2005

Characterization of the Zona Incerta

Heather Joyce Green
Virginia Commonwealth University

CHARACTERIZATION OF THE ZONA INCERTA

A Thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science at Virginia Commonwealth University.

by

HEATHER JOYCE GREEN
Bachelors of Science, Louisiana Tech University, 2001

Director: Paul A. Wetzel, Ph.D.
Associate Professor, Department of Biomedical Engineering

Co-Director: Mark S. Baron, M.D.
Associate Professor, Department of Adult Neurology

Virginia Commonwealth University
Richmond, Virginia
August 2005

Acknowledgement

I would like to thank Dr. Kathryn Holloway, Kelli Massey-Makhoul, R.N., Nanette Eubank, R.N., Mariann Haselman, R.N., Miriam Hirsch, R.N., and the OR staff at both the VCU Medical Center and the Hunter Holmes McGuire VA Medical Center for letting me take part in the procedures. Also, I would like to thank the members of the PADRECC in Richmond. You have provided an unexpected opportunity and helped guide this project along the way.

I would also like to thank my family, especially my parents, for their encouragement and support. You have all been enthusiastic supporters of my education. I could not have accomplished so much without you.

Finally, I need to express my gratitude to the co-chairs for this project, Dr. Wetzel and Dr. Baron. You both have provided guidance and support. I have greatly enjoyed the time I have spent working with each of you on this project.

Table of Contents

Page

List of Figures

Page

Abstract

CHARACTERIZATION OF THE ZONA INCERTA

By Heather J. Green, M.S.

A Thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2005

Director: Paul A. Wetzel, Ph.D.
Associate Professor, Department of Biomedical Engineering

Co-Director: Mark S. Baron, M.D.
Associate Professor, Department of Adult Neurology

Parkinson's Disease affects more than 1 million people in the United States with 60,000 new cases being diagnosed each year. Currently, there is no cure for Parkinson's Disease, but there are several treatment options available. Currently the most popular surgical option is Deep Brain Stimulation. Microelectrode recording helps identify nuclei as the microelectrode passes through them. While the firing frequencies of the target nuclei are well defined, other nuclei are not. This study will attempt to characterize the

Zona Incerta, which is the structure directly above the Subthalamic Nucleus, a target nucleus. Characterization of the firing frequency of the Zona Incerta will help aid Deep Brain Stimulation procedures. Looking at the Interspike Intervals for 25 files showed that the average firing frequency is 11.6 Hz. A file recorded in the STN was used for comparison and to validate the methods used. This yielded an average firing frequency of 37.5Hz.

Introduction

Parkinson's Disease (PD) is a progressive neurological disorder. While there are many treatments available to patients suffering from Parkinson's Disease there currently is no cure. The disease is the result of the malfunction and subsequent death of dopamanergic cells within the brain. With the loss of 80% of these dopamine-producing cells, parkinsonian symptoms begin to appear. Treatments range from therapy to pharmacologic to surgical. Currently there are more than a million adults living in the United States with Parkinson's Disease and about 60,000 new cases are diagnosed each year (pan.org).

There are several treatment options available for patients with Parkinson's Disease, including medication, physical therapy, and surgery. Currently, the surgical treatment chosen most often is Deep Brain Stimulation (DBS). During this procedure a microelectrode is advanced through the brain and microelectrode recording (MER) allows surgical personnel to listen and see the firing of the neurons. There are different firing patterns and frequencies associated with different areas of the brain. Once the microelectrode reaches the target nucleus a doctor or nurse moves the patients' limbs to see if any "driving" of the neuron can be observed. Driving is causing a change in the neurons firing due to limb movement. If the region appears to have a lot of motor inputs, observed by multiple places where driving is observed, then the region is stimulated. The

stimulation is done at different depths and voltages to observe efficacy and side effects. Once a placement yields good symptom control and little side effects, the lead is secured.

The target nuclei have been well defined so that when neurosurgeons and neurologists encounter them they are easily identified. Other nuclei, however have not been studied and observed to identify their characteristics. Better identification of the nuclei encountered during the procedure before reaching the target nucleus helps to improve targeting and should another pass need to be made, helps direct the direction (anterior vs. posterior and/or medial vs. lateral) and amount of movement.

The purpose of this study is to characterize the Zona Incerta (ZI) by characterizing its firing frequency. The Zona Incerta is the neuronal structure above the Subthalamic Nucleus (STN) and is about 2mm deep. Characterization of the ZI can assist neurosurgical personnel in better targeting the STN when using microelectrode recording.

Several topic areas must be understood in order to approach such a task. An understanding of Parkinson's Disease; it's symptoms, treatments, and possible causes is needed to better understand the patient population being treated. The surgery needs to be understood including the procedure and the theory behind the therapy. The Zona Incerta should also be examined to discover what is already known about the structure, since it is the focus of interest. Its connections to other neuronal structures helps to better determine what role it may play. Previous studies can provide a review of the mechanisms through which the signals were acquired during the surgery. This will help determine the best route to examine analyze the signals collected.

Parkinson's Disease

Fundamentals of Parkinson's Disease

Parkinson's Disease affects more than 1 million people with in the United States and is in a group of conditions called movement disorders. Dr. James Parkinson first described PD in 1817 in his work "An Essay on the Shaking Palsy." PD is a chronic and progressive neurodegenerative disease. Most patients are diagnosed in their 50's or 60's and with each decade increase in age, the likelihood of developing the disease increases (apdaparkinson.org). Currently the average age of onset is in the 60s. Some patients do develop PD at an earlier age, in their 30s or earlier, these patients are diagnosed with early onset PD.

The cause of PD is not currently known, however it is suspected that both genetic and environmental factors play a role. Less than 30% of PD patients report having a family member with the disease. (parkinsons.org) There are some environmental toxins which have been shown to cause Parkinson's symptom, an example is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). There are studies underway to establish links between rural living, exposure to herbicides and pesticides, and other factors that may contribute to the development of Parkinson's Disease. Other theories on the cause of PD involve the presence of substances that are involved in beginning or speeding cell death. (parkinsons.org) It is likely that all of these theories are involved in a person's development of PD to some degree. Parkinson's Disease can also be drug induced.

Certain antipsychotics, antiemetics, and antihypertensive have been shown to reversibly induce PD like symptoms by interfering with dopamine.

The mechanisms of PD are poorly understood. The symptoms of PD are thought to in part be related to STN hyperactivity (Benedetti et al, 2004).

