs, the cutoff frequencies were 200Hz and 2.5kHz. Additionally, in the electrically generated signals, the first millisecond of recording after stimulation onset is removed before filtering. This was done in order to minimize the effect of the electrical stimulation artifact, which would otherwise induce a waveform distortion into the signal. This step was not necessary with optically generated ABRs, due to the perceived absence an electrical stimulation artifact. Because of the different filtering parameters used in INS or electrically generated ABRs, the waveforms of these two types of signals were not directly compared.

3.9.1.2 Peak Detection and Review Algorithm

The ABR time-domain signal can be analysed to extract several parameters. However in order for these parameters to become evident, it was necessary to methodically detect the multiple peaks that exist within the signal.

The detection technique we used involved finding the specific points where the derivative of the signal is zero. These corresponded to local maxima and minima of the signal. The time and magnitude of these different peaks could then be extracted for identification and comparison.
One major peak was found to be consistently present in the rat optically generated ABR in the rat. This specific peak corresponds to the signal maximum, and was detected and labeled “P2” in the literature (Lasky et al., 2002). This terminology was also used here, so the highest amplitude peak was labeled P2. P4 was also identified and labeled, being the next large wave after P2. P3 was a small wave, not present in all the signals because it often merged with wave 2 or 4.

Figure 3.7: Typical example of a rat ABR demonstrating peaks (P) and waves.
The automatic detection and labeling of the peaks was manually reviewed to avoid and minimize detection errors, to ensure correct labeling of the waves, particularly when P3 is not present, and to identify signals with no response. The developed algorithm includes the possibility to manipulate the signal and to define the runs as “no signal”, which sets the amplitudes to zero and removes the latencies from the analysis. Visual confirmation of a signal that could be regarded as a P2 would allow the observer to consider that a response was generated.

At this stage of processing, a quality control mechanism was enacted. As previously described (see section 3.8) during each experiment ‘reference runs were inserted that were performed using a set of reference parameters at regular intervals. The parameters were pulse rate of 23 Hz, wavelength of 1849 nm, pulse width of 0.25 ms and laser peak power of 70%, which correspond to a radiant energy of 711 mJ/cm². We assessed the consistency of the P2 latency and p2 amplitude in these reference runs, allowing us to monitor how the responses within a single animal varied as well as allowing comparison between different subjects. The reference runs took place approximately every 5-6 runs. Whenever this latency varied by more than 5% from the previous reference run, the subsequent data was discarded. Indeed, the latencies of the first waves has been shown to be extremely reliable under normal conditions, (Hall, 2006) and an variability could indicate a diminished data quality. This could be due to several factors including physiological condition of the animal, a damaged cochlear nucleus, a damaged auditory pathway or problems with anesthesia including overdose.

3.9.1.3 Parameters extraction

Once the different waves were detected, several features could be identified from the signal to aid our analysis. Two different types of parameters are extracted, corresponding either to the waveform of the signal – wave amplitudes and latencies – or to the energy of
the signal – root mean squared (RMS) and percentage of energy in each wave (Wilson, 2004). The amplitude of a wave was defined, in keeping with standard practice, as the maximum of the amplitudes calculated with the previous and the following minima. The latencies were defined as the time between the stimulus onset and the peak of the wave. The RMS value of the signal is a measure of the mean signal energy and is calculated by taking the square root of the average value of the squared signal. The signal energy was calculated by the sum of the squared values in the interval. Once these parameters were extracted, a sub-group of parameters were selected for the subsequent analysis based on their reliability and their physiological significance. For example, P2 and P4 are reliably present in the signals, whereas P3 is not. Because of this, we judged the latency and amplitude of P2 and P4 to be more relevant for analysis than those of properties of P3. The list of analyzed parameters was:

- Amplitude P2
- Latency of P2
- Amplitude P4
- Latency P4,
- RMS energy of the signal,

Wave 2 is defined as the signal present in the time between the local minima directly before and after P2.

Normalization of the data through the use of reference runs as described above allowed comparisons to be made between different subjects and across different sites in the same animal (see section 3.8). The signal energy values are not normalized.
3.9.2 Inferior Colliculus Recordings

3.9.2.1 Initial Processing

The recorded signal from the IC is composed of three major signal types. First, the activity from the neurons directly adjacent to the electrodes can be recorded in the form of spikes. The evoked potentials are also recorded by the electrodes, reflecting the post-synaptic potentials due to the synchronous activation of large groups of neurons along the auditory pathways. The evoked potential frequency is lower, so it can be removed by high-pass filtering with a forward and reverse Butterworth filter of order 5, with a cutoff frequency of 500 Hz. (See figure 3.8)

Background noise from other sources is also present in the recorded signal. Its frequency is similar to the frequency of the spikes, so it cannot be removed by digital filtering. However, its amplitude is smaller than the spikes, so a proper spike detection method can minimize its influence on the data.
Figure 3.8: Examples of A) a signal with no significant activation and B) a signal with activation. In each plot, the raw signal is shown in blue and the low frequency component in red. The low frequency component, which mainly corresponds to the evoked potential, is filtered out.
3.9.2.2 Spike Detection Threshold Determination

The general principle of the developed spike detection algorithm is to fix a threshold and then count the number of times the signal passes this threshold (Lim and Anderson, 2006) (Richter et al., 2011). The threshold should be fixed at a level that is sufficiently high to be above basal recorded electrical activity (without spikes), yet low enough to be able to detect the spikes. We therefore chose to use a method based on the median of the absolute value of the signal, as it gave a reliable measure of the background noise without being too affected by either background electrical noise or spontaneous / random spikes that occur in the absence of stimulation.

For INS generated signals, we chose to use the initial 4ms of recording to calculate the detection threshold. It was noticed after early trial experiments that the activation starts after about 5 ms. In the case of electrically generated signals, this window cannot be used because it mainly contains the stimulation artifact. A 4 ms window at the end of the recording is then used. For a given run, the threshold used was calculated with the average of the medians on this interval for the 32 trials. The median on the first 4ms interval was multiplied by a factor of 4 to set the threshold. This factor was selected as the one giving the threshold with the most reliable spike detection, being low enough to detect the spikes and high enough to ignore the noise.
Figure 3.9: High-pass filtered signals (in blue) with threshold (in red) for a signal showing activation.
3.9.2.3 Determination Of The Analysis Time Window

Figure 3.10: Example of IC activation recorded following a 70dB stimulation at frequencies varying from 1 to 32 kHz. The main activation seems to occur between 5 and 25ms after stimulation onset.

To find the window in which the activation occurs, the PSTH (peri-stimulus time histogram) was used (Lim and Anderson, 2006, Shivdasani et al., 2008). The PSTH calculated the average number of spikes per time interval, over the different trials. The time interval was fixed to 1ms. Figure 3.10 is an example from the data showing the PSTHs in a colour plot for the different stimulation frequencies and the 16 recording electrodes. Following analysis of several datasets using the PSTHs, a window of 5 to 25ms was chosen for the analysis. The activation at each level, frequency and electrode was then defined as the average of the spike counts over this 20ms window.
3.9.2.4 Post-Processing
In order to extract reliable parameters from the IC data obtained, several post-processing steps were used. Firstly, the spontaneous activity was defined as the average of the spike counts at the zero stimulation level across the electrodes. The standard deviation of the baseline activity was also calculated, before subtracting the spontaneous activity from all the values. A threshold was then defined and all the values below this threshold are set to zero. This threshold represents the significant activation level and can be defined either with a multiple of the standard deviation of the spontaneous activity (Lim and Anderson, 2006), or by a percentage of the spontaneous activity (Barsz et al., 2007). For this analysis, the threshold was fixed to four times the standard deviation of the baseline. Since there was no objective way to determine the exact value of the multiplication factor of the standard deviation, it was determined by testing several different values on several datasets and choosing the value that provides the best discrimination between noise and signal. Critically, the same factor was used uniformly in every dataset, to ensure that the data was comparable.

3.9.2.5 Parameters Extraction
Several parameters were then extracted from these profiles. First, the activation threshold was defined as the lowest level of a peak power series triggering a significant activation in at least one electrode (Lim and Anderson, 2006). The characteristic frequency was defined as the frequency corresponding to the electrode with the highest activation two steps above the threshold (Snyder and Sinex, 2002). These two steps correspond to 10dB in the acoustic experiments and to 10% of the laser peak power in the INS experiments (Lim and Anderson, 2006). The width of activation at this level, which was calculated with the number of activated electrodes, was also obtained (Richter et al., 2011). The fourth parameter extracted is the mean spike count across the 16 channels for each stimulation level, which gives an indication of the averaged activation in the IC in a frequency-
independent way. This last parameter was obtained before applying the thresholding step in the post-processing algorithm. The baseline level was then removed, but the non-significant values are not set to zero.

**Figure 3.11:** Illustration of the extraction of the parameters from the processed spatial tuning curve.
Chapter 4: Does Infrared Neural Stimulation Applied To The Cochlear Nucleus Stimulate Central Auditory Pathways?

4.1 Hypotheses

H1. The application of pulsed infrared energy to the surface of the dorsal cochlear nucleus can generate responses in the central auditory system that can be recorded in the form of auditory brainstem responses and/or recordings from the inferior colliculus.

H2. The response to INS stimulation of the cochlear nucleus varies with parameters laser peak power, pulse width, and wavelength, and location on the cochlear nucleus.

In order to test these hypotheses, we designed experiments to identify whether such responses could be recorded and whether varying the stimulation parameters of the laser or its location would make any difference to the response. Altering the parameters (laser peak power, pulse width, wavelength) alter the total radiant energy per pulse delivered to the tissue. We therefore aimed to identify whether there was any change in the responses to INS if the parameters varied and if this variation could be accounted for by an overall change in the total radiant energy delivered as opposed to the result of the parametric variation itself. Therefore we designed a series of experimental test runs to test this hypothesis. Each cycle of testing is referred to as a ‘run’. The parameters for each of the runs are described in the tables below.

4.2 Laser Calibration Equations & Experimental Set-Up

In order to define the parameters for each of the runs, we must first appreciate how the stimulation parameters are related to each other. By understanding this relationship, we can construct series of runs that either keeps the total radiant energy constant when altering other variables, or we can calculate how altering a variable will alter the total radiant energy supplied per pulse. These equations were derived from unpublished work by
Dr Ken Hancock at Eaton Peabody Laboratories in collaboration with the manufacturers of
the laser.

Laser settings

P set power setting (%)

W pulse width (ms)

λ wavelength (nm)

We do not factor pulse rate into the power and energy calibration because we are interested
in the power and/or energy per pulse and not over a longer time scale.

Instantaneous power

Over most of the parameter space, the instantaneous power is given by:

\[ P_{\text{inst}} = (P_{\text{set}} \cdot P_0) \cdot \left[1 - 0.01(\lambda - \lambda_0)\right] \]  

(1)

The sensitivity \( S_L \) and offset \( P_0 \) characterize the calibration. They depend primarily on the
laser and optical fiber, and to some degree on ambient temperature and light. Thus, a
calibration should be performed for each specific setup, and from time to time to assess
stability. For the setup in our soundproof chamber (Capella laser S/N 9, and a 400-µm
fiber) the calibration parameters are \( S_L = 46.6 \, \text{W/cm}^2/\% \) and \( P_0 = -255 \, \text{W/cm}^2 \).

The second term in Eq. 1 is the wavelength correction and \( \lambda_0 = 1849 \, \text{nm} \).

Energy

The energy is the instantaneous power (equivalent to peak power) multiplied by the pulse
width, \( W \). (Note if the pulse width is in milliseconds, the energy comes out in mJ/cm\(^2\).)

\[ E = P_{\text{inst}} \cdot W \]  

(2)

Inverse equation

It may often be desirable to compute the laser settings necessary to achieve a specified
energy.

The most likely candidate is the power setting, in which case:

\[ P_{\text{set}} \]  

(3)

Where

\[ C_\lambda = 1 - 0.01(\lambda - \lambda_0) \]  

(4)
The animal was prepared in the manner described in the section 3.2. The location to place the tip of the laser for the parametric investigations was chosen to be A1 (see figure 3.5 & 3.6) In preliminary work by the group, this location on the cochlear nucleus consistently gave good responses (Lee, 2009). Our recording of ABR measured the response after each pulse of laser light, not after the whole pulse train.

4.3 ABR Recordings When Laser Parameters Are Varied

4.3.1 Effect Of Peak Power And Pulse Width

Effect of Radiant Energy Series

To investigate the effect of altering individual parameters on the ABR it was necessary to take into account that any effect on the ABR may be the direct result of the parametric change or could be due to a change in radiant energy caused by altering that parameter. Therefore experiments were devised and subdivided into ‘runs’ where the total energy delivered to the tissue could be held at a constant level whilst other parameters were varied. The laser calibration equations show that radiant energy delivered by each pulse was most heavily influenced by the pulse width and the laser peak power.

4.3.1.1 Methods: Effect Of Peak Power And Pulse Width

In order to investigate the effect on the ABR parameters, namely P2 & P4 latency and amplitude, of varying the radiant energy by altering peak power or pulse width, experiments comprising of the following runs were designed. In the first set of experiments, termed peak power series, the peak power was altered (16.3%– 69%) in order to generate different total radiant energies, between 100 and 700 mJ/cm². The remaining laser parameters were kept fixed at pulse rate 23Hz, wavelength 1849nm and pulse width
0.25ms. These parameters were chosen as preliminary work by Lee et al (2009) suggested reliable responses to INS could be generated. The exact parameters used are detailed in the table 4.1.

<table>
<thead>
<tr>
<th>Radiant energy (mJ/cm²)</th>
<th>Pulse Width (ms)</th>
<th>Peak Power (%)</th>
<th>Pulse rate (Hz)</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.25</td>
<td>16.3</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>200</td>
<td>0.25</td>
<td>25.1</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>300</td>
<td>0.25</td>
<td>33.9</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>400</td>
<td>0.25</td>
<td>42.7</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>500</td>
<td>0.25</td>
<td>51.5</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>600</td>
<td>0.25</td>
<td>60.2</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>700</td>
<td>0.25</td>
<td>69</td>
<td>23</td>
<td>1849</td>
</tr>
</tbody>
</table>

*Table 4.1:* Laser parameters for varying the radiant energy by adjusting laser peak power only.

In order to investigate the effect of varying radiant energy by altering pulse width, a further set of runs was devised. The laser peak power was fixed for all runs at 51.5%; therefore the any change in radiant energy resulted only from changing the pulse width. All other parameters remain fixed at the reference values (wavelength 1849nm, pulse rate 23 Hz the pulse rate at 23) Table 4.2 gives the exact laser parameters used for all runs in this series.
<table>
<thead>
<tr>
<th>Radiant Energy (mJ/cm²)</th>
<th>Pulse Width (ms)</th>
<th>Peak Power (%)</th>
<th>Pulse Rate (Hz)</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.05</td>
<td>51.5</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>200</td>
<td>0.1</td>
<td>51.5</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>300</td>
<td>0.15</td>
<td>51.5</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>400</td>
<td>0.2</td>
<td>51.5</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>500</td>
<td>0.25</td>
<td>51.5</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>600</td>
<td>0.3</td>
<td>51.5</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>700</td>
<td>0.35</td>
<td>51.5</td>
<td>23</td>
<td>1849</td>
</tr>
</tbody>
</table>

Table 4.2: Parameters for Pulse width Series, varying the radiant energy by adjusting the pulse width only.