Parkinson's Disease PD has some cardinal features including but not limited to tremor at rest, bradykinesia (slowness of movement), and muscle rigidity. These symptoms are caused by a loss of dopaminergic cells in the substantia nigra (SNr), which leads to a depletion of dopamine in the striatum (Deuschl et al, 2000). Dopamine, a neurotransmitter, sends signals to parts of the brain that control movement initiation and coordination. Parkinson's symptoms begin to appear when about 80% of the dopamine producing cells in the SNr are no longer functioning (parkinsons.org). The dopamine depletion provokes a cascade of functional changes in basal ganglia circuitry (Perier et al, 1999). PD is defined to be present, if two of the following four criteria are met:

- Rest tremor
- Bradykinesia
- Rigidity
- Postural instability

(Deuschl et al, 2000) The following is a more extensive summary of the symptoms experienced by patients with PD.

- Tremor
- Rigidity
- Bradykinesia
- Postural instability
- Stooped, shuffling gait
- Difficulty arising from a chair

- Micrographia
- Masked face
- Slowed activities of daily living (ADLs)
- Difficulty turning in bed
- Hypophonic speech
- Sialorrhea
- Anosmia
- Foot dystonia
- Depression
- Constipation
- Increased sweating
- Urinary frequency/urgency
- Male erectile dysfunction
- Difficulty swallowing
- Pain
- Dementia or confusion
- Skin problems
- Fear/anxiety
- Memory difficulties & slowed thinking
- Loss of energy
- Fatigue and aching

Compiled from apdaparkinson.org and pdf.org

Tremor is one of the most recognized symptoms of PD and it is generally seen in the early stages of the disease. Tremors initially manifest in the hand or the foot on one side of the body. The tremor occurs when the muscles of the body part are relaxed which is why it is referred to as resting tremor. Rest tremor is asymmetrical. Rest tremor is characterized by observing an increase in amplitude when the patient is under mental stress (Deuschl et al, 2000). In patients with idiopathic PD, approximately 95% of them have classical resting tremor (Deuschl et al, 2000). As the disease progresses the tremor doesn't necessarily become worse and the progression of the disease is also not

proportional to the severity of the rigidity and akinesia. This tremor generally has a frequency above 4Hz and up to 9Hz early in the disease. (Deuschl et al, 2000)

Another type of tremor that PD patients may also experience is postural tremor. Tremor of the muscles in the trunk of the body is called postural tremor. Postural tremor may occur alone or with rest tremor. The frequency of the postural tremor can vary from that of the resting tremor by as much as 1.5Hz (Deuschl, 1999). Postural tremor tends to be symmetrical.

Bradykinesia is a slowing of voluntary movement. This also encompasses the inability to complete movements, difficulty initiating movements, and halting of ongoing movements (pdf.org). Patients will have difficulty walking and develop short shuffling steps, which can lead to falling and balance problems (pdf.org). Patients also have freezing, becoming stuck and finding it difficult to start walking. Bradykinesia also affects the muscles of the face leading to a mask like expression and patients have difficulty showing emotion through facial expression (pdf.org).

Rigidity in PD patients leads to a decrease in range of motion due to the fact that the muscles are contracted when in a normal patient they would be relaxed. The contraction of the muscles can lead to pain and cramps (pdf.org).

Dystonia is the involuntary contraction of muscles, which forms parts of the body into unnatural and possibly painful positions and movements (dystonia-foundation.org). These contractions interfere with normal movements and functions.

Current Therapies for the Treatment of Parkinson's Disease

There are some effective therapies for the treatment of Parkinson's Disease. There currently is not any way to prevent or stop the progression of the disease, but there are ways to help ease the severity of the symptoms using pharmacological, surgical, physical therapy, and palliative care. As the disease progresses one or a combination of these methods and treatments are used to address the individuals' needs. With pharmaceutical treatments patients develop on and off periods. The on periods are when they are able to experience the full effect of the medication. The off periods are when the benefits of the drug are lost sooner than expected or are suddenly absent. Currently there is research being done using stem cells, from adult and other sources, and neuroprotective drug trials to find therapies to stop and possibly reverse the disease progression. This work will focus on the methods of treatment that are currently available and widely used.

Therapies – Drug Classes

Patients' have a wide range of responses to medications. They are considered being "on" when they are receiving predictable symptom control. They are considered "off" when they lose symptom control unexpectedly.

There are five drug classes used for the treatment of PD; dopaminergic agents, COMT inhibitors, MAO-B inhibitors, anticholinergics, and amantadine. The patients' reaction to the drug is usually varied, so the fine-tuning of medications may take a while. With disease progression, the dosage may be increased, drugs may be added to the regimen, and drugs may be removed due to loss of efficacy.

Dopamineric agents include drugs with levodopa or drugs that mimic the effects of levodopa. Sinemet (carbadopa/levadopa) is the drug commonly prescribed. Levodopa, an amino acid, is a precursor to dopamine and is converted in the brain (apdaparkinson.org). Levodopa was introduced as a part of PD therapy in the 1960s and remains the gold standard for all other treatments (apdaparkinson.org). There are some severe side effects to levodopa therapy, such as nausea, orthostatic hypotension, hallucinations, and dyskinesias, therefore it is usually combined with other drugs to lessen the side effects (apdaparkinson.org). The drugs that mimic the effect of levodopa are known as dopamine agonists. Examples of dopamine agonists are: Apomorphine, Bromocriptine, Pergolide, Pramipexole, and Ropinirole. These drugs directly stimulate the dopamine receptors, although they don't have the same degree of effectiveness as levodopa (apdaparkinson.org). Still, there are some side effects with this type of therapy. There is a greater tendency for edema and psychosis, and therefore the use of these types of drugs is generally limited to younger patients who are otherwise healthy (apdaparkinson.org). The use of dopamine agonists is generally paired with levodopa for these patients.

COMT (catechol O-methyltransferase) inhibitors are used to prolong the effectiveness of levodopa by preventing its breakdown. This decreases the amount of off time the patients experience. Another benefit to slowing the breakdown is that, generally, the dosage of levodopa can be lowered when COMT inhibitors are added to the medication therapy. Two COMT inhibitors used in the United States are Entacapone and Tolcapone. The main side effect of these drugs is diarrhea, which can become so severe

that the treatment must be stopped. However, this occurs in a very small percentage of patients.

MAO-B (Monoamine oxidase B) inhibitors also slow the breakdown of dopamine in the brain. MAO-B acts in the brain to degrade dopamine (apdaparkinson.org). Generally this is prescribed for patients who are early in the progression of the disease since the effects are mild (apdaparkinson.org). The MAO-B drug approved for use in the United States is Selegiline. Side effects of MAO-B inhibiters include but are not limited to insomnia, hallucinations, and orthostatic hypotension. There is a serious side effect for patients who are also taking antidepressant medication as the patients' blood pressure may be dangerously raised.

Anticholinergics are mainly effective against tremor and rigidity. Anticholinergics do not affect the dopaminergic system; instead they decrease the activity of acetylcholine. This has been shown to be affective therapy for patients who are not responding well to dopamine therapy. Examples of Anticholinergics are Trihexyphenidyl, Benztropine, and Ethopropazine. However, there are significant side effects of memory loss, dry mouth, urinary retention, constipation, sedation, delirium, and hallucinations (apdaparkinson.org).

Amantadine has its most significant affect on dyskinesias. Amantadine activates the release of dopamine from storages sites, blocks the re-uptake of dopamine into nerve terminals, and blocks glutamate receptor activity. The dopamine actions help ease dyskinesias brought about by levodopa. As with all Parkinson's drugs there are some serious side effects, which may limit the duration of its use. These include

hallucinations, insomnia, and skin mottling. Usually Amantadine is prescribed for patients early in the pharmaceutical therapy as the effectiveness wears off quickly in more advanced patients. The benefit to patients with dyskinesias generally wears off within about 8 months.

Therapies – Physical Therapy

Physical therapy is important in the treatment of Parkinson's patients. These patients not only have motor control difficulties, but also develop balance problems due to the inability to make postural adjustments. Regular exercise and/or physical therapy is essential for maintaining and improving mobility, flexibility, balance, range of motion, and warding off additional parkinsonian symptoms (pdf.org). With physical therapy training PD patients are better able to control their muscles and learn ways to adapt to the progressive nature of the disease to maintain as much independence as possible. Exercise provides an adjunct therapy to medication and helps prevent problems due to inactivity and muscle atrophy. PD patients who receive physical therapy have improved activities of daily living (ADLs) and mobility (Miyai et al, 2002). Physical activity helps maintain flexibility, muscle strength and tone, and improve circulation. One trial has shown that there is a beneficial effect in the short-term from physical therapy (Comella et al, 1994). Also, regular exercise can have psychological benefits as well, improving the patients' mood, sense of well-being, and reducing stress. Still, today there are not any specific physical therapy routines given to patients, although the suggestion of daily exercise is strongly recommended.

One area of interest is therapy involving treadmills and their use with or without body weight support and the effect on gait disturbance. The gait disturbance is primarily due to a reduction in stride length, while the steps per minute are slightly enhanced (Faist et al, 2001). Pharmacological treatment increases stride length, but does not change steps per minute (Faist et al, 2001). Studies have been done that show that treadmill training with body weight support may be more effective than physical therapy alone (Miyai et al, 2000). The subjects of this study had Hoehn and Yahr stages 2-3.5, bilateral disease without impairment of balance to mild to moderate bilateral disease some postural instability physically independent, and were not demented. In the first study Miyai and collaborators examined the short-term effects of treadmill training and found that body weight support with the training gave the best short-term effects. Subsequently, a long-term study was performed that showed treadmill training with body weight support has a lasting effect on gait disturbance in PD patients (Miyai et al, 2002).

While there is a vast array of treatments available for patients suffering from Parkinson's Disease; none of these treatments are able to stop, reverse, or prevent the progression of the disease. Eventually, medications and other forms of therapy may no longer allow the patient to have a satisfactory quality of life and surgical treatments maybe considered.

Therapies – Surgical Interventions

Currently there are two different types of surgical treatments for the treatment of Parkinson's Disease. Surgery is used as a last resort for patients who are no longer have a well managed response to pharmaceutical treatments. Generally these patients have

severe motor complications. There are two different types of surgeries for Parkinson's patients; ablative and deep brain stimulation. As with any surgery there are serious risks involved including morbidity. Patient selection is performed with great care to make sure that the patients chosen are the best candidates for these procedures. While age is not necessarily an exclusion criteria, patients with impaired cognition, brain atrophy, and conditions that lead to increased surgical risk are generally not surgical candidates.

There are two main targets in ablative procedures; the thalamus and the internal segment of the globus pallidus (GPi). These ablative procedures use radiofrequencies to create heat and destroy a pea-sized area in the target nucleus. Thalamotomy (ablation of the thalamus) is most successful in the treatment of tremor. The effects on other Parkinsonian symptoms, however, are not as successful. For this reason thalamotomy was generally only used to treat patients whose most disabling symptom is tremor.

Prior to deep brain stimulation, pallidotomy was the most performed surgical procedure to treat Parkinson's Disease. Macroelectrodes were used to locate the GPi and avoid the optic tract. Radiofrequencies are used to ablate the identified portion of the GPi. Pallidotomy was effective to varying degree in the treatment of dyskinesias, dystonia, tremor, rigidity, bradykinesia, and gait.

Deep Brain Stimulation has become a popular surgical treatment for PD patients. The main advantage of DBS over ablative procedures is the fact that the stimulator settings can be adjusted to get the best therapeutic results. The energy consumption of the DBS device is proportional to the area under the pulse curve (pulse width * voltage) and the rate (Krack et al, 2000). Energy consumption is also depends on monopolar or

bipolar stimulation (Krack et al, 2000). Bipolar stimulation has a more focused current delivery, but at a higher energy cost (Krack et al, 2000). Monopolar stimulation has a more radial diffusion (Krack et al, 2000). There are three possible targets Nucleus Ventrointer Medius (Vim), Subthalamic Nucleus, and GPi; of these the STN and GPi are classically chosen as the target. Typically Vim is only used to treat patients whose only Parkinsonian symptom is tremor or patients suffering from Essential Tremor. Patients who have DBS placed in the GPi have marked benefits in the cardinal features of PD and levodopa induced dyskinesia (McIntyre and Thakor, 2002).

The STN is the other major target for PD patients. Patients with DBS placed in the STN have strong clinical effects on all PD features including gait disturbance (McIntyre and Thakor, 2002). A PET study examining patients who were undergoing STN stimulation showed increased blood flow to the supplementary motor area, the cingulated cortex, and the dorsolateral prefrontal cortex (Krack et al, 2000). A study examining the effects of STN stimulation on gait has shown that there is almost a threefold increase in walking velocity through stride length and little effect on steps per minute (Faist et al, 2001). There is a close link between walking velocity and kinematic measures, which are improved with STN stimulation (Faist et al, 2001). A combination of both levodopa and STN stimulation lead to further improvement in gait (Faist et al, 2001).

Most side effects due to stimulation can be adjusted with programming and medication adjustments. Stimulation induced dyskinesias require the adaptation of stimulation parameters and medication (Krack et al, 2000). Bilateral stimulation can

cause both behavioral and cognitive deficits (Krack et al, 2000). Another side effect from stimulation is weight gain (Krack et al, 2000). Speech disturbance may result from current spreading into neighboring structures (Krack et al, 2000).