4.3.1.2 Results Effect Of Peak Power And Pulse Width

Figure 4.1a shows an example of superimposed ABRs recorded from a single animal. In this example, the radiant energy was varied by altering the laser peak power only (16.3%-69%) whilst the pulse width remained constant at 0.25ms. Wavelength 1849nm and pulse rate 23Hz were held constant throughout. The blue line represents a total radiant energy of 100 mJ/cm², with the laser peak power set at 16.3%. The yellow line represents a total radiant energy of 400 mJ/cm² with peak power set at 42.7%. The red line represents a total radiant energy of 700 mJ/cm² with laser power set at 69%. These values are summarized in Table 4.3.

<table>
<thead>
<tr>
<th>Line Colour</th>
<th>Radiant Energy (mJ/cm²)</th>
<th>Peak Power (%)</th>
<th>Pulse Width (ms)</th>
<th>Pulse Rate (Hz)</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>100</td>
<td>16.3</td>
<td>0.25</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>Yellow</td>
<td>400</td>
<td>42.7</td>
<td>0.25</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>Red</td>
<td>700</td>
<td>69</td>
<td>0.25</td>
<td>23</td>
<td>1849</td>
</tr>
</tbody>
</table>

Table 4.3: Laser parameters for data plotted in figure 4.1, where radiant energy is varied by altering peak power and pulse width is kept constant
Figure 4.1a shows that at the lowest radiant energy (blue), there is no evidence of a multi-peaked response that could accurately be characterized as an ABR. When radiant energy increased to 400 mJ/cm² and then to 700 mJ/cm², a well-defined, multi-peaked response developed. The latency of P2 and P4 was shorter at 700 mJ/cm² when compared to 400 mJ/cm², suggesting a stronger ABR response. This figure supports the hypothesis that an increase in either peak power or radiant energy applied by the laser pulses leads to a greater responses to INS. Figure 4.1b Shows Figure 4.3 shows an expanded view of a full set of ABRs, when radiant energy is adjusted by increase in laser peak power. Radiant energy levels levels from 200-700mJ/cm² are plotted on a single expanded figure. The laser parameters used are as detailed in table 4.1.
Figure 4.1a: Examples of ABR responses when the radiant energy is adjusted by varying peak power, keeping pulse width constant.

Figure 4.1b: Expanded view of full set of ABR responses from 1 subject when radiant energy is adjusted by varying peak power, keeping pulse width constant.
Figure 4.2a. shows examples of ABRs from one animal when the total radiant energy is varied by adjusting the pulse width, whilst peak power is held constant. Wavelength and pulse rate were kept constant at 1849nm and 23Hz respectively. The blue line represents 100 mJ/cm² and a pulse width of 0.05 ms, the yellow line represents 400 mJ/cm² at a pulse width of 0.2ms and the red line represents 700 mJ/cm² with a pulse width of 0.35ms. The laser parameters for this figure are summarized in Table 4.4

<table>
<thead>
<tr>
<th>Line Colour</th>
<th>Radiant Energy (mJ/cm²)</th>
<th>Peak Power %</th>
<th>Pulse Width (ms)</th>
<th>Pulse Rate (Hz)</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>100</td>
<td>51.5</td>
<td>0.05</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>Yellow</td>
<td>400</td>
<td>51.5</td>
<td>0.2</td>
<td>23</td>
<td>1849</td>
</tr>
<tr>
<td>Red</td>
<td>700</td>
<td>51.5</td>
<td>0.35</td>
<td>23</td>
<td>1849</td>
</tr>
</tbody>
</table>

**Table 4.4:** Laser parameters for data plotted in figure 4.2 where radiant energy is varied by adjusting pulse width with peak power kept constant

Figure 4.2b shows a full set of ABRs recorded from a single animal using the parameters described in table 4.2.

Figures 4.2a & 4.2b show that at all the pulse widths tested in this animal, there was clear evidence of a multi-peaked response, consistent with an ABR. All of the plotted waveforms have similar P2 & P4 amplitudes and latencies. From this figure it can be seen that whilst the radiant energy varied with the pulse width, there was little difference in the ABR waveforms recorded in this series. The peak power remained constant throughout this series. The lack of alteration in response with radiant energy in this series, compared to the change observed in Fig. 4.1a & 4.1b, suggest that it was the peak power, not the radiant energy that was causing the change in ABR response in Fig. 4.1a & 4.1b.
**Figure 4.2a:** Examples of ABR responses when adjusting the pulse width varies the radiant energy, keeping laser peak power constant.

**Figure 4.2b:** Expanded view of full set of ABR responses from 1 subject when radiant energy is adjusted by varying pulse width, keeping radiant energy constant.
Figure 4.3 plots the P2 and P4 amplitudes against total radiant energy. Both the peak power series and the pulse width series are represented on the same figure for comparison purposes. The black lines represent the pulse width series, with the red lines representing the peak power series. The triangles represent the P2 amplitudes, with the squares representing the P4 amplitudes. The error bars represent the standard error from our sample of 8 animals. There is an intersection of the red and black lines at radiant energy level of 500 mJ/cm². This is because at this specific radiant energy, the stimulation parameters for both the peak power series and the pulse width series were identical (Pulse width 0.25 ms, peak power 51.5%, pulse rate 23 Hz, wavelength 1849 nm). Therefore only a single point is seen as the triangles and squares overlap and the same set of error bars are used.

Analysis using a repeated measures one way ANOVA show there is no significant effect at the p=0.05 level of the pulse width on P2 amplitude (F(6,49) = 0.372, p=0.892), nor pulse width on P4 amplitude (F(6,49) = 0.114, p=0.994). However, an effect of peak power on P2 amplitude is clearly seen with increasing amplitudes recorded as peak power increases. This effect is shown to be statistically significant using a one-way ANOVA at the p<0.05 level. (F(6,46) = 4.751, p=0.001). It also appears that P4 amplitude increases with peak power, but at the p<0.05 level, the result is not statistically significant, (F(6,46) = 0.878, p=0.519). Despite the appearance from the figure it is possible that the effect of peak power on p4 has a similar pattern to the p2 effect but the amplitudes were too low and variable to see the significance.
Figure 4.3: The effect of varying peak power (red) and pulse width (black) upon amplitude of P2 and P4 waves. N=8

Figure 4.4 shows the P2 and P4 wave latencies plotted against radiant energy, with the peak power series and pulse width series shown. Black lines represent the pulse width series, red lines the peak power series. Triangles represent the P2 latencies and squares represent the P4 latencies. There was a significant effect of peak power on p2 latency upon analysis with a one way ANOVA with p<0.05, F (6,46) = 23.961, p=<0.001). No significant relationship at the p, 0.05 level was identified using the same statistical analysis for peak power and p4 latency, F (6,45) = 1.446, p= 0.222). Pulse width did not have any significant effect (p<0.05) on P2 latency, (F (6,49)= 0.671, p= 0.673), nor on P4 latency, (F (6,48) = 0.107, p= 0.995).
Figure 4.4. Effect of laser peak power (red) and pulse width (black) on the latency of P2 and P4. N=8

4.3.1.3 Variability Of Data Between Subjects

As described in 3.9.1.3 and demonstrated above, the raw ABR waveforms were analysed and parameters of the waveforms extracted to aid in comparison between subjects for different stimulation conditions. The figures above include error bars representing the standard error to highlight that the original data was not without variation. Below are examples of ABR waveforms from different subjects with normal hearing who are exposed to identical test conditions. Figure 4.5a depicts the optically evoked ABRs in 2 subjects highlighting the maximum and minimum P2 amplitudes gained from each animal when identical stimulation conditions were used. These were peak power 70%, pulse width 0.25ms, pulse rate 23 Hz and wavelength 1849 nm. The waveforms are similar for both animals at both maximal (top pair) and minimal (lower pair) P2 amplitudes.
ABRs From 2 Subjects Exposed To Identical Optical Stimuli

Figure 4.5a. Examples of optically evoked ABRs recorded from 2 subjects. The top pair represent the maximal P2 amplitudes for the animal whilst the bottom pair represent the minimum P2 amplitudes for the animal. Laser parameters: peak power 70%, pulse width 0.25ms, pulse rate 23 Hz wavelength 1849 nm.

Figure 4.5b shows examples of ABRs after broadband clicks in 2 normal hearing subjects. This figure further highlights that whilst the extracted waveform parameters could vary between subjects when identical stimuli are presented, the waveforms themselves retain the same characteristics and therefore can be used for analysis even when responses in one animal may seemingly be small.

Examples of ABRs to Broadband Clicks From 2 Subjects

Figure 4.5b: ABRs to broadband clicks from 2 subjects
4.3.1.4 Discussion And Conclusions: The Effect Of Altering The Peak Power And Pulse Width On The ABR

The results above demonstrate that as the radiant energy increased with peak power there was a change in the ABR, with lower P2 latencies and greater P2 amplitude seen. However, when radiant energy was adjusted by changing the pulse width only, the extracted ABR parameters for P2 and P4 did not change significantly.

This suggests that when using different radiant energy levels, it is the peak power component that dominates the change in response seen in the optically evoked ABR.
4.3.2 Effect of Altering Wavelength On ABR

4.3.2.1 Methods: Effect of Altering Wavelength On ABR

The Capella laser (Lockheed Martin Acculight, Bothell, WA, USA) used for these experiments was a diode laser and therefore generates a limited range of wavelengths. The functional range of the laser is 1849nm to 1865nm. Whilst these different wavelengths would in theory deliver different amounts of energy to the tissue, in these differences are small. As the mechanism of INS is unclear, the possibility that there is a specific wavelength of light that may optimally stimulates tissues cannot be discounted. Therefore sets of experiments were designed to investigate the effect of adjusting the wavelength. In order to control for any effect of wavelength changes varying radiant energy, the first set of experiments involved altering the wavelength whilst we held the radiant energy constant. This was achieved by making small corrective changes to the peak power. Lower wavelengths deliver more energy to the tissue per pulse; hence increasing the wavelength was compensated by a small increase in peak power.

A second set of experiments was devised where only wavelength was adjusted, keeping the peak power constant (therefore allowing the radiant energy to vary freely). As wavelength increased, the depth of penetration of the laser energy increases. It was hypothesised that increasing the wavelength of infrared light applied increase the strength of the optically evoked ABR as more of the CN tissue is potentially exposed to infrared light.

Table 4.5 shows the different laser parameters used for experiments where wavelength was varied between 1849nm and 1865nm while the radiant energy kept constant at 597 mJ/cm². As described, the peak power was adjusted to maintain the total radiant energy at 597 mJ/cm². The pulse width was held constant at 0.25ms, the pulse rate held at 23Hz.
Table 4.5: Wavelength series where radiant energy remained constant as wavelength and peak power were altered.

An additional set of experiments was undertaken where wavelength alone was adjusted, allowing the total radiant energy to vary, whilst the peak power was held constant at 60%, pulse rate at 23 Hz and pulse width at 0.25 ms. Consequently, the radiant energy values for this series varied between 492.1 mJ/cm$^2$ and 597 mJ/cm$^2$. The laser parameters are summarized in Table 3.4

Table 4.6: Energy Series where radiant energy varies as wavelength is altered
4.3.2.2 Results: Effect of Altering Wavelength On ABR

Figure 4.6 shows the effect of wavelength upon the P2 and P4 amplitudes in conditions when the radiant energy was held constant (black lines) and in the condition where the peak power is held constant whilst the wavelength is altered, allowing the radiant energy to change (red lines). The data is presented for 8 subjects. The triangles represent the P2 amplitude, whilst the P4 amplitude is represented by squares. These results are taken from 8 subjects.

Analysis using a one-way ANOVA showed that there was no significant effect of wavelength across the range of wavelength tested. Specifically looking at the P2 amplitudes (triangles), there was no significant effect of varying wavelength when radiant energy is held constant (red line + red triangles), $F(8,71)= 0.499, p=0.852$ and when the radiant energy was allowed to vary as the wavelength was changed (black line + black triangles), $F(8,53) = 1.86, p=0.090$. Similarly, the P4 amplitudes (squares) did not significantly vary when the radiant energy was held constant (with peak power altering in small amounts to maintain constant radiant energy) $F(8,71) = 0.256, p= 0.977$ and when the radiant energy was allowed to drift as wavelength changed $F(8,53) = 0.086, p=0.999$ (with peak power remaining constant at 60%)
Figure 4.6 The effect of varying wavelength whilst keeping radiant energy constant (in black) and varying wavelength whilst allowing radiant energy to vary freely (in red) on a) the amplitudes of P2 and P4. N=8

Figure 4.7 shows the effect of the wavelength upon the P2 and P4 latencies. The laser parameters for these results, taken from the same 8 animals as used in figure 4.4 are summarised in tables 4.3 and 4.4. Black lines represent the conditions where the radiant energy was held constant (and peak power altered). Red lines represent the conditions where the total radiant energy was allowed to vary with the wavelength. The triangles represent P2 latency, whilst the squares represent the p4 latency. As clearly shown in this figure, and by one-way ANOVA, the flat lines show there to be no substantial difference across the range of wavelengths, nor any statistically significant effect of wavelength on P2 latency when the wavelength varied but radiant energy was held constant (F (8,71)= 0.633, p= 0.746). Neither was there an effect of wavelength on P4 latency using the same conditions of stimulation (F (8,71) = 0.044, p= 0.999).
When the wavelength varied and the total radiant energy was allowed to drift, one way repeated measure ANOVA showed that there was no significant effect \((p<0.05)\) of wavelength on P2 latency \((F(8,53) = 1.232, p=0.302)\), nor any significant effect on P4 Latency, \((F(8,53) = 0.042, p= 0.999)\).

![Diagram showing the effect of varying wavelength on P2 and P4 latencies](image)

**Figure 4.7:** Effects of the altering the wavelength at constant radiant energy (in black) and constant peak power (in red) on the latencies of P2 and P4. \(N=8\)

### 4.3.2.3 Discussion And Conclusion: The Effect Of Wavelength On ABR

These experiments show that there is no statistically significant effect of wavelength upon the extracted ABR features. It has been demonstrated that peak power drives changes in ABR whilst radiant energy itself does not have a significant effect. Therefore, in the condition where laser peak power was kept constant and radiant energy allowed to drift, an effect of wavelength that was mediated by radiant energy changes would not necessarily be identified. If there was a specific wavelength of light that optimally stimulated tissues, then we may have seen an effect of varying wavelength.
In the series where radiant energy was held constant, with small adjustments of peak power an effect on ABR could be anticipated, as an effect of peak power has already been demonstrated. No such effect was seen in this series.

This result could be explained by examining the range of peak powers used in the series where wavelength was altered and peak power was adjusted in order to keep a constant radiant energy. In this series, a range of peak power from 60%- 70.2% was used. This represents not only a narrow range (11.2%), but also a range of peak powers that is at the top of the range of peak powers previously tested. Therefore, it is possible that the response to INS may become asymptotic above a certain level of peak power. The relationship between peak power and P2 and P4 amplitudes appears to be non-linear.