Bilateral stimulation of both the GPi and STN show significant motor benefits and a reduction in dyskinesias and motor fluctuations for patients with advanced PD (McIntyre and Thakor, 2002). Stimulation of the STN generally produces a decrease in dyskinesias by permitting a decrease in medications.

Basal Ganglia

The Basal Ganglia (BG) consists of the following interconnected structures caudate nucleus, putamen, globus pallidus, substantia nigra, and subthalamic nucleus (McIntyre and Thakor, 2002). The output of the BG comes from the GPi and the SNr and is projected to the ventral lateral motor and intralaminar nuclei of the thalamus and then projected to the frontal cortex and striatum (McIntyre and Thakor, 2002). There are changes in the communication pathways with the development of PD. The BG is the primary locus of pathology in PD (Deuschl et al, 2000). The root of the neurologic dysfunction underlying PD is thought to be the abnormal synchronous neural activity within the BG (McIntyre and Thakor, 2002). The BG mediates neuronal activity from the striatum to the thalamus through direct and indirect pathways (Deuschl et al, 2000). The direct pathway is putamen – internal portion of globus pallidus. The indirect pathway is external portion of the pallidum (GPe) – subthalamic nucleus – internal portion of the pallidum.

Zona Incerta

The Zona Incerta (ZI) was first described by Auguste Forel in 1877 as a "region of which nothing certain can be said". Still today not much is certain for this "zone of uncertainty". Work has been done to identify connections and place theory on possible roles for this nucleus. By identifying connections and projections the role that the ZI plays in maintaining life may be better narrowed. The ZI lays between the Thalamus and the Subthalamic Nucleus in the diencephalon. Figure 1 shows the location of the ZI and the STN.

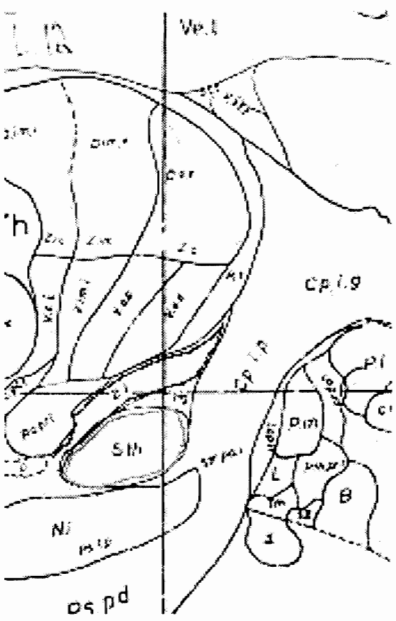

Figure 1: Location of ZI and STN, taken from Schaltenbrand Wahren

Anatomy-Cell types

The ZI is formed by a heterogeneous collection of cells. Studies have grouped these cells into different regions, the number of which vary by author and species studied. Primate studies have shown between 2 and 4 regions and rat studies have shown between 2 and 6. There are several ways to determine the boundaries of these sub-regions, although none is best at defining all of them. Each method may give a more defined border for one region, but not for others. Observing the results of each of these methods provides the reader with the best overall picture of the sub-regions.

One way to define the borders of the regions within the ZI is to look at the cells histologically. The following major sections have been described: ZIr – rostral sector, ZId – dorsal sector, ZIv – ventral sector, and ZIc – caudal sector. (Mitrofanis, 2005) Each of these sections has a different type and density of cells. The ZIr has densely packed spindle-shaped cells which have larger ovid-shaped cells scattered within them (Mitrofanis, 2005, Kolmac and Mitrofanis, 1999). The ZId has medium oval cells (Mitrofanis, 2005). ZIv has densely packed medium multipolar and fusiform cells (Mitrofanis, 2005). ZIc has a variation of cells including medium somata, which are multipolar, fusiform, and rounded, and a group of multipolar cells located medially in the region (Mitrofanis, 2005, Kolmac and Mitrofanis, 1999).

Anatomy – Immunohistochemical Differentiation

Immunohistochemical differentiation provides another way to define the borders of the ZI sub regions. The boundaries of both the Zlv and ZId are well defined using this method (Mitrofanis, 2005, Kolmac and Mitrofanis, 1999). ZIv contains cells, which can be marked using Parvalbumin (Pv)+ (Mitrofanis, 2005, Kolmac and Mitrofanis, 1999). The ZId is marked with nitric oxide sythase (Nos)+ (Mitrofanis, 2005). While these zones are marked well, there are other zones that may have one main marker, but the marker may be present in another region to a lesser extent. Among these markers are glutamatergic cells found mainly in the ZId but also present in all other regions, somatostatin which marks cells found in ZId, ZIv, and ZIr, and tyrosine hydrolase in the ZIr, ZId, ZIv (Mitrofanis, 2005).

Anatomy – Connections

Observing the connections the ZI makes with other parts of the central nervous system is an additional way to try to define sub-regions. Although many sections of the ZI connect to similar areas within the central nervous system, some of these connections can help define boundaries. Using tracer injections these borders can be defined.

The ZI receives connections from the cerebral cortex; primarily from the cingulate, frontal, and parietal areas (Mitrofanis, 2005). Animal studies have shown the cingualte projections terminate in the ZId and ZIr (Mitrofanis, 2005, Mitrofanis and Mikuletic, 1999). Projections from the parietal and frontal cortical areas are organized topographically with the head having the largest region (Mitrofanis, 2005). There are also projections from the ZI to the cortex primarily from the ZIr and ZId (Mitrofanis, 2005).

The ZI also projects to other areas of the diencephalon; especially the dorsal thalamus and the hypothalamus (Mitrofanis, 2005). The connections to the dorsal thalamus affect the intralaminar and higher-order nuclei via reciprocal connections (Mitrofanis, 2005, Power et al., 1999). The areas of the ZI where the majority of these connections are made are the ZId and ZIv, which contain both excitatory and inhibitory synapses (Mitrofanis, 2005). The projections to the hypothalamus primarily arise from the ZIr and are mostly dopaminergic (Mitrofanis, 2005).

In the basal ganglia the ZI interconnects with the substantia nigra, pedunculopontine tegmental nucleus, entopeduncular nucleus, and the globus pallidus (Mitrofanis, 2005). The majority of these connections are to the substantia nigra; both

pars compacta and pars reticulate, and the pedunculopontine temental nucleus in the pars dissipata (Mitrofanis, 2005). Most of the projections to these nuclei are Glu with a very few being GABAergic (Mitrofanis, 2005). This means that the ZI has an excitatory affect on these nuclei (Mitrofanis, 2005).

The ZI receives extensive input from the brainstem and sends projections back in return. In particular the pedunculopontine tegmental, periaqueductal gray, raphe, reticular nuclei, superior colliculus and the substantia nigra relay information to the ZId and ZIr (Mitrofanis, 2005). These regions of the brainstem are associated with arousal, locomotion, and emotion (Mitrofanis, 2005). The projections to the superior colliculus show obvious topography that arises principally from the ZIv (Mitrofanis, 2005).