4.3.3 The Effect Of Altering Pulse Rate On ABR

4.3.3.1 Methods: The Effect Of Altering Pulse Rate On ABR

In order to determine whether increasing the pulse rate had an effect upon the ABR, experiments were designed using increasing pulse rates, 3Hz- 83 Hz in steps of 10 Hz. By increasing the pulse rate, more pulses of light were delivered over the time course of stimulation; therefore the total energy delivered to the tissue over a fixed time interval increased. In order to prevent a 60Hz noise contamination from electrical phase interference in the evoked response, the pulse widths selected for testing were chosen so that they were not whole multiples of 60. It is important to reiterate at this stage that the ABR was recorded after each pulse from the laser, not after a train of pulses. Exogenous electrical stimulation of neural tissues can lead to a loss or reduction of response as the tissue enters a refractory period due to excessive stimulation (Glass, 1985). This set of experiments was undertaken to investigate whether there was any similar effect exerted by
INS with demonstration of accommodation or adaptation. It was hypothesised that there would be no effect of pulse rate upon the ABR parametrics as the pulse rates we are able to use are significantly less than those currently used by ABIs. This would suggest that the CN was robust enough to withstand such stimulation. However, as the mechanisms underpinning INS remain unproven, a cumulative effect of INS upon tissues cannot be discounted. Therefore the quantity of energy deposited over time could prove more important for laser stimulation. It is also possible that at higher pulse rates, energy was less able to dissipate, resulting in a heating effect on the tissue that could manifest as either an increase in ABR activity, or a decrease, reflecting damage to the CN induced by heating effect of infrared light.

Table 4.7 describes the laser parameters for each of the pulse rate series runs. Laser peak power was fixed at 70%, pulse width at 0.25ms, and wavelength was held steady at 1849nm. Pulse rate does not factor into the equations that define the radiant energy of each pulse, therefore each pulse has a constant radiant energy of 711mJ/cm², which is independent of the pulse rate.

<table>
<thead>
<tr>
<th>Laser Peak Power (%)</th>
<th>Pulse Rate (Hz)</th>
<th>Pulse Width (ms)</th>
<th>Wavelength (nm)</th>
<th>Radiant Energy (mJ/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>3</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
<tr>
<td>70</td>
<td>13</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
<tr>
<td>70</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
<tr>
<td>70</td>
<td>43</td>
<td>0.25</td>
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<tr>
<td>70</td>
<td>63</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
</tbody>
</table>

Table 4.7: Laser settings and calculated radiant energy for each run in the pulse rate series.
4.3.3.2 Results: The Effect of Altering The Pulse Rate On ABR

Figure 4.8 shows the effect of altering the pulse rate upon the P2 and P4 latencies of the optically evoked ABRs in 6 animals. The black line represents the P2 amplitudes, whilst the red line demonstrates the P4 amplitude. As the pulse rate increased, a decrease in P2 amplitude was observed, whilst there was no clear effect on P4 amplitude. Statistical analysis using a one-way ANOVA demonstrated the effect of pulse rate upon P2 was statistically significant. (F (6,40)= 5.229, p= 0.001). It appeared from the figure that there may have been a similar effect of pulse rate on P4 amplitude, but this was shown to be statistically insignificant at the p<0.05 level, (F (6,40) = 0.394, p= 0.878).

Figure 4.8 Effects of pulse rate on the amplitudes of P2 and P4. The effect of pulse rate on P2 amplitude was significant N=6,
Figure 4.9 shows the effect of altering pulse rate on the P2 and P4 latencies of resultant ABRs for the same 6 subjects as used for figure 4.7. The black line represents the p2 latency, with the red line showing P4 latency. An increasing pulse rate significantly decreased the P2 latency. This was assessed using a one-way ANOVA test (F (6,40) = 5.999, p= 0.0002). There is no significant effect upon the P4 latency at the p<0.05, (F (6,40)= 0.525, p= 0.785) level. Therefore we can observe that P4 latency is not affected by an increasing pulse rate.

![The Effect Of Altering Pulse Rate On P2 and P4 Latencies](image)

**Figure 4.9:** Effects of the pulse rate series on a) the amplitudes of P2 and P4, and b) the latencies of P2 and P4. The variations of the latencies of P2 are significant (p<0.05). N=8

### 4.3.3.3 Discussion And Conclusion: The Effect Of Pulse Rate On ABR

The results of the pulse rate series suggest that between 3 and 63 Hz, there is a significant effect of the pulse rate upon the amplitude and latency of P2, and the total amplitude of the signal. The decrease in P2 amplitudes with increasing pulse rate does suggest some
fatiguing of the response, which could be in keeping with an accommodation or adaptation response. However, the decrease in P2 latency does not fit with adaptation or accommodation. In fact, the decrease in p2 latency would suggest that the response to INS is keeping pace with increase pulse rates.

4.3.4 Summary & Discussion: The Effect of Altering Laser Parameters on ABR.

The above experiments demonstrated that the peak power has a significant effect on the ABRs parametrics. In addition, there is a statistically significant effect of pulse rate on P2 latency and P2 amplitude. Altering the pulse width, or the wavelength of infrared light applied to the cochlear nucleus does not have any significant effect on the ABR.

These experiments investigated whether varying the laser parameters would have an effect on the ABR. The initial investigations looking at varying the radiant energy by altering the pulse width and peak power respectively showed that radiant energy itself did not have an effect upon the ABR. We can deduce this because at the exposing the tissue to the same radiant energy does not result in the same ABR. Increasing the pulse width to increase the radiant energy had no effect, whilst increasing the radiant energy by increasing the peak power did have an effect. Infrared light exposure will cause a heating effect on tissues as energy is deposited into the tissues. Although there was a putative model for INS that suggested that the mechanism was down to heating of the tissues,(Wells et al., 2007a) (Wells et al., 2007a), work by Izzo et al (Izzo et al., 2007b) suggested that a heating effect alone cannot account for the change in optical responses observed with parametric variation. Therefore, we can anticipate that if heating is not the mechanism of activation, then radiant energy is unlikely to be a driver of the response to INS.
Izzo et al also identified that at smaller pulse durations e.g. higher pulse rates, a higher energy level (governed by peak power) was required to elicit responses in their guinea pig model. Whilst we did not explicitly test for this condition, it is interesting to note that an effect of pulse rate was also identified in INS of the peripheral auditory system. This group similarly had limitations in the pulse rate they could test at (100Hz).

The same group also identified that there was an effect of altering wavelength on CAP measurements. The lack of effect of wavelength in our experiments is inconsistent with the results of Izzo et al (Izzo et al., 2007b). This group calculated the optical penetration depths at different wavelengths for their laser (which had a functional range of 1844-1873nm). At lower wavelengths, there was a greater penetration than higher wavelengths. This finding was of particular significance in the cochlear as increasing penetration means more of the spiral ganglia would be exposed to infrared light as the wavelength decreased and perhaps a higher likelihood of stimulation. However, in the dorsal cochlear nucleus, it remains unclear which of the cell types may actually respond to infrared light. There was no reason to suspect that superficial stimulation is more or less likely to generate a response to INS than deeper tissues, which are accessed by lower wavelengths of light. However, the lack of influence of wavelength on the ABR coupled with the almost constant P2 and P4 amplitudes found when wavelength was varied and peak power was held constant suggests that across all wavelengths in this series, the stimulus applied to the CN was almost identical. This suggested that once again, it was the peak power that was driving the response in the wavelength series. No effect was seen in the condition where peak power was adjusted to keep radiant energy constant when wavelength was varied. This may be because by a peak power of 60%, a maxima of stimulation was being reached, with peak powers over 60% resulting in smaller incremental changes in response when measured by ABR. The parametric studies clearly demonstrate that peak power drove the response. The absence of influence of other parameters, with the exception of pulse rate,
does however raise a suspicion that responses to INS may not solely be down to the infrared light alone. An artifact response that is governed primarily by peak power could account for the results seen thus far.

The presence of an artifact response is further supported by comparing the ABR waveforms for acoustic and infrared stimulation. Figure 4.10 shows ABRs generated in the same animal with normal hearing when acoustic stimuli in the form of a broadband click electrical stimuli is applied directly the cochlear nucleus and also infrared stimuli to the cochlear nucleus. It can be seen from this example that the acoustic and infrared evoked ABRs have similar waveforms and latencies. The electrically evoked ABR is difficult to compare with optically and acoustic evoked responses as the electrical stimulus artifact interferes with initial waveform. This would further support a hypothesis that the peak power driven artifact may have been acoustic in nature.

**Figure 4.10:** Comparison of A) acoustic, B) electrical and C) infrared evoked ABRs in the same animal. Stimulus parameters- acoustic, 2KHz broadband click at 40 dB. Electrical Bipolar stimulation, 0.4 mA at 5 Hz. Laser parameters Peak power 70%, pulse width0.25ms, pulse rate 23 Hz
4.4 The Effect Of Location Of Stimulation on ABR

4.4.1 Methods: The Effect Of Location Of Stimulation on ABR

4.4.1.1 Dividing The DCN Into A Virtual Grid

The parametric studies were all undertaken with the laser fibre located over the rostral/lateral border of the CN, site A1 (see Fig 3.5 & 3.6). In order to investigate the effect of varying the site of stimulation on the surface of the DCN, Experiments were devised to test whether there was any effect of moving the site of stimulation.

Section 3.7 describes how that we the exposed surface of the DCN was subdivided into 12 territories. The Rostral caudal axis was divided into 3 rows, labeled A, B & C. The lateral-medical axis was divided into 4 columns, 1,2,3,4. This led to the development of a virtual grid. The Rostral caudal axis was divided into 3 rows, labeled A, B & C. The lateral-medical axis was divided into 4 columns, 1,2,3,4. This allowed the surface of the DCN to be subdivided into 12 territories. The exposed surface of the DCN was approximately 16mmx 12mm. Therefore, using a 400-micron fibre, 12 discrete locations with minimal overlap could be identified and spatially defined. Figure 4.9, is a photograph of the left DCN taken down the microscope, with the virtual grid superimposed on top. The IVth ventricle is seen medially and the temporal bone laterally. The cerebellar peduncle marked the rostral border, the remaining brainstem is caudal to the DCN.

Figure 4.11: Virtual grid super-imposed on a photograph of the DCN taken through our operative microscope.
Figure 4.12 shows a simplified cartoon of the virtual grid. The 2 dimensions can be seen on this grid, the 1-4 axis (or lateral to medial) and the A-C axis, that represents Rostral to Caudal. We defined the Lateral to Medial axis as Dimension 1 and the Rostral to caudal axis as Dimension 2. The 2 Dimensions are labelled on figure 4.11A and 4.11B also.

**Figure 4.12**: Cartoon of virtual grid subdividing the DCN into 12 sub-sites. Dimension 1 and Dimension 2 are also shown.

In order to help identify the presence of any location dependence, the DCN could be considered in terms of its 2 dimensions only. This allowed the creation of a system of Rows and Columns. Columns 1-4 represent progression along dimension 1 i.e. lateral to medial. Moving from Row A - Row C represents movement along Dimension 2 (the rostral caudal axis. A cartoon representation of the rows and columns is demonstrated in Figures 13a & 13b.
Column 1 contained A1, B1 and C1. Column 2 Contains A2, B2 & C2, Column 3 was comprised of A3, B3 and C3. Column 4 was A4, B4 and C4.

Using a similar principle Row A contained A1, A2, A3 & A4, Row B was B1, B2, B3 and B4. Finally, Row C was C1, C2, C3 and C4.
4.4.1.2 Stimulation Parameters For Location Studies

Experiments were designed to identify whether there was any effect on the ABR of moving the site of stimulation to different points across the DCN. All the parametric studies were undertaken with the laser fibre at site A1. For the sake of comparison, the laser stimulation parameters were kept fixed for all of the location studies. These parameters were peak power 70%, pulse width 0.25ms, pulse rate 23Hz and wavelength 1849nm. The specific stimulation parameters for each site are detailed below in table 3.8.

<table>
<thead>
<tr>
<th>Location</th>
<th>Laser Peak Power (%)</th>
<th>Pulse Rate (Hz)</th>
<th>Pulse Width (ms)</th>
<th>Wavelength (nm)</th>
<th>Radiant Energy (mJ/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
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<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
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<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
<tr>
<td>A3</td>
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<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
<tr>
<td>A4</td>
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<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
<tr>
<td>B1</td>
<td>70</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
<tr>
<td>B2</td>
<td>70</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
<tr>
<td>B3</td>
<td>70</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
<tr>
<td>B4</td>
<td>70</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
<tr>
<td>C1</td>
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<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
<tr>
<td>C2</td>
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<td>0.25</td>
<td>1849</td>
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</tr>
<tr>
<td>C3</td>
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<td>0.25</td>
<td>1849</td>
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<tr>
<td>C4</td>
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<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
</tbody>
</table>

Table 4.8: Location study laser settings

4.4.2 Results: The Effect Of Location On ABR

Figure 4.14 shows a typical example of the waveforms we recorded from a single animal across all 12 sites, using the laser stimulation parameters shown in table 4.8. The 12 squares each represent one of the sub sites described above and shown in figures 3.5 and 3.6. Each square is labeled to show which of the sites on the DCN it represents. These sites
are analogous to those shown in figure 3.9. In order to generate this type of figure, we have to take 12 recordings, one form each site. This was done by moving the laser fibre using a micro-manipulator.

![Example of ABR signals obtained from a single animal at different stimulation sites on the CN surface following INS, using the laser parameters as defined by Table 4.8.](image)

**Figure 4.14** Example of ABR signals obtained from a single animal at different stimulation sites on the CN surface following INS, using the laser parameters as defined by Table 4.8.

Figure 4.14 demonstrates that in the most lateral sites (e.g. A1, B1 and C1, corresponding to Column 1), multi-peaked responses, consistent with ABRs were present. At locations A2, B2 and C2 i.e. Column 2, there were still multi-peaked waveforms, but they were less recognizable as ABRs. It was observed that the waveform response differed markedly at the extreme ends of a Row e.g. A1 and A4. However, no such difference occurred at the two end sites of a column (e.g. A2 and C2). It was anticipated based on these observations
that the distance along dimension 1 (lateral to medial axis) had a larger influence on ABR presence than the distance along Dimension 2 (the rostral-caudal axis).

Figure 4.15 plots the P2 amplitudes for all the sites within each column for 7 animals. Each column is comprised of the cumulative data from 3 sites e.g. Column 1 data represents A1, B1 and C1. It can be seen that the amplitudes of P2 diminished as the stimulation site moved along Dimension 1 e.g. from lateral to medial, or from column 1 to column 4. This trend was statistically significant at the p<0.05 level using a one-way ANOVA test (F (3,53)= 7.44, p= 0.0003). Dimension 2 did not have any effect upon the P2 amplitude at the p<0.05 level (not plotted).

(F (2, 53)=1.7, p= 0.194). This analysis strongly supported the initial observation that it was Dimension 1 that had the greatest on an ABR being present at any given location. This finding also showed that the sites of stimulation that are most lateral resulted in the higher amplitudes ABRs.

![Figure 4.15: Effect of Dimension 1 (Lateral to Medial Axis) upon the P2 amplitude. N=7](image)
Figure 4.16 demonstrates an effect of Dimension 1 on the P2 latency. The data plotted comes from the same 7 subjects illustrated in figure 4.13. The effect was statistically significant at the p<0.05 level using a one-way ANOVA test, (F (3,53)=5.96, p= 0.0015). There was no significant effect of Dimension 2 upon the P2 latency at the p<0.05 level, (F (2,53) = 0.11, p= 0.897), (not plotted) These findings further supported our observation from Figure 3.11 that it was position along the Lateral-Medial axis that had the greatest influence on the ABR.

Figure 4.16: Effect of different column (lateral to medial) upon the P2 latency. N=7
This pattern of results was repeated for P4 amplitudes and P4 latencies. Using one way ANOVA tests, we showed that Dimension 1 had a statistically significant effect at the p<0.05 level upon P4 amplitude, (F (3,53)= 4.25, p= 0.0096), and also a significant effect on P4 latency, (F (3,53)= 5.05, p= 0.004). No significant effect of Dimension 2 on P4 amplitude was demonstrated at the P<0.05 level, (F (2,51) = 1.91, p= 0.159). Similarly, there was no statistically significant effect at the 5% level of Dimension 2 on P4 latency, (F (2,53) = 0.18, p= 0.840).

Figure 4.17 shows the average RMS for each of the 12 sites recorded from 7 animals. As already demonstrated, sites that are located laterally showed ABRs and these ABR’s had higher P2 and P4 amplitudes when compared to responses generated at more medial sites. Therefore the mean RMS of the responses for each site was plotted. The rationale was that in some of the sites, no ABR was evident; however there appeared to be some form of activity. Though RMS is a crude measure, it gives some indication of activity. Blue bars represent Row A, red bars represent Row B and yellow bars represent Row C. The error bars show the standard error. The horizontal axis is the Column numbers 1-4, corresponding to the Dimension 1 (Lateral to Medial axis). Similarly to the P2 and P4 analysis above, the RMS data in Figure 3.13 showed that sites placed more laterally resulted in larger RMS and by inference a larger response. This observation for the effect of Dimension 1 on RMS is significant at the p<0.05 level in a one-way ANOVA test, (F (3,53)= 29.77, p<0.0001).
Figure 4.17: Averaged RMS values of the ABR signals at each stimulation site (N=7)
4.4.3 Discussion and Conclusion-The Effect Of Location Of Stimulation On The ABR

The data generated demonstrated there was a location dependence of ABR results, with stronger responses generated at the lateral border of the DCN. The rostral-Caudal axis did not have any significant effect on the ABR. The basis of this location dependence was unclear if INS was truly acting by stimulating cells within the cochlear nucleus. There does not appear to be any population of cells that has a distribution within the DCN that would correspond to this spatial pattern of responses (Hackney et al., 1990, McCreery, 2008, McCreery et al., 1998). It was possible that that INS is acting directly on the auditory nerve as it entered the cochlear nucleus complex. Exogenous stimulation of the auditory nerve has been successfully demonstrated for both electrical and INS, though the INS was applied to the auditory nerve within the cochlear itself (Littlefield et al., 2010, Simmons, 1966). This would explain why responses are strongest at the lateral border of the DCN and weaker towards the midline. However, in order to directly stimulate the nerve directly, it would require the infrared light to pass through all of the dorsal cochlear nucleus tissue which would be 2-3mm in thickness. At the wavelengths used for these experiments, it was calculated that the infrared energy penetrates to a depth of around 700 micron (Hale and Querry, 1973).

It is feasible that the location dependence seen with responses to INS arises from an artifact mechanism. An optophonic artifact has been described (Teudt et al., 2011). If the artifact was within the subject’s hearing range, then the artifact being placed closer to the acoustic site of hearing may cause the location dependence we observed. A potential mechanism for the optophonic artifact activating the peripheral auditory pathways would be direct stimulation of the cochlear caused by vibration of hair cells, rather than as an auditory stimulus involving the tympanic membrane and movement of the ossicles.
4.5 The Effect of Varying Laser Parameters On Inferior Colliculus Recordings

4.5.1 Methods

4.5.1.1 Placement Of IC Electrode And Development Of Acoustic Map.

The first stage in recording from the IC was confirming the IC electrode was placed perpendicular to the ICCN, to take full advantage of the tonotopic organization of the iso-frequency laminae.

Figure 4.18a) shows a typical example of the acoustic map we derived after IC electrode placement. Each of the 11 boxes represents a different acoustic tone frequency presented to the animal. The vertical axis is the dB SPL of the presented tone, the horizontal axis refers to the electrode number (15 to 1). IC neuron firing rates above the base line are represented by the red end of the spectrum with lower firing rates, or those closer to spontaneous firing rate represented by colours at the blue end of the spectrum. Figure 4.18 a) shows that for the low frequencies, the maximal activity was seen in the high electrode numbers, whereas high frequencies cause maximal activity at the low electrode numbers. Figure 4.18b) shows how the characteristic frequency varies with the electrode number. High frequency laminae correspond to low electrode number and deeper insertion of the electrode, low frequencies are represented by high electrode number and more superficial penetration.
**Figure 4.18 a)**: Typical example of acoustic map derived after placement of IC electrode.

**Figure 4.18 b)** Representation of characteristic frequency for each electrode in this acoustic map with cartoon representation of IC showing correct dorsal-ventral alignment.

Figure 4.19 shows the electrode mapping for 9 subjects used for our IC data. We can see that although there was some variation in the electrode that was maximally activated at each acoustic frequency, the downward slope of each line confirms that in each case, the IC electrode was correctly placed within the ICCN, taking advantage of the tonotopic axis.
Figure 4.19: Electrode-frequency mapping for 9 subjects confirming the correct placement of the 16-electrodes array into the central nucleus of the IC.

The software developed in house for IC recording ran each experiment as a collated series of steps, requiring the operator to increase the peak power of the laser sequentially in fixed increments. Therefore all the series can all be considered as energy series. However, it was possible to independently vary all the other parameters (pulse rate, wavelength, pulse width), despite the requirement to conduct the series as a set of runs where the peak power of the laser was increased sequentially.

As with the ABR studies, the effect of altering radiant energy by adjusting peak power, the effect of radiant energy by adjusting pulse width, the effect of wavelength alone and the effect of pulse rate alone were all investigated.

IC recordings were made in the form of spike counts. The method of spike detection is described in section 3.9.2.
The IC experiments take longer to record than the ABR experiments, therefore the tissue was exposed to the potential heating effects of radiant energy for a longer period of time in total as compared to the time it takes to required to generate data for the ABR experiments. This was of specific relevance during the pulse width series. Therefore, for wavelengths > 0.25ms the peak power was raised to levels less than 70% (see table 4.11). The specific parameters are detailed below.

4.5.2 The Effect of Varying Peak Power And Pulse Width on IC Recordings

4.5.2.1 Methods: The Effect of Varying Peak Power And Pulse Width on IC Recordings

As described, the IC experiments all contained a series of runs with the peak power increasing incrementally.

For the peak power series, and pulse width fixed at 0.25 ms, the laser parameters were set as described in table 4.9

<table>
<thead>
<tr>
<th>Laser Peak Power (%)</th>
<th>Pulse Rate (Hz)</th>
<th>Pulse Width (ms)</th>
<th>Wavelength (nm)</th>
<th>Radiant Energy (mJ/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>1849</td>
<td>711</td>
</tr>
</tbody>
</table>

Table 4.9: The laser parameters for each of the runs for the peak power series.
Experiments were performed where the radiant energy was varied by adjusting pulse width. A range of pulse widths between 0.05ms and 0.4 ms was tested. To test each pulse width, the same steps of peak power as described in table 4.9, were used but increased the pulse width after the end of each 15 steps of the run. For example, to test at pulse width 0.10ms, the runs were as described in table 4.10.

<table>
<thead>
<tr>
<th>Laser Peak Power (%)</th>
<th>Pulse Rate (Hz)</th>
<th>Pulse Width (ms)</th>
<th>Wavelength (nm)</th>
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<tbody>
<tr>
<td>0</td>
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<td>0.10</td>
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</table>

**Table 4.10:** Laser parameters to test for effect of pulse width of 0.10 on IC activity

The full range of pulse widths tested and laser parameters are summarized in table 3.12. At pulse widths greater than 0.3, the maximum peak power used was reduced in order to reduce the chance of thermal damage to the cochlear nucleus.
Table 4.11: Table summarising the laser parameters for studying the effect of varying radiant energy by adjusting pulse width. The peak power increased in steps of 5% as described in table 4.10.

### 4.5.2.2 Results: The Effect Of Varying Peak Power And Pulse Width.

Figure 4.20 plots the effect of varying peak power (red lines) and varying the pulse width (black lines). The error bars represent the standard error for maximal IC activity recorded during that particular condition for the 8 subjects that are included in this sample. There was an effect of varying the peak power on the IC activity above the baseline. This effect was significant at the 5% level using a one-way ANOVA test ($F (14,308) = 13.06$, $p<0.0001$). There was no significant effect of varying the pulse width on IC activity, ($F (7,55) = 0.267$, $p= 0.963$).
4.4.2.3 Discussion And Conclusion

This result was consistent with the ABR recordings that demonstrated a statistically significant effect of varying radiant energy by adjusting peak power, but not by adjusting pulse width. This further underlines the peak power was the dominating parameter in responses to infrared neural stimulation.

Figure 4:20. The effect of varying peak power (red lines) and the effect of varying pulse width (black lines) N=8
4.5.3 The Effect of Varying Wavelength On IC Activity

4.5.3.1 Methods: The Effect of Varying Wavelength On IC Activity

In order to investigate the effect of varying the wavelength on IC activity, experiments were devised where wavelength was altered. As described above, the IC experiments consisted of testing each parameter at a series of peak power levels. For example in order to test wavelength 1849nm, the runs were set out as in table 4.12.

<table>
<thead>
<tr>
<th>Laser Peak Power (%)</th>
<th>Pulse Rate (Hz)</th>
<th>Pulse Width (ms)</th>
<th>Wavelength (nm)</th>
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</thead>
<tbody>
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<td>0</td>
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</tr>
<tr>
<td>40</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>45</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>50</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>55</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>60</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>65</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
</tbody>
</table>

Table 4.12: Laser parameters for measuring the IC response with a wavelength of 1849nm.

Testing was done across the entire wavelength range of the laser (1849-1865nm). The runs and laser parameters are summarized in table 4.13.
<table>
<thead>
<tr>
<th>Laser Peak Power (%)</th>
<th>Pulse Rate (Hz)</th>
<th>Pulse Width (ms)</th>
<th>Wavelength (nm)</th>
<th>Radiant Energy (mJ/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-70</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>483.5</td>
</tr>
<tr>
<td>0-70</td>
<td>23</td>
<td>0.25</td>
<td>1851</td>
<td>472.9</td>
</tr>
<tr>
<td>0-70</td>
<td>23</td>
<td>0.25</td>
<td>1853</td>
<td>462.2</td>
</tr>
<tr>
<td>0-70</td>
<td>23</td>
<td>0.25</td>
<td>1855</td>
<td>451.6</td>
</tr>
<tr>
<td>0-70</td>
<td>23</td>
<td>0.25</td>
<td>1857</td>
<td>441</td>
</tr>
<tr>
<td>0-70</td>
<td>23</td>
<td>0.25</td>
<td>1859</td>
<td>430.3</td>
</tr>
<tr>
<td>0-70</td>
<td>23</td>
<td>0.25</td>
<td>1861</td>
<td>419.7</td>
</tr>
<tr>
<td>0-70</td>
<td>23</td>
<td>0.25</td>
<td>1863</td>
<td>409</td>
</tr>
<tr>
<td>0-70</td>
<td>23</td>
<td>0.25</td>
<td>1865</td>
<td>398.4</td>
</tr>
</tbody>
</table>

Table 4.13: Laser parameters for effect of varying wavelength on IC activity. The peak power steps for each wavelength are the same as shown in table 3.13, with 5% increments.

4.5.3.2 Results: The Effect Of Varying Wavelength On IC Activity

Figure 4.21 shows the collated results for the series investigating the effect of wavelength on IC activity. The data is derived from mean spike counts taken at all power levels from 0%-70% in steps of 5%. The horizontal axis represents the wavelength, the vertical axis is the IC activity in spike counts above the baseline. The error bars show the standard error across subjects. There was no apparent relationship between the wavelength and an increase in IC activity. A one-way ANOVA at the p <0.05 level shows there to be no statistically significant relationship between wavelength and IC activity at the p<0.05 level, (F (8,61)= 0.999).
3.3 Discussion & Conclusion: The Effect Of Varying Wavelength On IC Activity

These results demonstrated that there is no significant effect on IC activity when the wavelength was varied. These findings are consistent with our previous experiments that showed no effect on the ABR when wavelength was varied. The consistency of the responses suggests that it is the peak power that is driving this response.

Figure 4.21: The effect of varying wavelength on IC activity (N=8)
4.5.4 The Effect Of Varying Pulse rate on IC Activity

4.5.4.1 Methods: The Effect Of Varying Pulse rate on IC Activity
In order to test the effect of pulse rate upon the IC activity runs were devised as follows.

The runs were organised as a power series. An example of the laser parameters for testing 33Hz is given in table 4.14.

<table>
<thead>
<tr>
<th>Laser Peak Power (%)</th>
<th>Pulse Rate (Hz)</th>
<th>Pulse Width (ms)</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>10</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>15</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>20</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>25</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>30</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>35</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>40</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>45</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>50</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>55</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>60</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>65</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>70</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
</tbody>
</table>

Table 4.14: Laser parameters for investigating the effect of pulse rate on IC activity

The summarized laser parameters for the entire pulse rate series from 3Hz- 83Hz is detailed in Table 4.15.
### Table 4.15: Summarized laser parameters for the effect of varying pulse rate on IC activity

<table>
<thead>
<tr>
<th>Laser Peak Power (%)</th>
<th>Pulse Rate (Hz)</th>
<th>Pulse Width (ms)</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-70</td>
<td>3</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>0-70</td>
<td>13</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>0-70</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>0-70</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>0-70</td>
<td>33</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>0-70</td>
<td>53</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>0-70</td>
<td>63</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>0-70</td>
<td>73</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>0-70</td>
<td>83</td>
<td>0.25</td>
<td>1849</td>
</tr>
</tbody>
</table>

#### 4.5.4.2 Results

Figure 4.22 shows the results for the effect of pulse rate on IC activity. The error bars show the standard error for the 8 subjects that are represented. The data is derived from mean spike counts taken at all power levels from 0%-70% in steps of 5%. We can see that there is no clear relationship between the pulse rate and the IC activity. A one-way ANOVA test at the 5% level does not demonstrate any statistically significant effect of pulse rate on IC activity, \( F(8,58) = 0.211, p = 0.987 \).
There was no significant effect of varying the pulse rate on the IC activity. The pulse rates used were much lower than physiological levels and lower than pulse rates used in cochlear implants or ABIs. Therefore, although an effect was not observed at the rates tested, an effect may not be evident unless much higher pulse rates are used. The results are not consistent with our ABR recordings, which showed lower P2 latencies and amplitudes as pulse rate increased.