There is also reciprocal innervation between the ZI and parts of the spinal cord. ZI afferents terminate within the spinal gray matter, specifically the ventral horn (Mitrofanis, 2005). Projections back to the ZI originate from the dorsal horn and intermediate gray (Mitrofanis, 2005). The information relayed contains both somatic and visceral receptors (Mitrofanis, 2005). Most of these projections appear to be in register somatotopically in the ZIv, ZId, and ZIc (Mitrofanis, 2005).

Physiology

Several attempts have been made to determine the function(s) performed by the ZI using various methods including; electrophysiological, pharmacological, and ablation. These studies have revealed four possible main functions that the ZI performs; namely controlling visceral activity, influencing arousal, shifting attention, and maintaining posture and locomotion.

The visceral functions associated with the ZI are similar to the hypothalamus. Chemical and electrical lesions of the ZI have shown a possible role in controlling visceral activity, specifically ingestion, sexual cycles, and cardiovascular activity (Mitrofanis, 2005). These studies are not conclusive since the ablation may not have been limited to the ZI, and surrounding areas or fibers may have been affected leading to the observed affects (Mitrofanis, 2005). Most of the effects are linked to the ZIr, the cardiovascular system is an exception (Mitrofanis, 2005). Lesions and injections of lidocaine result in an increase in intake of water and food, which leads to the conclusion that typically the ZI inhibits satiety (Mitrofanis, 2005). There are also studies that have shown that injections of dopamine or D_2 agonists further inhibit satiety (Mitrofanis, 2005, Tonelli and Chiaraviglio, 1995). Studies have shown when dopamine is released into the ZI of females production of luteinizing hormone is stimulated resulting in ovulation (Mitrofanis, 2005, James et al, 1987). In males, when stimulated oxytocin$^+$ cells become apparent in the ZI and other locations (Mitrofanis, 2005). The cardiovascular system influence is a lowering of arterial pressure and heart rate (Mitrofanis, 2005). Rat studies have shown that microinjections of L-glutamate into the ZI decrease both blood pressure and heart rate (Benedetti et al, 2004). Most of the cardiovascular control is linked to the ZIv (Mitrofanis, 2005). Electrical and glutamate stimulation of the ZI induces long lasting cardiovascular responses in rats (Benedetti et al, 2004).

The association of the ZI with arousal comes from the many interconnections with the major arousal centers, the brainstem and thalamus (Mitrofanis, 2005). Also, the ZI contains many Enk cells, which are associated with arousal nuclei (Mitrofanis, 2005).

There have been no functional studies to elaborate on this possible function; all the evidence is gathered from anatomical evidence (Mitrofanis, 2005). One study, which examined the effects of bilateral lesioning of the ZI, showed no effect on the sleep/wakefulness cycle of rats (Mitrofanis, 2005). This can lead to the assumption that the ZI does not play a major role, but may play a role in shifting from alert to non-alert and vice-versa.

The ZI projections to the superior colliculus are topographically organized and primarily initiate from the ZIv (Mitrofanis, 2005). Feline studies have shown that when the ZI is stimulated there are distinct eye and head orientating movements (Mitrofanis, 2005). This leads to the theory that the ZI is involved in the initiating orientative eye and head movements. Electrophysiologic studies have shown that GABAergic cells have pauses in their ongoing activity before the start of a saccade and the activity resumes at the end of a saccade (Mitrofanis, 2005). Muscimol and bicuculline/picrotoxin injections into the ZI generate characteristic head tilt movements (Mitrofansis, 2005).

Functional studies using electrical and chemical stimulation have shown that the ZI generates locomotor activity and particular limbic-related movements, generally associated with defense orientation. Anatomical studies have shown multiple ZI connections with motor related centers, the cerebellum, the red nucleus, and the cervical and lumbrosacral regions of the spinal cord (Mitrofanis, 2005). The majority of these connections are associated with the ZIc (Mitrofanis, 2005).

Relevance

While the exact role of the ZI is still unclear, there does appear to be some relevance to the treatment of Parkinson's Disease. A rat receiving either electrical or chemical stimulation of the ZI is more likely to develop generalized seizure (Mitrofanis, 2005). This shows the extensive connections with forebrain and brainstem regions (Mitrofanis, 2005). Some studies have shown that electrical stimulation of the ZI abolishes catalepsy in humans, illuminating the role of the ZI in postural control (Mitrofanis, 2005, Ossowska et al., 1993). The results from the study performed by Benazzouz and associates give some evidence that the ZI may directly or indirectly control the basal ganglia activity (Benazzouz et al, 2004).

Chapter 4: Deep Brain Stimulation

Theory

As stated earlier Deep Brain Stimulation has become a popular surgical intervention for patients suffering from PD. Surgical treatment is normally considered when the patient is no longer receiving a beneficial effect from the medication, or when the side effects from the medication are too debilitating to the patient. The two most common targets for this surgery are the STN and the GPi. While this procedure is a recognized therapy, there is still a lack of knowledge as to why and how this is beneficial for PD patients, essential tremor patients, and patients with obsessive compulsive disorder. There is a lack of understanding whether the stimulation produces an excitatory response or inhibitory response on the target structure and surrounding tissues.

Theory – What Is Being Effected

The stimulus can possibly affect multiple neuronal elements in the target area and each of these would yield different affects. The amount of structures affected by the stimulation is dependent upon the strength of the current intensity. Large myelinated axons are highly excitable and it is possible that the prominent affects of DBS are due to their activation (Dostrovsky and Lozano, 2002). Stimulation which activates projection neurons and/or afferent inputs to the nucleus would evoke both orthodromic and antidromic action potentials (Dostrovsky and Lozano, 2002). The effects of this

activation, depending on location in the target nucleus, would be seen clinically by the activation of afferent inputs onto neurons in the nucleus, direct effects on the output neurons, and/or effects mediated in other regions by the initial antidromic activation (Dostrovsky and Lozano, 2002).

Theory – Frequency Effects

Firing rates of peripheral and central neurons code the magnitude of the signal, and increased firing rates result in increased postsynaptic effect due to temporal summation (Dostrovsky and Lozano, 2002). Therefore increasing the stimulation frequency leads to an increased effect of the target structure of the neurons stimulated (Dostrovsky and Lozano, 2002). The frequencies used during DBS are generally over 100Hz (Dostrovsky and Lozano, 2002). Using frequencies at or above 100Hz produces effects similar to those received after an electrical or chemical lesion (Dostrovsky and Lozano, 2002). There have been observations that stimulation with low frequency can have the opposite effect (Dostrovsky and Lozano, 2002).