**Figure 4.22:** The effect of pulse rate on IC activity (N=8)

### 4.5.4.3: Discussion and Conclusion

There was no significant effect of varying the pulse rate on the IC activity. The pulse rates used were much lower than physiological levels and lower than pulse rates used in cochlear implants or ABIs. Therefore, although an effect was not observed at the rates tested, an effect may not be evident unless much higher pulse rates are used. The results are not consistent with our ABR recordings, which showed lower P2 latencies and amplitudes as pulse rate increased.
4.5.5 Summary Of Results: The Effect of Parametric Changes on IC Activity

The studies we undertook suggested that only varying peak power has a significant effect on IC activity. The other tested parameters of wavelength, pulse width and pulse rate had no effect on IC activity. This is consistent with the data we gathered from our ABR experiments, which also suggested that peak power was the dominating parameter, rather than radiant energy. The lack of effect of wavelength and pulse width and ongoing domination of peak power indicated at this stage that the response to INS could be artifact, if an artifact can be described which is affected by peak power only and is independent of pulse width and wavelength.
4.6 The Effect Of Location of Stimulation On IC activity

4.6.1 Methods

Using the same virtual grid as shown in Figure 4.12 to subdivide the exposed CN into 12 sub-sites, a series of experiments was designed to identify whether any relationship existed between Dimensions 1 & Dimension 2 and IC activity (see figures 4.11 & 4.12 & 4.13).

The stimulation parameters for each run in the location series is detailed in table 4.16. As in the previous IC experiments, the runs were organised as a power series, with increments in the peak power up to a maximum of 70%.

<table>
<thead>
<tr>
<th>Laser Peak Power (%)</th>
<th>Pulse Rate (Hz)</th>
<th>Pulse Width (ms)</th>
<th>Wavelength (nm)</th>
<th>Radiant Energy (mJ/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>25</td>
</tr>
<tr>
<td>10</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>50</td>
</tr>
<tr>
<td>15</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>85.4</td>
</tr>
<tr>
<td>20</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>142.2</td>
</tr>
<tr>
<td>25</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>199.1</td>
</tr>
<tr>
<td>30</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>256</td>
</tr>
<tr>
<td>35</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>312.9</td>
</tr>
<tr>
<td>40</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>369.8</td>
</tr>
<tr>
<td>45</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>426.6</td>
</tr>
<tr>
<td>50</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>483.5</td>
</tr>
<tr>
<td>55</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>540.4</td>
</tr>
<tr>
<td>60</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>597.2</td>
</tr>
<tr>
<td>65</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>654.4</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
<td>711</td>
</tr>
</tbody>
</table>

*Table 4.16:* Laser parameters for the location studies
4.6.2 Results: The Effect Of Location On IC Activity

Figure 4.23 a) shows a typical example of the IC recordings from our twelve sub sites. Each of the horizontal axes of each blue square represents the electrode number and the horizontal axis represents the firing rate of the IC neurons, graded against a colour scale. The results represent responses from all peaks powers tested (0-70%) as detailed in Table 4.16. Figure 4.23b shows the ABR responses in the same animal for the same locations. The ABR recordings for each location were taken immediately after the IC data was collected for that location. Therefore, the optical fibre was not moved, so these tiles are providing data for exactly the same location. It can be seen from this example in 4.23a that there was broad activation (signified by numerous squares coloured yellow, orange and red indicating firing activity and hence greater IC neuron activity) in Column 1 and to a lesser extent column 2. There was also broad activation across Row A. This map can be contrasted with the more sharply defined acoustic map shown in figure 4.18a). These data suggested that there was a relationship between Dimension 1 and IC activity, with greater activity closer to the lateral edge of the CN and diminished activity towards the medial edge of the CN. This observation was previously shown to be statistically significant at the 5% level in ABR studies.
**Figure 4.23 a & b:** A) shows IC responses at all 12 locations, B) shows the ABR responses in the same animal for the same locations.

Figure 4.24 shows the effect of dimension 1 (lateral to medial axis) upon the IC activation for 7 subjects. The error bars represent the standard error. Sites in column 1 produced a higher average activation when stimulated using the parameters set out in table 4.16 than sites located within columns 2, 3 or 4. This effect of dimension 1 on IC activity was statistically significant at the 5% level on a one-way ANOVA test, $(F (3, 60)= 5.08, p= 0.004)$. There was no statistically significant effect of Dimension 2 (rostral-caudal axis) on IC activity at the 5% confidence level, $(F (2,60)= 1.24, p= 0.296)$. 
4.6.3 Discussion & Conclusion: The Effect Of Location On IC Recordings.

These experiments suggested that there was a statistically significant relationship between IC activity and Dimension 1, with the greatest activity seen towards the lateral border of the CN in column 1. These findings were consistent with the findings previously described for ABR recordings where we also found a statistically significant relationship between Dimension 1 and P2 amplitude and latency.

**Figure 4.24:** Effect of different locations from the lateral-to-medial axis on the IC averaged activation across the electrodes. N=7
4.7 General Discussion: ABR And IC Response To INS In Subjects With Normal Hearing

The ABR and IC results together suggest that when INS is applied to sites at the lateral border of the CN, it is more likely to stimulate the central auditory pathway than at sites that are more medially located.

The reasons for this location dependence are as yet unclear as little is known about the specific cell types that may be targeted by INS. However, these results alone would indicate that if INS does stimulate the central auditory pathways and that there is such a statistically significant effect of the lateral to medial axis then either there is a population of INS sensitive cells, similar in physiology to the bushy cells that generate the ABR, that is in greater concentration in these lateral borders of the DCN.

In view of the ABR and IC results, the possibility that the responses recorded in hearing animals were due to an optophonic artifact must be considered.

The optophonic artifact described in Teudt’s paper (Teudt et al., 2011) manifested as a broad click that would be within the audible range of our rat subjects.

Examining table 4.1 and figure 4.3 we can see that the lowest peak power that results in an identifiable ABR with extractable parameters is 25.1%. All of our ABR studies, with the exception of the peak power series detailed by table 4.1 and figures 4.3 & 4.4 were conducted with laser peak power levels in excess of 25.1%. A laser peak power of 25.1%, with pulse width of 0.25ms, wavelength of 1849nm and pulse rate of 23Hz corresponds to a calculated radiant energy of 200 mJ/cm².

Examining inferior colliculus responses, it can be seen that if the radiant energy was kept low, by keeping the peak power low, then there was an effect on the IC responses. However, varying the radiant energy by altering pulse width has no significant effect.
Figure 4.20 shows that as long as the peak power above 25.1%, corresponding to a radiant energy level above 200 mJ/cm², then there is a consistent IC response across all parameters.

The IC responses to INS were broad responses, with several of the IC channels stimulated at the same time as demonstrated in figure 4.23a. In other studies of INS, responses to INS were shown to be spatially specific, with only a small population of cells activated at the tip of the fibre.

These IC results however point to a much broader type of activation suggesting that stimulation was not focal, or that cell types in the CN that are sensitive to INS generate broadband responses. Analysis of the cyto-architecture of the CN does not suggest that there is a population of cells distributed across the CN in such a way that they would result in broad activation when stimulated (McCreery, 2008). IC responses of this type are consistent with a broadband stimulus, such as a click, rather than a narrow band pure tone stimulus as shown in Figure 4.18a.

If the response were in fact due to an auditory artifact, then it would be anticipated that the responses to INS would be stronger if the source of stimulus was closer to the acoustic hearing activations sites that are located within the temporal bone. The location studies strongly support this hypothesis with both ABR and IC responses strongly influenced by the lateral to medical axis. Locations closer to the lateral border of the cochlear nucleus were closer to the temporal bone and gave stronger responses than stimulation sites located further from the temporal bone. Again, examination of the cyto-architecture of the mammalian CN does not suggest that there is a distribution of cell types that would support this location dependence (McCreery, 2008, McCreery et al., 1998, Hackney et al., 1990).
Therefore, in order to characterise whether responses of the central auditory system to INS were the result of an optophonic artifact or genuine neural excitation, the degree of optophonic artifact in our experimental set up was characterised and then further experiments were done in acutely deafened subjects. These experiments are detailed in Chapter 5.
Chapter 5: The Effect Of Acute Deafening On Auditory Responses to INS

5.1 The Optophonic Artifact

In chapter 4 we identified that there were auditory responses to INS in hearing animals, the dominating parameter was peak power and the responses were strongest at locations closest to the temporal bone. The parametric results are not in keeping with the results of other groups using INS, particularly Dr Richter’s group using INS in the cochlear. Furthermore, there is no cyto-architectural rationale for the location dependence. Therefore, we must consider that the responses seen are down to the optophonic artifact described by Teudt (Teudt et al., 2011). We therefore measured the optophonic artifact in our laboratory set up. This is described below.

5.1.1 Method: Measuring the acoustic artifact

These experiments also took place in our sound-attenuated booth. The same 400-micron optical fibre used for all our experiments was secured using a micromanipulator (Narasinhe International, East Meadow, NY, USA). The tip of the fibre was facing in a horizontal plane, in mid air. We positioned a microphone (built by Eaton Peabody Laboratory Engineering Department) perpendicular to the tip and at a distance of 3-4mm from the tip. The perpendicular orientation was undertaken to reduce any photoelectric effect that could have interfered with our recordings. The microphone was connected to our amplifier (see section 3.5.3).

In order to measure the optophonic artifact, we used the same parametric variations as we had used for the ABR parametric studies. We recorded the sound produced when varying the peak power, pulse width and wavelength. Pulse rate was not tested as it merely reflects
how quickly the pulses come within a pulse train, there is no intrinsic change in the pulse itself, and therefore we would not anticipate any change in the optophonic artifact recorded.

Using in house software, we were able to record the sound levels of any acoustic artifact generated at the tip of the optical fibre when the laser was firing pulses.

5.1.2 Results: The Effect Of Varying Laser Parameters On The Optophonic Artifact

5.1.2.1: The Effect of varying peak power on the optophonic artifact

Table 5.1 details the parameters used for measuring the optophonic artifact.

<table>
<thead>
<tr>
<th>Peak Power %</th>
<th>Pulse Rate Hz</th>
<th>Pulse Width ms</th>
<th>Wavelength nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>10</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>20</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>30</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>40</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>50</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>60</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
</tbody>
</table>

Table 5.1: Laser parameters for measuring effect of peak power on acoustic artifact

Figure 5.1 plots the sound level recorded against the peak power. This clearly demonstrates that there is an acoustic artifact. Furthermore, as peak power increased above 20%, we recorded sound levels in excess of 40dB SPL. At 70% peak power, the sound level had reached 53dB SPL.
5.1.2.2 The Effect Of Varying Wavelength On The Optophonic Artifact

Table 5.2 details the laser parameters for investigating the effect that varying the wavelength had on acoustic artifact.

<table>
<thead>
<tr>
<th>Peak Power %</th>
<th>Pulse Rate Hz</th>
<th>Pulse Width ms</th>
<th>Wavelength nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.25</td>
<td>1854</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.25</td>
<td>1858</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.25</td>
<td>1862</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.25</td>
<td>1866</td>
</tr>
</tbody>
</table>

Table 5.2: Laser parameters for measuring the effect of varying wavelength on acoustic artifact

Figure 5.2 plots the wavelength plotted against the recorded sound level. We observed that there is an acoustic artifact across the range of wavelengths. There is no change in the sound levels as wavelength varies. Furthermore, the acoustic level is 53 dB SPL, the same acoustic level as generated by a peak power of 70% as shown in figure 5.1. This results suggests that not only is the optophonic artifact present, but it is driven by the peak power level.
5.1.2.3 The Effect of Varying Pulse Width on the Optophonic Artifact

Table 5.3 details the laser parameters used to investigate the effect of varying the pulse width on the optophonic artifact.

<table>
<thead>
<tr>
<th>Peak Power %</th>
<th>Pulse Rate Hz</th>
<th>Pulse Width ms</th>
<th>Wavelength nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>23</td>
<td>0.05</td>
<td>1849</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.1</td>
<td>1849</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.15</td>
<td>1849</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.2</td>
<td>1849</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.25</td>
<td>1849</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.3</td>
<td>1849</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.35</td>
<td>1849</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>0.4</td>
<td>1849</td>
</tr>
</tbody>
</table>

Table 5.3: Laser parameters for investigating the effect of varying pulse width on the optophonic artifact

Figure 5.3 plots the acoustic artifact sound level in dB SPL against the pulse width. These experiments demonstrated that for all the pulse widths tested, there was an optophonic artifact. Furthermore, the sound level of the artifact was consistent at a level of 53 dB SPL.
5.1.3 Discussion & Conclusion: The Effect Of Parametric Variation On The Optophonic Artifact

The results shown in figure 5.1-5.3 demonstrated that at laser parameters used for experiments detailed in chapter 4, there is an optophonic artifact generated. In addition, the optophonic artifact increases with the peak power. Furthermore, the acoustic artifact was at a consistent level of 53 dB SPL across the ranges tested. This sound level was identical to the sound level recorded in the peak power series when the laser was set at a peak power of 70%. The artifact itself manifests as a broadband click.

These findings suggest that the optophonic artifact drives the responses recorded in the parametric studies from chapter 4. In addition, the location dependence identified would also correlate with an optophonic artifact as the locations with strongest responses were also closest to the temporal bone and the acoustic hearing mechanisms.

In order to identify whether the optophonic artifact drove the responses to INS shown in chapter 4, it was necessary to perform further experiments to control for the optophonic artifact.
artifact. We re-performed the same experiments as described in chapter 4 using animals that were acutely deafened by sectioning of the VIIIth nerve (see section 3.3.4).

5.1.4 Hypothesis: The Effect Of Acute Deafening On INS Evoked Responses Of The Central Auditory Pathway

H1: Based on the findings in Chapter 4 and the characterization of the acoustic artifact in this preparation, Responses to INS are driven by the optophonic artifact. Therefore, it is expected that acute deafening of the subject to lead to an absence of any responses to INS in terms of ABR or increases in IC activity above baseline.

5.2 Subject Selection, Acute Deafening And Verification Of Deafness

5.2.1 Subject Selection

A total of 15 subjects were used for all the deafening experiments. As the process of deafening is inherently physically and physiologically traumatic, we were not able to gather high quality ABR and IC data for both parametric and location studies from each subject. This would have subjected each subject to 6-7 hours of experimentation. Therefore we strategically chose which of the types of experiments to undertake on each subject, depending on how much high quality data we had already gathered for a particular test condition. In some subjects, we were able to perform experiments both pre and post deafening. For these subjects, their data contributes to both chapter 4 results and chapter 5. However, we must clarify that in chapter 5 when pre and post deafening results are displayed on the same figure, they are not necessarily pre and post deafening in exactly the same subjects. We are presenting the most reliable data we have across a range of subjects. The results shown for hearing subjects are the same as those from Chapter 4.
5.2.2 Acute Deafening Technique and Acoustic Verification

The subjects were deafened in the manner described in section 3.3.4. Deafening was confirmed by attempting to record ABR responses to acoustic stimuli presented to the deafened ear. The optophonic artifact at 70% was measured at 53 dB SPL, therefore a shift in hearing thresholds to click responses above 60 dB SPL was taken as evidence of deafening and disconnection of the peripheral and central auditory pathways. If the hearing threshold was raised relative to the initial hearing thresholds measured at the start of animal preparation, VIIIth nerve sectioning was reattempted and acoustic thresholds re-measured.