One study by Drostrovsky and Lozano looked at the effects of different stimulation frequencies in the target nucleus' and the resulting effects of the stimulation. Low frequency stimulation, about 50Hz, did not produce an affect on the spontaneous firing of neurons in the STN (Dostrovsky and Lozano, 2002). Similar stimulation of the GPi produced a short duration inhibition of about 25msec (Dostrovsky and Lozano, 2002). Some studies have used cytrochorme oxidase subunit I (CoI) mRNA, a metabolic marker of global changes in neuronal activity, to perform analysis of global changes in functional activity since it is not dependent upon markers of neurotransmission (Perier et

al, 1999). In normal rats, stimulation of the STN at 20Hz did not induce a change in the level of CoI mRNA in the substantia nigra pars riticulata, and globus pallidus (Benazzouz et al, 2004). In rats treated with a 6-hyroydopamine (6-OHDA) lesion to induce parkinsonian symptoms the stimulation produced higher levels of CoI mRNA in the STN and SNr (Benazzouz et al, 2004). This study also examined the effects of stimulation on the ZI. Low frequency, 20Hz, stimulation of normal rats had no effect the level of CoI mRNA in the STN, SNr, or GP (Benazzouz et al, 2004). The same stimulus given to 6-OHDA rats produced normalization of CoI mRNA in the SNr, but showed no change in the STN and GP (Benazzouz et al, 2004).

High frequency stimulation of the GPi, about 200Hz, produced inhibition after the termination of the pulse train, which lasted a few hundred microseconds (Dostrovsky and Lozano, 2002). Similar stimulation of the STN also produced inhibition following the stimulus train that lasted between 50msec to over 500msec. However, the stimulation initially produced inhibition, rebound excitation, and a further inhibitory period (Dostrovsky and Lozano, 2002). In normal rats, stimulation of the STN at 130Hz induced a significant reduction in the level of cytochrome oxidase subunit I (CoI) mRNA, a metabolic marker of global changes in neuronal activity, expression in the STN and substantia nigra pars riticulata (SNr) (Benazzouz et al, 2004). In the 6-OHDA rats the high frequency stimulation of the STN induced normalization of CoI mRNA in the STN, and the SNr reversed and decreased levels to below normal (Benazzouz et al, 2004). Similarly to the low frequency, the effects of high frequency stimulation to the ZI were studied. In normal rats there was a significant decrease in the level of CoI mRNA in the

SNr, a decrease in the STN, and a significant increase in the GP (Benazzouz et al, 2004). In the 6-OHDA rats high frequency stimulation of the ZI resulted in a normalization of CoI mRNA expression in the STN, a reversal of levels leading to a decrease in the SNr, and a normalization of the levels in the GP (Benazzouz et al, 2004). This leads to the conclusion that high frequency stimulation of the ZI in parkinsonian rats reverses dopamine denervation induced metabolic changes in the SNr and GP, which is similar to those induced by high frequency stimulation of the STN (Benazzouz et al, 2004).

Theory – Mechanisms

There are several theories as to how DBS helps ease some of the symptoms of PD. There are different proposed mechanisms, while no one theory seems to deliver all of the clinically evident results. Neural modeling, neural recording, and functional imaging are being used to determine the mechanisms of DBS (McIntyre and Thakor, 2002). Neural modeling is used to help determine the action potential generation resulting from stimulation (McIntyre and Thakor, 2002). Neural recording experiments have examined changes in baseline activity during and following the high frequency stimulus (McIntyre and Thakor, 2002). Functional imaging is used to address the effects of stimulation from a systems level perspective (McIntyre and Thakor, 2002). The most accurate statement available about DBS is that an unknown number of cells, in an unknown volume of tissue, are affected in an unknown manner, and produce a therapeutic effect where stimulus frequency is an important factor in the outcome (McIntyre and Thakor, 2002). There are three major theories for the mechanism through which DBS works.

The simplest hypothesis is stimulation reduces or inactivates neurons in the vicinity of the electrical stimulation (Lozano, 2001). Synaptic inhibition leads to the indirect regulation of neuronal output via activation of axon terminals that make synaptic connections with neurons near the stimulating electrode (McIntyre and Thakor, 2002). The synaptic inhibition could occur as a direct effect on the cell body or through synaptic mechanism involving the enhancement of inhibitory transmission (Lozano, 2001). This theory indicates that stimulation activates inhibitory afferents causing the release of GABA onto neurons leading to the inhibition of the structure receiving stimulation (Dostrovsky and Lozano, 2002). Additionally, the effectiveness of cathodic over anodic stimuli and the short strength-duration time constant of the therapeutic effect, both suggest that the stimulation target neural elements are axonal in nature (McIntyre and Thakor, 2002).

Depolarization blockade causes stimulation-induced alterations in the activation of voltage-gated currents which leads to blocked neural output near the stimulating electrode (McIntyre and Thakor, 2002). The persistent membrane depolarization inactivates sodium channels, which further prevents action potentials from being generated (McIntyre and Thakor, 2002). In vitro experiments have demonstrated that following 1 minute of extracellular stimulation from 100-250Hz produced a blockade of subthalamic activity for up to 6 minutes (McIntyre and Thakor, 2002). Stimulation of the STN seems to induce post-synaptic depolarization block and/or activation of the afferent inhibitory fibers (Benedetti et al, 2004). The frequencies used create a depolarization block that leads to the depressed output of the structure; but studies have not been able to

support this theory (Dostrovsky and Lozano, 2002). In the mounting evidence to disprove this theory is the electrophysiological study showed the cells fire at a rate over 200Hz and that the stimulation does not produce a block of frequencies that are clinically effective (McIntyre and Thakor, 2002).

Desynchronization of the network oscillatory activity underlying the disease state by stimulation is the final theory (McIntyre and Thakor, 2002). Neural modeling suggests that neurons directly affected by high frequency stimulus trains are excited by each stimulus pulse (McIntyre and Thakor, 2002). PET and fMRI imaging studies have shown that effective stimulation was associated with an increase in synaptic activity of the motor cortex and BG structures; meaning that DBS has an excitatory effect (McIntyre and Thakor, 2002). Simply, DBS could have the effects of lesioning by overriding abnormal electrical activity (McIntyre and Thakor, 2002).

Patient Inclusion

As with any surgery there are many risks associated with Deep Brain Stimulation. For this reason, there are extensive inclusion criteria to be considered for the procedure. Patients have to be over 21 years of age with idiopathic PD. Patients also should have clearly defined "on" periods in response to levodopa, their PD symptoms need to be disabling, and three or more hours of "off" periods. Pharmacological treatment should have been stable for more than one month. Previous surgeries for PD do not prevent this treatment. The patients preoperative CT and MRI are examined to make sure that there is nothing discovered through imaging which would preclude the procedure. Pregnancy,

alcohol abuse, drug abuse, Parkinson's Plus, and other implanted pulse generators such as pacemakers and defibrillators contraindicate surgery.

Procedure

The surgery consists of a number of stages. Patients do not take their PD meds for a minimum of 12 hours before the surgery. The patient is first prepped for the surgery, while under anesthesia, and the burr holes are drilled into the scull. A local anesthetic is provided at the sight of the incision into the scalp. The patient is then brought out from under anesthesia. The patient undergoes the procedure without any Parkinson's medication and awake due to the fact that either the drugs used to treat PD or the drugs used to anesthetize the patient might cause changes in the MER and stimulation testing. Next, MER is performed to target the nuclei effectively. The microelectrode and cannula can be used to stimulate the target nucleus to test for efficacy and side effects. Depending on the results observed from the microelectrode recording and the test stimulation, the system maybe repositioned and another microelectrode track done to determine the optimum placement. Once the best position for the lead has been located, the microelectrode is removed and the stimulator lead is secured in place. Testing with the stimulator is repeated to verify the results obtained previously. Once the testing has been done the lead is secured and the incisions in the scalp are closed. The pulse generator and extension are typically implanted in a second procedure after about a week.

Localization of subcortical nuclei is based on internal references, like the line between the anterior and posterior commissures (AC-PC line), along with a stereotactic atlas (Krack et al, 2000). MRI provides very precise imaging, which enables direct

visualization of the target nuclei, AC-PC points, and other relevant structures (Krack et al, 2000). A CT scan is also performed and then merged with the MRI. The CT shows the external markers, either the frame or the fiducials. Postoperative CT is used to check for any bleeding. A postoperative MRI may be done to check lead placement.

Since the STN is closer to the midline and the third ventricle creates less interference during an MRI the spatial variability is quite low (Krack et al, 2000). The microelectrode passes through the Thalamus into the ZI and finally into the STN. Upon entering the STN there is an increase in background noise due to the increased density of active neurons (Krack et al, 2000). The typical firing rates of the STN are 25-45Hz with irregular firing patterns, which may have movement or tremor activity (Krack et al, 2000). There are three types of cells that have been described in the STN. The tonic cells have a mean frequency of 49 Hz and are modulated by passive and voluntary movement. The pausing cells have an irregular discharge and high firing rates for a few milliseconds and then a pause, similar to the pausers in the GPe. Tremor cells have a burst pattern of about 4–5 Hz with frequency of 9–66 Hz, and are sensitive to kinesthetic stimulation. (Deuschl et al, 2000).

For placements in the GP the microelectrode passes through the striatum into the external segment of the globus palladus and finally into the internal segment of the globus palladus. Microelectrode recording and stimulator testing are used throughout the GPi and continue on to identify the top of the optic tract to avoid placement of the lead in or near it. Confirmation of the optic tract is verified by the use of a flashing light and the corresponding signal change in the microelectrode recording.

Microelectrode recording helps determine precisely the boundaries of the target area (Krack et al, 2000). Depending on location the waveform and sound give an indication as to which nucleus the microelectrode is passing through. The transition from the ZI to the STN is characterized by an abrupt increase in background noise, since the density of cells is higher in the STN than in the ZI (Falkenberg, McNames, and Burchiel, 2003).

Macrostimulation results in larger current diffusion, which provides more clear-cut responses (Krack et al, 2000). However, placement of a macroelectrode into a small target can induce reversible lesion-like effects, making intra-operative evaluation extremely difficult (Krack et al, 2000). The stimulation is used to observe the effects before securing the placement of the lead. The lead has four contacts near its tip; of these there are several configurations of the contacts to be selected as the cathode. Modeling shows that the current density is concentrated at the edges of the electrode contacts (McIntyre and Thakor, 2002). The electrical field created by the lead is dependent of the shape of the electrode and electrical conductivity of the tissue (McIntyre et al, 2004). The field of influence is dependent upon the location of the lead, since the CNS is inhomogeneous, and dependent upon direction, due to anisotropic nature of the tissue (McIntyre et al, 2004). Depending on placement, stimulation at therapeutic levels can spread outside the target and into surrounding structures (McIntyre et al, 2004).

DBS Effects on Tissue

A few postmortem examinations have been done to examine the effects of DBS on the tissue surrounding the leads to look for deleterious effects. The cause of death for

the patients whose brains were examined was unrelated to the surgery and subsequent stimulation (Henderson et al, 2002 & Haberler et al, 2000). The brains prepared using a formalin fixation (4% - 15% depending on study) and slices were done coronally, parallel to the lead track, and perpendicular to the lead track (Henderson et al, 2002 & Haberler et al, 2000). Tissue blocks were paraffin embedded and stained (Henderson et al, 2002 & Haberler et al, 2000). The brains showed morphological substrate of PD, including loss of pigmented neurons, Lewy bodies, and gliosis in the substantia nigra (Haberler et al, 2000). The results showed mild cell loss, gliosis, some tissue vacuolation associated with the electrode tracts, and a mild inflammatory response associated with the electrode penetration, which breaches the blood-brain barrier (Henderson et al, 2002). There was a development of a thin inner capsule of connective tissue (Haberler et al, 2000). There was no significant neuronal loss associated with the surgery and the minor tissue changes were unlikely to have significant functional effects (Henderson et al, 2002). This supports the theory that the procedure is essentially reversible (Henderson et al, 2002).

Signal Processing

Neuronal structures are typically classified using three parameters: kinesthetic activity (response to movement), phasic activity (spike pattern), and tonic activity (firing rate) (Farve et al, 1999). Kinesthetic and tonic responses can be evaluated based on objective characteristics of the spike train (Falkenberg, McNames, Burchiel, 2003). Currently the phasic activity is dependent upon a human observer to describe or interpret the spike pattern, this creates error and inconsistency when considering different sources (Falkenberg, McNames, Burchiel, 2003).

Single unit neuronal activity is generally studied in two ways: 1) performance during a behavioral task, or 2) under natural conditions without extraneous stimuli (Kaneoke & Vitek, 1996). Spontaneous neuronal discharge patterns may change with varying physiological and pathological conditions (Kaneoke & Vitek, 1996). Changes from normal have been observed in parkinsonian monkeys in numerous subcortical structures (Kaneoke & Vitek, 1996).

There are two types of activity observed from the neuronal recordings. Bursts are periods in which a significantly higher number of discharges occur in comparison to other times in the spike train (Kaneoke & Vitek, 1996). Oscillations are patterns of neuronal activity in which discharges occur periodically (Kaneoke & Vitek, 1996). Bursts and oscillations may occur with different mechanisms and have different

physiological meanings (Kaneoke & Vitek, 1996). Bursts may have specific effects on its target cells while oscillations may reflect synchronized discharges of several adjacent neurons (Kaneoke & Vitek, 1996).