5.2.3 Confirmation Of Integrity Of Cochlear Nucleus After Deafening

To ensure the process of deafening had not damaged the CN itself, electrically evoked ABR (eABR) responses were recorded (see 3.6.2). The electrically evoked ABRs were inspected and compared to examples of eABRs the lab had generated in other work. If there was no clear multi-peaked response consistent with an eABR, the animal was rejected for further testing with an assumption that the CN was likely damaged and responses further attempts to elicit responses may lead to inaccurate results. Figure 5.8 gives examples of eABR pre and post deafening.
5.3 The Effect of Acute Deafening on ABRs when Laser Parameters Were Varied

5.3.1: Methods
The animals were prepared in exactly the same way as described in section 3.2

Laser parameters are detailed below

5.3.1.1: Methods: The Effect Of Acute Deafening On ABR When Peak Power Is Varied

The laser parameters for this set of experiments was the same as described in section 4.3.1.1 and table 4.1

5.3.2.1: Methods: The Effect Of Acute Deafening On ABR When Pulse Width Is Varied

The laser parameters for this set of experiments was the same as described in section 4.3.1.1 and table 4.2

5.3.1.3: Methods: The Effect Of Acute Deafening On ABR When Wavelength Is Varied

The laser parameters for this set of experiments was the same as described in section 4.3.1.1 and table 4.6
5.3.2 Results & Discussion: The Effect Of Acute Deafening On ABR

5.3.2.1 Results: The Effect Of Acute Deafening On ABR When Peak Power Is Varied

Figure 5.1 shows the results for the effect of varying peak power upon the mean RMS of the resultant signal. The red line shows the data for animals with normal hearing (N=8). These are the same animals as used in Chapter 4. The black lines show the data for subjects after acute deafening (n=3). We observed that in the acutely deafened animal, there was no obvious multi-peaked ABR response generated by INS. Therefore, it was impossible to extract parameters such as P2 and P4 latencies and amplitudes. In order to quantify the difference in the responses between hearing and non-hearing animals, we chose to use the RMS of the response on the grounds that a multi-peaked response would generate a higher RMS than a noise response. We can see from figure 5.1 that in subjects with normal hearing, there was a significant effect of varying peak power on the RMS of the signal, F (6,52) = 3.740, p= 0.004 in the hearing animals. However, the RMS in deafened animals does not appear to increase with peak power. The mean RMS of the deafened subjects across all peak powers tested is in this series is 0.1234 +/- 0.0061 (standard error). In conditions where we recorded the RMS of the signal with the laser at peak power 0%, a mean RMS of 0.151 +/- 0.025 standard error was recorded. A paired t-test of the RMS (deafened power series) vs. RMS (laser peak power 0%) reveals p= 0.3415. This suggests that there is no significant difference in RMS between conditions when there is no INS applied and when the animal is acutely deafened. A one way ANOVA for the highest tested energy level tested, tested 700mJ/cm² shows a significant effect of deafening on the RMS (F (1,9) 9.467) p=0.015). At 100 mJ/ cm², an energy level that had previously not demonstrated a significant response in hearing animals (see figure 4.3), the ANOVA test suggests there is no significant effect of deafening on the response at this peak power level. (F (1,10)= 0.039, p= 0.847).
Figure 5.4: The effect of acute deafening on the responses to INS when radiant energy is varied by adjusting peak power. Deafened (black) N=3, Hearing (red) N=8
5.3.2.2 Results: The Effect Of Acute Deafening On ABR When Pulse Width Is Varied

Figure 5.5 shows the results for the effect of varying pulse width upon the mean RMS of the resultant signal. The red line shows the data for animals with normal hearing (N=8) These are the same animals as used in Chapter 4. The black lines show the data for subjects after acute deafening (N=3). Once again, we observed that in the acutely deafened animal, there were no obvious multi-peaked ABR response generated by INS, therefore P2 and P4 parameter extraction was not possible. Therefore RMS was calculated for the responses. We know from our experiments in hearing animals that at the laser parameters used, there were ABRs generated across the range of pulse widths, we also know that that there was no significant effect of changing pulse widths on the P2 and P4 extracted parameters. Therefore it was not unexpected to find the RMS values across the range of pulse widths to be consistent. However, acute deafening reduces the mean RMS across the range of pulse widths tested. The mean RMS in the deafened subjects was 0.1235 +/- 0.0067 (standard error). The mean RMS for conditions where the laser was switched off and ABRs recorded was 0.151 +/- 0.025 standard error A two-tailed paired t-test of these 2 conditions demonstrated p= 0.3415. Therefore there is no statistically significant difference between conditions when the laser is at peak power level of 0% and the conditions when the animal was acutely deafened and exposed to INS.

ANOVA testing demonstrates that at a radiant energy level of 700mJ/cm², deafening has a significant effect on the RMS, F (1,9)= 8.94, p = 0.017. At a radiant energy level of 100mJ/cm² deafening also has a significant effect on the RMS, F (1,9) = 6.237, p= 0.004. Figure 5.5 shows that in hearing subjects, the RMS values were higher than those in the acutely deafened animals.
The Effect Of Deafening On ABR Responses When Pulse Width Is Varied

**Figure 5.5:** The effect of acute deafening on the responses to INS when radiant energy is varied by adjusting pulse width. Deafened (black) N=3, Hearing (red) N=8
5.3.2.3 Results: The Effect Of Acute Deafening On ABR When Wavelength Is Varied

After acute deafening, we were unable to identify any multi-peaked waveform response that could be identified as an ABR. Mean RMS values were calculated for animals with normal hearing and acutely deafened subjects. Figure 5.5 plots the RMS values against wavelength for subjects, with data plotted for both deafened and un-deafened subjects. The red line represents the effect seen in hearing animals (n=8). The black line represents results from animals that have been acutely deafened (n=4). The mean RMS across all values was 0.425 +/- 0.063 standard error, in animals with normal hearing. However, in acutely deafened animals, the RMS values were reduced, with a mean RMS of 0.096 +/- 0.0015. A two tailed paired t-test comparing the mean RMS in deafened animals to the Mean RMS in zero stimulation conditions (laser peak power at 0%), 0.151 +/- 0.025 standard error) shows an insignificant result (p= 0.04613).

One-way ANOVA tests demonstrate that at each wavelength, deafening has a statistically significant effect on the RMS response (p<0.05). For example, for deafened subjects at 1849nm F (1,9)=15.09, p=0.004.

The Effect Of Deafening On ABR Responses When Peak Power Is Varied

![Figure 5.6: The effect of acute deafening on the responses to INS when wavelength is varied. Deafened (black) N=4, Hearing (red) N=8](image-url)
5.3.3 Discussion: The Effect of Acute Deafening On ABR When Laser Parameters Are Varied

5.3.3.1 The Effect Of Acute Deafening On ABR When Peak Power Is Varied
In acutely deafened subjects there was no increase in RMS seen when peak power was increased. There was also no significant difference seem between the mean RMS in conditions when the laser was switched off i.e. no infrared energy applied to the cochlear nucleus and the mean RMS in acutely deafened subjects. These findings suggest that there was no response to INS observed in acutely deafened subjects and supports the hypothesis that responses generated in hearing animals were entirely due to the optophonic artifact.

5.3.3.2 The Effect Of Acute Deafening On ABR When Pulse Width Is Varied
Acute deafening removed the response to INS across all pulse widths tested. We can be confident that the responses seen in hearing animals were the result of the optophonic artifact.

5.3.3.3 The Effect Of Acute Deafening On ABR When Wavelength Is Varied
Acute deafening reduces the RMS of the response to the same level as background noise. Responses in the form of ABR were not identifiable after disconnection of the peripheral and central auditory systems. This suggests that the optophonic artifact drives the responses recorded in the form of ABR and demonstrated in Figure 4.5.
5.4 The Effect of Deafening On INS Evoked ABRs: Location Studies

5.4.1 Methods: The Effect Of Acute Deafening On ABR - Location Studies

The subjects were prepared in the same manner as described in section 3.2.

The location studies in deafened animals were undertaken in the same manner as described in section 4.4.1. Laser parameters were identical to those in table 4.8.

5.4.2 Results: The Effect Of Acute Deafening On ABR - Location Studies

Figure 5.7 shows typical results pre and post deafening. This particular example shows pre and post deafening results for the same animal. The laser parameters used are the same as described in table 4.8. The locations tested on the CN, identified on the figure, are B1, B2, B3 & B4. In addition, the tip of the optical fibre was placed on the temporal bone directly and in the IVth ventricle (see figures 3.5 & 3.6). The figure demonstrates that in hearing animals, there was a clear ABR response in B1, with diminishing responses as the fibre is moved laterally. There is no ABR response in B4. The response in B4 was also very similar to the response at the IVth ventricle. There is a strong response seen when the fibre is placed on the temporal bone. This finding alone would have supported the hypothesis that responses to INS were in fact acoustic responses to the optophonlic artifact and not driven by stimulation of the cochlear nucleus itself. After deafening, we can see that there are no clear multi-peaked waveforms generated. The clear responses previously seen when the fibre was placed on the temporal bone or at location B1 were absent, replaced with noise.
**Figure 5.7:** Typical example of pre and post deafened ABR responses at different locations from the same subject.

Figure 5.8 shows examples of an electrically evoked ABR from the same animal from figure 5.7. The stimulation parameters are detailed in section 3.6.2. The stimulation amplitude is 0.3mA in this example. We can see multi-peaked responses in pre and post deafening, confirming that the deafening technique has not paralysed the central auditory pathway’s ability to generate ABRs.

**Figure 5.8:** Example of electrically evoked ABRs pre and post deafening.
Figure 5.9 plots the mean RMS at locations B1, B2, B3 & B4. In Blue, the values for hearing subjects are shown (n=8). In Red are the results from 4 deafened subjects (n=3).

The reduction in RMS is clearly shown at Sites B1 & B2. The Mean RMS value for all the values in these data sets for deafened subjects is $0.032 \pm 0.011$. The mean RMS for Site B1 in hearing animals is $0.405 \pm 0.12$. A paired t test 1 tail test shows a significant difference between these RMS values ($p<0.005$). At site B4, the mean RMS in hearing animals is $0.067 \pm 0.077$. In acutely deafened subjects, the mean RMS is $0.058 \pm 0.052$. A two-tailed T-test shows that these results were not significantly different ($p=0.746$).

There was also no significant difference between the RMS at sites B1 or B4 in deafened subjects and the RMS in conditions where the laser was switched off and recording is made of background noise only ($p>0.05$)

RMS Values Across Row B in Hearing and Deafened Subjects

![RMS Values Across Row B](image)

**Figure 5.9** RMS values for Locations B1, B2, B3 & B4 in hearing subjects (n=8) and deafened subjects (N=3)
Figure 5.10 displays the mean RMS energies for all of the sites for both hearing (n=8) and acutely deafened subjects (n=3). Data was aggregated from a total of 15 different subjects to ensure all data sets were complete. There were no multi-peaked responses consistent with ABRs in the deafened subjects making parameter extraction impossible. Therefore, RMS was used as a means of comparing hearing and deafened subjects. The legend identifies which colours represents the different rows (rostral to caudal axis) the numerical figures on the horizontal axis represent the columns (lateral to medial axis). The mean RMS values for hearing animals in column 1 are higher than in any of the other columns as would correspond with our earlier findings that the strongest responses tend to the lateral border of the CN (see section 4.4.2). An ANOVA test of the effect of deafening upon the lateral to medial location dependence shows a statistically effect of acute deafening F (3,126)= 14.67, p<0.001. A statistically significant effect of deafening on the RMS values for all locations was demonstrated, (F (1,126)= 24.50, p<0.001).

**Comparison Of RMS Values At All Locations Pre and Post Deafening**

![Comparison Of RMS Values At All Locations Pre and Post Deafening](image)

**Figure 5.10:** RMS values for all sites tested on the CN. Hearing (n=8), Deaf (n=3)
5.4.3 Discussion: The Effect Of Acute Deafening On ABR - Location Studies

Acute deafening has a significant effect on the ABR responses at all sites. The RMS of resultant signals has been shown to be the same as conditions where no stimulation is present i.e., the same as the background noise of the system. Theses studies further highlight the association between responses to INS in hearing animals and the optophonic artifact. The responses in hearing animals were strongest at locations closest to the acoustic detection apparatus housed within the temporal bone. Nerve sectioning, without damage to the CN itself (see figure 5.8) removed the response to INS at all locations. The location dependence previously seen was no longer evident and ABR responses absent from all tested locations.

5.5: Discussion: The Effect Of Deafening On Auditory Brainstem Responses To INS

In conditions where an animal was acutely deafened, we were unable to elicit an INS evoked ABRs. There was no effect of peak power variation seen, contrasting with the results seen in hearing subjects. In addition, there was no effect of location on the responses.

It can be seem from the recordings of the optophonic artifact that at the peak powers used for these studies, there was an audible optophonic artifact, manifesting as a broadband click. The devastating effect of disconnecting the peripheral and central auditory pathways on the ABR strongly implicates the optophonic artifact as being solely responsible for response to INS recorded in subjects with normal hearing. If INS was unable to elicit ABRs, it almost entirely precludes the possibility that an infrared driven ABI would be possible. However, as the function of the DCN is likely that of a processor, with extensive
internal circuits and few outputs (McCreery, 2008) it was appropriate to identify whether there was any response to INS, in form of changes in baseline Inferior colliculus activity. It was already shown that INS has an effect on IC activity (measured as a change spike count over the baseline) in the hearing subject. The effect of acute deafening on the acutely deafened subject is described below.
5.6 The Effect Of Acute Deafening on INS-Evoked Inferior Colliculus Responses

In Chapter 4, it was demonstrated that responses to INS in subjects with normal hearing could be recorded from the central nucleus of the inferior colliculus (ICCN) using a multi-channel penetrating electrode. Investigations in hearing subjects showed that IC activity in response to INS were sensitive to the peak power, with a statistically significant effect (p<0.05.) There was no statistically significant effect of varying the pulse width, pulse rate or the wavelength. However, for all these parametric conditions, IC activity above the baseline was recorded, suggesting activity in the IC in response to INS. It was suggested that the responses could have been driven by the optophonic artifact. In 5.1 it was shown that at laser peak powers we used in experiments, there was an audible acoustic artifact. Acute deafening removed the response to INS across all parametric variations and at all locations.

In order to investigate whether there was any residual effect of INS after acute deafening, we repeated the experiments detailed in section 4.4. The rationale was that whilst ABRs are produced when bushy cells are stimulated, there may be cell types within the DCN that respond to INS but may not generate ABRs. These cells may activate neural pathways that lead to the IC, resulting in a change in baseline activity in the IC, measured as spike counts.
5.6.1 Methods: The Effect Of Acute Deafening on INS-Evoked Inferior Colliculus Responses When Laser Parameters Were Varied

The animals were prepared in the same manner as described in section 3.2. Laser parameters are detailed below.

5.6.1.1 Methods: The Effect Of Acute Deafening On IC Responses When Peak Power Is Varied

The laser parameters for this set of experiments were as described in 4.5.2.1 and table 4.9.

5.6.1.2 Methods: The Effect Of Acute Deafening On IC Responses When Pulse Width Is Varied

The laser parameters for this set of experiments were as described in 4.5.2.1 and table 4.10.

5.6.1.3 Methods: The Effect Of Acute Deafening On IC Responses When Wavelength Is Varied

The laser parameters for this set of experiments were as described in 4.5.3.1 and table 4.10.

5.6.2 Results: The Effect Of Acute Deafening On IC Responses When Laser Parameters Were Varied

5.6.2.1 Results: The Effect Of Acute Deafening On IC Responses When Peak Power Is Varied

Figure 5.11 displays the average spike counts above baseline, averaged across all the 16 channels in the electrode at different peak powers. The results in hearing subjects (n=8) are displayed in red, whilst the results for deafened subjects (n=3) are in red. The data shows that in acutely deafened animals, there is no significant response above baseline IC activity. One-way ANOVA tests demonstrate that acute deafening has a significant deleterious effect upon the IC responses to INS at all peak power levels above 10%. At peak power of 5% & 10%, there is no significant effect of deafening p>0.05. At these levels, there is no evidence of IC activity in hearing subjects, hence an insignificant effect of deafening is not unanticipated. At a peak power of 50% equivalent to a radiant energy level of 483.5 mJ/cm², F (1,31)= 54.82, p<0.001. At 70% peak power, equivalent to radiant energy of
The Mean baseline IC spike count activity was at 0% laser peak power for hearing subjects was 0.0139 +/- 0.002. Mean spike count activity at 70% peak power in deafened subjects was 0.0132 +/- 0.02. A two-tailed T-test demonstrates there is no significant difference between these baseline values (p=0.337).

These results indicated that there is effect on IC baseline activity when peak power is varied in acutely deafened animals.

**Figure 5.11:** The effect of acute deafening on the IC response to INS when radiant energy is varied with respect to peak power. Deafened subjects (black) N=3. Hearing subjects (red), N=8.
5.6.2.2 Results: The Effect Of Acute Deafening On IC Responses When Pulse Width Is Varied

Figure 5.12 shows the effect of pulse width on IC activity in hearing subjects (n=8), plotted in red and deafened subjects (n=3) plotted in black. The effect of adjusting peak power and pulse width of INS in hearing subjects has already been described in 4.4.2.2 and is shown in figure 4.19.

In hearing animals, ANOVA tests demonstrated no significant effect of varying the radiant energy upon IC activity (p>0.9). In deafened animals, there was no increase in mean IC activity (above the baseline activity (measured as laser power 0% in hearing animals). A two tailed T-test to compare the mean IC activity in deafened subjects and the mean activity at 0% laser power reveals p>0.05, indicating there was no significant difference between these stets of values. ANOVA tests at all the tested pulse widths confirm that acute deafening has a significant (p<0.05) on IC activity in response to INS. For example, at pulse width 0.25 ms, corresponding to radiant energy of 483.5 mJ/cm², F (1,10)=24.34, p=0.0001. At pulse width 0.4ms, corresponding to radiant energy 773.6 mJ/cm² F(1,9)=8.95, p= 0.015.

These results indicate that there was a significant effect of deafening on the IC activity and we have also demonstrated that the IC activity after deafening is fell to a level that is the same as when no INS is applied. Therefore conclusions can be drawn that acute deafening removed any response to INS when pulse width was varied.
The Effect Of Acute Deafening On IC Activity When Pulse Width Is Varied

Figure 5.12: The effect of acute deafening on the IC response to INS when pulse width is varied. Deafened subjects (black) N=3. Hearing subjects (red, n=8). Peak Power = 50%

5.6.2.3 Results: The Effect Of Acute Deafening On IC Responses When Wavelength Width Is Varied

Figure 5.13 shows the effect of acute deafening on IC responses to INS. The peak power for all these data points was 50%. The results in hearing animals are displayed for comparison in red (n=8), the deafened subjects are displayed in black (n=3) Acute deafening has a statistically significant effect on IC activity at all wavelengths. One-way ANOVA tests show that at all wavelengths tested, acute deafening had a significant effect on the IC response. At 1849nm,
At wavelength 1865nm, the same effect was evident, 
$F(1,11)=21.81, p<0.001$. Mean IC activity across all deafened subjects was 0.021 +/- 0.0017. A two-tailed paired t-test comparing the mean IC activity in deafened subjects with the mean IC activity at 0% laser power in hearing subjects revealed no significant difference between these means( $p=0.568$). These results support the hypothesis that acute deafening removes the response to INS. It appeared that any responses previously recorded in hearing subjects were due to the optophonic artifact.

**5.6.3 Discussion: The Effect Of Acute Deafening on INS-Evoked Inferior Colliculus Responses When Laser Parameters Were Varied**

These results demonstrated that after acute deafening, there was no increase in IC activity when INS was presented to the cochlear nucleus. There was no effect of varying any of the parameters on IC activity. Furthermore, the mean IC activity after deafening in each experiment was not significantly different from the mean IC activity in conditions when the laser was turned off and no infrared energy was applied to the cochlear nucleus.

These results support the hypothesis in 5.1.4 that INS responses seen in hearing animals were in fact responses to acoustic artifact generated at the tip of the optical fibre and detected by the normal acoustic detection mechanisms within the temporal bone.

In order to complete the investigation, we performed location study experiments to identify if there was any response to INS seen after acute deafening when the tip of the laser fibre was moved onto different sites across the cochlear nucleus.
5.7 The Effect Of Acute Deafening On IC Responses To INS-Location Studies

5.7.1 Methods: The Effect Of Acute Deafening On IC Responses To INS-Location Studies

The subjects were prepared in the same manner as described in section 3.2.

The location studies in deafened animals were undertaken in the same manner as described in section 4.6.1. Laser parameters were identical to those in table 4.16. The locations on the CN were subdivided as demonstrated in figures 4.9, 4.10 & 4.11.

5.7.2 Results: The Effect Of Acute Deafening On IC Responses To INS-Location Studies

Figure 5.13 gives a typical example of IC responses before and after deafening in the same subject. The locations illustrated are B1, B2, B3 & B4 in addition to the IVth ventricle and temporal bone as controls. The top row represents results before deafening, the bottom row represents results after deafening.

The horizontal axis for each of the squares is depth of electrode. Figure 3.4 shows the correlation between depth and electrode number. Higher depth correlates to iso-frequency laminae that respond to higher frequency auditory stimuli, low depth corresponds to low frequency auditory stimuli. The vertical axis represents the peak power level. As previously described, each IC experiment is undertaken as a power series, requiring increments in the peak power to build the data set. The colour scale indicates spike count activity above the baseline. The baseline for this example was set as the level of IC activity when the laser was set at 0% in the pre-deafened subject. Colours at the red end of the spectrum represent increased IC activity in terms of spike count. If a square has a deep blue colour, it represents no increase in activity above baseline activity.
Examining square B1 before deafening, we can see IC activity across almost the entire horizontal axis. This shows that the stimulus is broadband, consistent with a click. In addition, the colours start shifting from blue towards the red end of the spectrum at a peak power level of around 40%. Together, these results suggest that the IC is responding to a broadband stimulus when the laser fibre is placed over site B1 in a hearing subject. This broad pattern of activation can be compared to the IC responses to pure tones in figure 4.17A. Activation increase as the peak power increases. These findings are consistent with the response in hearing animals being the result of acoustic artifact. The laser produces a broadband click and increase in sound level as the peak power of the laser increased. These properties of the optophonic artifact would explain the pattern of activation seen in the hearing animal.

There is also strong activation at the temporal bone. Moving along the lateral to medial axis to sites B2, B3, B4, and IVth ventricle, responses waned, then disappeared by B4, with no response seen in the IVth Ventricle. This is confirmed by the predominance of the darkest blue colour.

The lower row of tiles represents the results after acute deafening. There is no site where there is any indication of an increase in IC activity. The darkest blue colour predominated across all IC depths and at all peak power levels. These results show that after acute deafening, there was no increase in IC activity when infrared energy was applied to different sites across the cochlear nucleus.
IC responses Pre and Post Deafening Along the Lateral To Medial Axis

Figure 5.13: Example of IC responses pre and post deafening in the same animal at different locations across the Lateral-Medial axis. The top row represents results pre-deafening, the bottom row represents results post deafening.

Figure 5.14 shows the averaged IC activity from 8 subjects with normal hearing and 3 deafened subjects when Infrared energy was applied to sites B1, B2, B3 & B4. There is a clear effect of deafening on the IC activity.
The Effect Of Acute Deafening On IC Activity Across Row B

Figure 5.14: IC activity across the lateral-medial axis pre and post deafening. (deafened n=3, un-deafened n=8)

Figure 5.15 plots the IC activity after INS for all 12 sites in both hearing and deafened subjects. The activity is averaged across all 16 channels for laser peak power level of 50%. The results pre and post deafening are displayed and highlights the change in IC activity after deafening. The error bars represent the standard error for the mean activation. For hearing animals there are 8 data points for each site, for deafened results, there are 3 data points per site. This figure re presents collated results from a total of 15 different subjects.

The horizontal axis represents the Columns 1-4 (lateral to medial axis). The legend identifies which colour belongs to which row (rostral-caudal axis). We can see that in the un-deafened subjects, there is IC activity above the baseline in Column 1. These findings are consistent with our results for ABRs, which also indicate that the lateral edge of the CN is where responses are greatest. After deafening however, the IC responses to INS fall to baseline or in some cases below baseline. This indicated that after deafening, no response to INS was identifiable.
Focusing on column 1, where the strongest ABR and IC responses were previously seen in hearing animals, we can say that deafening has a significant effect (p<0.05) on IC responses to INS. One-way ANOVA shows F (1, 27) = 13.31, p= 0.001. We were unable to identify any other columns that demonstrated a significant effect of deafening on the IC activity. However, the error bars indicate that the range of values recorded varied substantially, so there was no clear separation between deafened and non-deafened subjects. We would view this not as evidence of preservation of IC responses, but further support that INS in the un-deafened animal works by an optophonic mechanism. In the absence of responses to INS in the hearing animal in columns 2,3 &4, we would not anticipate deafening to have any significant effects as there was no clear stimulatory effect seen in the hearing condition.

When all of the mean activations levels for deafened subjects in column 1 were aggregated a single mean activation of 0.03 +/-0.012 was derived. The mean baseline activity, when the laser was set to 0% for these subjects was 0.023 +/- 0.068. A two-tailed paired t test does not show any significant difference between these samples (p= 0.12).

We can conclude that acute deafening not only reduces the IC response to INS, but returns them to a level consistent with background noise levels.
5.7.3 Discussion: The Effect Of Acute Deafening On IC Responses To INS-Location Studies

After acute deafening, there was no further IC response to INS at any site on the cochlear nucleus. Sites that gave strong responses in the un-deafened animal, closer to the lateral border gave no responses, even at the highest laser peak powers. This loss of response at all location after deafening fitted with hypothesis stated in 5.1.4 that the responses identified in hearing animals were all the result acoustic stimulation resulting from the optophonic artifact.

Figure 5.15: IC responses to INS at all sites pre and post deafening (Deafened N=3, un-deafened N=8)
5.8 Conclusions: Infrared Neural Stimulation Of The Cochlear Nucleus

These experiments have demonstrated that in animals with normal hearing, it is possible to generate ABRs and IC responses when Infrared energy is applied to the cochlear nucleus. However, once dividing the VIIIth nerve controlled for the optophonic artifact, no responses to INS could be elicited. The effect of peak power and location so clearly seen in hearing animals disappeared.

Our characterization of the optophonic artifact offers an explanation for the effect of peak power and location.

At peak powers over 20-30% an optophonic artifact was generated that was audible as an acoustic stimulus to the subjects with normal hearing. Varying the remaining laser parameters of pulse width and wavelength did not have any effect on the sound level of the optophonic artifact and hence no effect on the response in terms of ABR and increases in IC activity.

The location dependence is also a feature of the optophonic artifact. Locations that were laterally placed on the CN corresponded to the strongest ABR and IC responses. These sites were also the closest sites to the temporal bone that houses the acoustic detection apparatus of the peripheral auditory system. The closer the stimulus (laser tip) was to the temporal bone and the stronger the response. Cutting the VIIIth nerve effectively controlled for any optophonic artifacts, as the cochlear was no longer able to activate the central auditory pathway. This resulted in a loss of all responses to INS after nerve sectioning, with no responses no longer seen at the lateral border of the cochlear nucleus.
Chapter 6: Discussion & Conclusion

6.1 The Optophonic Artifact

When the optophonic artifact was controlled for, Infrared light applied to the DCN did not elicit auditory responses in the form of ABRs or recordings from the inferior colliculus.

INS has, however, been shown to generate neural responses in other tissues, namely facial nerve (Teudt et al., 2007a), peripheral nerve (Wells et al., 2005b, Wells et al., 2007b) and auditory nerve (Izzo et al., 2006, Izzo et al., 2007a, Izzo et al., 2007b, Littlefield et al., 2010, Moreno et al., 2011, Richter et al., 2011). Therefore, either our method of application of INS was ineffective, or there was an intrinsic property of the cochlear nucleus tissue structure that leads to a non-response to INS. A third possibility exists: that infrared light may act on the cochlear nucleus tissue not in a stimulating fashion, but in an inhibitory fashion.

Figure 4.10 compares the waveforms for acoustically generated ABR with electrical and INS evoked ABR. It is difficult to compare the electrically evoked ABR as the artifact from the bipolar stimulus interferes with the early waveforms. However, it is evident that the acoustic and INS evoked waveforms are very similar. At an early stage it was identified that INS evoked ABR in hearing animals had a wave configuration that included a wave II. In rodents and humans, wave II is generated by the proximal VIIIth nerve as it enters the brainstem. This fact alone could have raised early suspicion that the auditory responses generated in hearing animals were acoustic in origin. However there was no published data on INS evoked ABR therefore it was unknown how an INS evoked waveform may appear. In addition, it was unclear exactly what tissue in the path of the beam of infrared energy was actually being stimulated. Location A1 had been shown to give consistently good responses to INS. The working theory during the experiments on undeafened animals was that it was possible that the auditory nerve itself was being
directly stimulated by the INS as it passed into the cochlear nucleus complex or as the branches spread within the complex. This hypothesis could explain both the presence of a wave II and the strong location dependence. Littlefield et al (2010) had already demonstrated that the auditory nerve itself could be stimulated by INS applied to the cochlea. However, as the effect of deafening demonstrated, the wave 2 was in fact being generated by a more conventional means; stimulation of the cochlea by the click generated at the tip of the laser fibre. Location A1 gave the strongest responses because it is the point on the surface of the DCN that was closest to the temporal bone and the inner ear organs located within the bulla of the rodent temporal bone.

6.2 INS And The Auditory system

6.2.1 Mechanism of INS

It is useful then to consider what is known about how INS putatively works. Although little is known about the mechanism, Shapiro et al (Shapiro et al., 2012) have provided the most comprehensive mechanism explanation to date. This paper suggested that INS functions by causing a change in the capacitance properties of the lipid bi-layer, resulting in a capacitance current. Whilst the initial experiments were done in oocytes, Shapiro et al then demonstrated that that INS induced capacitance currents can take place in artificial lipid by-layers that are bereft of membrane proteins. This finding implies that there is no specific target protein or channel responsible for INS and therefore any cell with a lipid bi-layer could be a target for INS. Therefore, if all cells could in theory respond to INS with an induced capacitance current, why does INS applied to the cochlear nucleus not appear to give an auditory response?

6.2.2 Generators Of Auditory Responses

At this point, it would be helpful to review what type of auditory responses we were looking to identify. The auditory brainstem response is a well described far field response,
used in audiological testing clinically and experimentally (Otto et al., 1998). In order for an ABR to be generated, several cell types in the cochlear nucleus complex must be stimulated synchronously. The bushy cells, in particular the sub-set of globular bushy cells, are considered to be the generators of the P2 wave of the ABR. The P2 wave typically represents cochlear nucleus activity (Melcher and Kiang, 1996). The cellular basis of the ABR is summarized in (McCreaery, 2008) and in chapter 2 of this thesis.

The dorsal cochlear nucleus is surgically the most accessible part of the cochlear nucleus complex in rodents, therefore it was chosen as the target for these experiments. Functional studies have shown that the dorsal cochlear nucleus has a low ratio of output to input pathways. Though the DCN contains several types of neurons, only two types of output neurons are thought to be present: Fusiform (or Pyramidal cells) are the most numerous whilst there are a small number of giant cells. The fusiform cells have a primary target of the contralateral inferior colliculus, although there are some projections to the medial division of the medial geniculate nucleus. Therefore, it was reasonable to assume for these experiments that stimulation of neurons within the DCN with INS may have generated responses that could be recorded as changes in IC activity.
6.2.3 The DCN As A Therapeutic Target

The exact function of the DCN is not clearly understood. It is thought that the DCN has a role more in keeping with an auditory processor, given the density of internal circuits with seemingly few outputs. Young et al studied the physiology of the DCN, identifying that neurons within the DCN may possess inhibitory properties on the pathways that pass through the DCN (Young, 1980). Other authors have commented on how the anatomical arrangement of the DCN is more complex than the ventral subdivisions of the cochlear nucleus. Specifically, the rodent DCN may have an architecture that has more in common with cerebellum than brainstem (Reiss and Young, 2005).

It is important to note that the cyto-architecture of the DCN in humans is significantly different compared to most other mammals. The human DCN has a less layered structure, although in keeping with other mammalian DCNs, there is a relatively small output pathway relative to the inputs and internal size of the structure. This would enhance the opinion that the DCN’s major role is as a processor.

The paucity of output pathways from the DCN has implications for this structure as a potential target for auditory prostheses, as stimulation of neurons within the DCN may not necessarily result in an ABR.

For our experiments, the relevance of the cyto-architecture of the DCN is that as we are unsure which cell types may respond to INS, we cannot be sure that a response from cells in the DCN would necessarily translate into an ABR. In addition, although the outputs from the DCN are mainly to the IC, these output pathways are relatively small given the size of the DCN and its structural complexity. Therefore, we cannot be certain that a response to INS would be recordable as an increase in IC activity.
6.2.4 Infrared Neural Inhibition

Recent work by Duke et al (Duke et al., 2013) has shown that contrary to much of the published literature that shows infrared light to have a stimulatory effect (Izzo et al., 2006, Izzo et al., 2007a, Izzo et al., 2007b, Littlefield et al., 2010, Moreno et al., 2011, Richter et al., 2011, Wells et al., 2005a, Wells et al., 2007a, Wells et al., 2005b, Wells et al., 2007b, Cayce et al., 2011), there may also be an inhibitory effect when Infrared light is applied.

This group defined inhibition as “transient elimination of action potential initiation”. This inhibitory effect is thought to be thermally mediated and their proposed model is consistent with the thermo-modulated change in membrane capacitance that is described by Shapiro et al (2012). Infrared induced stimulation is related to a brief spatio-temporal gradient of temperature whilst infrared induced inhibition (the degree of suppression) is related to the change in baseline temperature, thus reducing the spatiotemporal gradient. Changes in their laser parameters generated either large but brief changes in temperature, or smaller increases in temperature for inhibition to occur.

The responses in Duke et al (2013) were recorded in the form of electromyograms. These are local responses and are inherently less susceptible to background electrical or electrophysiological noise. In addition, the laser used by this group was significantly more powerful than the Capella laser we used for our experiments. The typical laser wavelength used by Duke et al was 1450 nm, with the laser capable of a maximum power of 25W. The laser used in the experiments described in chapters 4 & 5 had a functional wavelength range of 1848-1863nm, corresponding to a maximal power of around 5W. In addition, the difference in wavelengths generates a large difference in the absorption coefficients, with lower wavelengths penetrating the tissues more deeply. Duke et al calculated that the absorption coefficient of water for 1860nm was 2.5 times less than that at 1450nm.
This difference in laser parameters between Duke’s set-up and our own means that simple extrapolation from Duke’s results would be inaccurate. It cannot be assumed that infrared radiation in the experiments described in chapters 4 & 5 resulted in infrared induced inhibition.

6.2.5 INS in the Peripheral Auditory System: Dr Richter’s Results

Dr C-P Richter has championed INS work in the auditory system, with several papers clearly demonstrating that INS can stimulate responses when applied to the auditory nerve in the cochlea. Richter’s experimental set up is analogous to our own. Guinea Pigs were used and responses were recorded as compound action potentials (CAP) recorded from the round window, or as Inferior Colliculus recordings. There are several methodological differences between Dr Richter’s set up and our own. Notably, Dr Richter’s experiments were all conducted using radiant energy levels that are far lower than the typical levels used in the experiments described in chapters 4 and 5. Dr Richter’s experiments were generally undertaken at radiant energy levels of <200mJ/cm$^2$. The optophonic artifact that would be generated at this energy level would be approximately <30dB SPL. This is much less than the 53dB SPL that was typically generated in our experiments. Dr Richter has recently published further work in genetically deaf cats that demonstrates similar findings to his work in guinea pigs, therefore we must assume that this new experimental model has fully controlled for the optophonic artifact (Matic et al., 2013).

6.2.6 Central Nervous System & INS

Whilst other groups have focused their attention on the peripheral nervous system, Cayce et al have undertaken work in the central nervous system. Building on their work in cortical slices that appeared to demonstrate an inhibitory effect of infrared light(Cayce et al., 2011), they have recently published in-vivo work that demonstrates both a stimulatory and inhibitory effect of infrared light when applied to the visual cortex of macaque.
monkeys (Cayce et al., 2014). Using single unit recording techniques, they were able to demonstrate that using a narrow optical fibre (100-200 µm) resulted in a stimulatory effect whilst use of a 400-µm fibre resulted in an inhibitory response. These responses were highly localized, underlying the highly spatially specific effect of infrared light acting on neural tissue.

Other groups have published preliminary work using infrared light to stimulate other parts of the central nervous system, including retinal and vestibular neurons (Bec et al., 2012). The Bec et al study demonstrated the small magnitude of responses as the responses were measured in terms of ionic exchange using whole cell patch-clamping techniques. Temperatures of the tissues also varied from 20°C to over 55°C. Whilst it is heartening to see positive results, the long term effects of heating tissues to this sort of temperature suggest that infrared laser light may be a non-viable option for neuroprosthetic development in retinal or vestibular prostheses given the small degree of response relative to the large increase in temperature. The issue of temperature-induced damage in the cochlea of guinea pigs has been investigated using histological and functional measurements (CAP) and found to be negligible at the energy levels employed to stimulate the cochlea with INS (Goyal et al., 2012).

6.2.7 Cochlear Nucleus Stimulation: Anatomical Considerations

In order to fully investigate whether infrared light can successfully stimulate the auditory system by applying it to the cochlear nucleus, we believe that a fundamental change in approach may be required. We need to consider both the target of stimulation and the actual method by which responses are recorded.

If we work on the assumption that the DCN acts largely as a processor, with most of its cells involved in local circuits and few outputs, then it would be reasonable to suggest an
alternative part of the cochlear nucleus complex as the target for exogenous stimulation. The Ventral Cochlear nucleus, composed of anterior (AVCN) and posterior (PVCN) segments, could be a more productive target. The CN VIIIth nerve enters the cochlear nucleus complex at and then subdivides into distinct branches travelling into the AVCN, PVCN and DCN respectively. It is the author’s opinion, based on the cyto architecture of the cochlear nucleus and the different ratio of input to output pathways in the DCN and VCN respectively, that attempting to stimulate the AVCN or PVCN, or in fact the auditory nerve itself is more likely to result in meaningful auditory responses (McCreery, 2008, McCreery et al., 1998).

In particular, accessing the auditory nerve would allow more of the processing capabilities of the cochlear nucleus complex to be brought into play and may result in a more meaningful auditory perception in response to stimulation of the central auditory pathway using a prosthesis. Teudt et al demonstrated that CN VII nerve could be successfully stimulated using infrared light (Teudt et al., 2007b). In that study, cranial nerves themselves were stimulated. Cranial nerves have different morphologies compared to peripheral nerves, cerebrum or cerebellum, which have been the other targets for INS. Therefore, it could be possible to stimulate the CN VIIIth nerve itself. The advantage of stimulating at this level of the auditory pathway is that the processing and coding functions of the whole cochlear nucleus complex come into play far more effectively if a native stimulation pathway is accessing them, as opposed to by an exogenous stimulus. If the auditory nerve is stimulated exogenously, then the processing, filtering and coding strategies that are intrinsic to the cochlear nucleus complex should also be applied to the signal as it passes toward the inferior colliculus and onwards towards the auditory cortex. One possible reason that ABI users seem to experience worse hearing on average when compared to CI users is that whilst the CI stimulates the auditory nerve and takes advantage of the cochlear nucleus function, the ABI user does not have the benefit of such processing on the signals.
sent higher up the central auditory pathway (McCreery, 2008, McCreery et al., 1998, Otto et al., 2008).

If stimulating the auditory nerve itself does not prove fruitful, then stimulation of the AVCN or PVCN could be the next target. As previously discussed, globular bushy cells are intrinsic to the formation of the ABR and need to be stimulated synchronously for the ABR to be generated. Therefore, if the aim is stimulate the central auditory pathway, and we know an ABR reflects activation of this pathway, then it would be logical to attempt to stimulate the cells responsible for ABR generation. The rostral portion of the mammalian AVCN contains a cluster of spherical cells, a central region of spherical and globular bushy cells in addition to multi-polar cells and a caudal region that contains octopus cells (Cant and Benson, 2003). In the rodent, the Multi-polar cells are replaced with T-stellate cells and their function seems to amplify and extract envelope information from an acoustic signal. Stimulation of these cell types could result in activation of the central auditory pathway.

Stimulation of the ventral cochlear nucleus in rats poses unique challenges. Surgical access to the AVCN and PVCN is extremely limited. The temporal bone and significant cerebral vasculature shields the lateral portion of the cochlear nucleus complex, making access by increasing the posterior craniotomy treacherous and likely to lead to significant blood loss or iatrogenic damage to the cochlear nucleus itself. Accessing the ventral cochlear nucleus by drilling through the lateral temporal bone is also restricted by an artery that runs through the arch of the stapes that may supply part of the cochlear nucleus. In addition, drilling out the cochlea results in contact with a large artery that is very difficult to control once opened due to the narrow access. There may be an opportunity to access the cochlear nucleus using a ventral approach. This technique is not commonly described but was used with some success my colleague Dr N Durakovic in a project that required a minimally
invasive access to the cochlear nucleus in non-fatal experiments. The particulars of the technique are yet to be published.

Even after the ventral cochlear nucleus is accessed, it is unclear how much space will be available for the optical fibre to be placed and whether this would be under direct vision. Discussion with colleagues who work with similar animal models suggests there is no gold-standard approach to this particular technical conundrum.

The human cochlear nucleus has been measured as having a length of approximately 12.8mm (from auditory tubercle to entry of VIIIth nerve. Of that total length, approximately 7.6mm is exposed in the lateral recess (Quester and Schröder, 1999, Quester et al., 2004) In the human, visualization of the cochlear nucleus is very limited during ABI placement. The ABI paddle is slid into position along the foramen of Luschka and covers most of the cochlear nucleus complex that is located within the lateral recess. This portion of the CN is likely to be the VCN, however surgical factors including iatrogenic damage to this exposed portion of the CN during VS removal means it is impossible for the surgeon to predict with any degree of certainty exactly what part of the CN is being covered by the ABI paddle. One method of overcoming this difficulty in visualization of the cochlear nucleus itself may be the with the use of an endoscope during surgery (Friedland and Wackym, 1999). However, the exact choice of target for the ABI remains controversial (Abe and Rhoton, 2006).
6.2.8 INS and the Auditory Brainstem Implant: Future Directions

Whilst Richter’s group has been able to demonstrate INS in terms of CAPs and IC responses, other groups working with INS have used more subtle measures of activity as responses to infrared light may be too small to be picked up by far field responses such as ABR, or too small to generate a significant increase in baseline activity levels in the inferior colliculus. Techniques employed by other groups include the use of patch clamping and measurement of EMG. If infrared light generates tiny responses, then it may be possible to use intracellular or single unit recording techniques to record responses. Our own lab possessed neither the equipment nor the specific expertise for such techniques. However, investigating local responses to infrared light application could prove a rich vein of research. If local responses to infrared light can be demonstrated, it may be possible to make use of this in a hybrid optical/electrical ABI. One of the challenges faced with the ABI in its present form is the inevitable current spread and lack of focus of stimulation (see section 2.5). If infrared light can be shown to inhibit neuronal activity then it may be possible to surround an electrode with a ring of infrared light emitting diodes that fire with the electrode. The resultant ring of neural inhibition around the electrode would reduce interaction between adjacent electrodes and could theoretically improve the focal resolution of electrodes in an ABI array. If a true INS response can be demonstrated, this could be of clinical relevance in a hybrid device, or perhaps even a penetrating ABI where a small population of neurons require stimulation. However, the results from the experiments in this thesis, coupled with the small responses reported by other groups in the CNS (see section 6.2.6) suggest that it is that an ABI array that is driven by infrared alone is unlikely to be clinically relevant.
6.2.9 Improving the Auditory Brainstem Implant:

A better understanding of the functions and interconnectivity of the numerous cell types within the cochlear nucleus complex would be beneficial as it may help future ABI devices to target specific population of cells. Specific activation of cell types may be possible using optogenetic techniques, though much work is still to be done in this field before human trials are feasible (Chow and Boyden, 2013).

Improvements in the tissue/electrode interaction may yield more focal stimulation that requires lower electrical thresholds for activation (Lacour et al., 2010). New electrode designs may also wish to re-visit the penetrating ABI. Accessing the tonotopic axis within the cochlear nucleus with surface electrodes is complicated due to the 3-D nature of this axis. A penetrating ABI, which takes advantage of bipolar, tripolar or multipolar stimulation techniques may give more focused stimulation within the cochlear nucleus.
6.2 Conclusion: Towards A New Generation Of Auditory Brainstem Implants

Infrared Neural Stimulation offers the possibility of finely focused stimulation of neural tissues. It is a modality that has shown promising results for cochlear implants and may have a role in other neuroprosthetic devices (Bec et al., 2012, Cayce et al., 2014), though preliminary results have struggled to show neural responses that are as readily clinically applicable as those demonstrated in cochlear implants. Application of infrared energy, delivered by an optical fibre, to the surface of the DCN does not generate recordable ABRs, nor increase in IC activity when the optophonic artifact has been fully controlled for by mechanical deafening of the subject. Whilst INS may not be the innovation that signals a paradigm shift for the ABI device, opportunities for improving this device do exist.

Though the ABI has provided many with some degree of hearing, rehabilitative options for patients with NF2, or those unable to receive cochlear implants, remain extremely limited. However, the ongoing success of the cochlear implant acts as a clarion call driving clinicians, engineers and scientists in their efforts to improve a device that could potentially reconnect people with their auditory environment.
References