Analyses used

Several approaches have been used to characterize patterns of spontaneous neuronal activity; mean discharge rates, instantaneous discharge rates, interspike interval (ISI), and autocorrelation (Kaneoke & Vitek, 1996). For the purposes of this study the number of spikes per second and the bursting pattern were of interest. Burst detection has classically been done in one of two ways.

First, by using the Poisson surprise method to find groups of neuronal discharges in which several successive ISI do not follow a Poisson process based on mean discharge rate of the spike train (Kaneoke & Vitek, 1996). The Poisson surprise (S) is calculated by:

$$S=-\log P$$

where P is

$$P=e^{-rt} \Sigma (rt)^i/i!$$

(Legendy & Salcman, 1985)

The Poisson method is useful when analyzing a continual random-looking spike train to detect an occasional segment having a momentary deviation from randomness (Legendy & Salcman, 1985). This does not mean that the spike train has a Poisson distribution (Legendy & Salcman, 1985).

Second, ISI histograms are used to differentiate the distribution of burst ISI from others occurring in the spike train (Kaneoke & Vitek, 1996). Neither of these methods is perfect, the Possion method fails to detect patterns of bursting activity due to the fact it relies on variance in ISI inside a burst, if there is not a variance the burst is not detected (Kaneoke & Vitek, 1996). The histogram method does not detect bursts when a spike train has prominently varying ISI (Kaneoke & Vitek, 1996).

Oscillatory activity, sometimes confused with bursts, is usually detected by autocorrelation (Kaneoke & Vitek, 1996). Analyzing a signal to detect oscillatory properties is typically done by autocorrelation and the Lomb periodogram (Kaneoke & Vitek, 1996). This allows for the detection of the presence of multiple frequencies and the assessment of the significance of the detected frequencies (Kaneoke & Vitek, 1996).

Relevance

Identifying single neurons within structures of the brain during surgery is currently done by observation of a displayed waveform and listening to the signal played through speakers for characteristic firing patterns. Repetition and experience is the primary way that a person becomes familiar with identifying neurons. Providing a less subjective technique for identification is ideal.

Detecting bursts is done with a few assumptions. First, that the spike train is composed of two periods, bursting and non-bursting. Second, there are fewer burst periods than non-burst periods. Third, the bursting periods contain significantly more spikes than the non-burst periods. (Kaneoke & Vitek, 1996)

Research Methodology

Surgical Procedure

Patients who underwent DBS have an extensive work up mentioned in Chapter 3. All patients receive a neuropsychological evaluation. This evaluation is done to uncover any sub-clinical dementia. While DBS does not cause dementia, it can aggravate the dementia if it is already present.

The patients' CT & MRI were merged using the Stealth Station (Medtronic) and the targeting was calculated. If the case was a frameless case, the equipment was secured to the skull following the burr hole and targeting was verified. Figure 2 shows the equipment that is secured to the scull during a frameless case. Figure 3 shows the placement of the equipment.

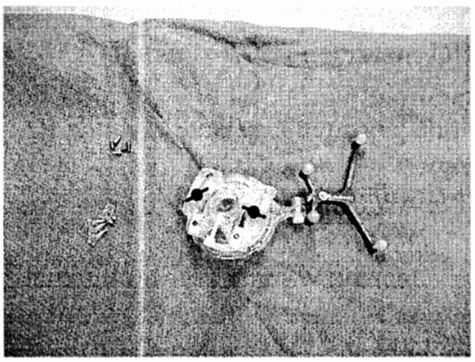

Figure 2: Frameless equipment

If the case was a framed case, the stereotactic frame was set up and the settings were confirmed.

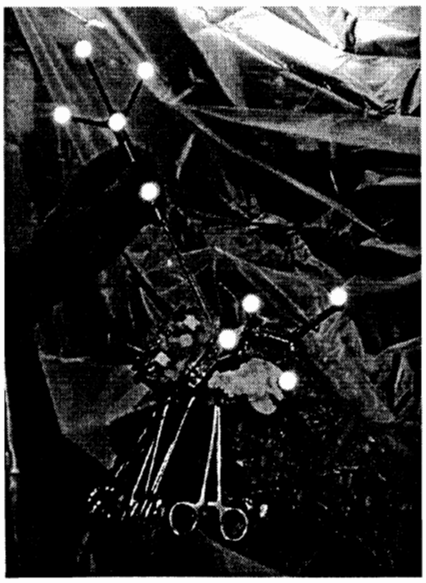

Figure 3: Frameless set-up

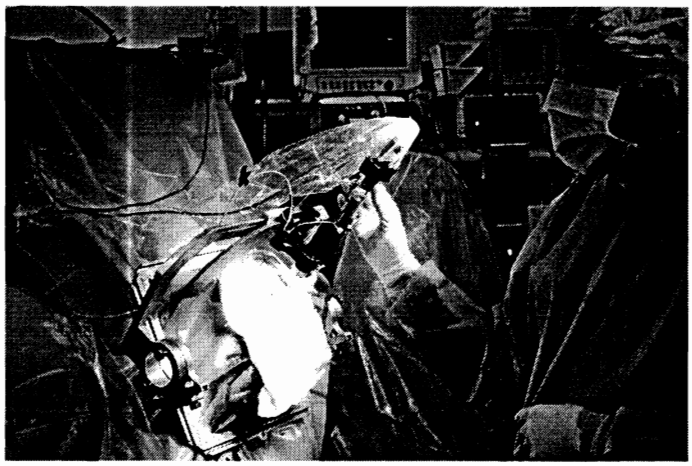

Figure 4: Framed procedure

Figure 4 shows the set up for the framed cases. The stereotactic frame is attached to the patients' skull and to the operating table to limit the movement of the patient. The surgeon is turning the microdrive to advance the microelectrode through the brain.

The microelectrode (Medtronic 34680) was placed inside the microcannula (Medtronic 9033G0611) and MER commenced. A cable (Medtronic 9013C0501) connecting the microelectrode to the Leadpoint system was connected. A second cable connected the microdrive to the Fred-Haer digital display unit. The target location for the STN surgeries was 12mm lateral from the midline, 4mm posterior from mid anterior commissure – posterior commissure plane, and 4mm deep from the anterior commissure – posterior commissure plane. This targets the bottom of the STN. The depth to target was displayed using a Fred-Haer digital display unit, which encoded the movement of the microelectrode and gave a digital read out of the distance traveled. Once the ideal placement was located, the lead was secured in place. Medtronic Model 3389 was used for STN procedures and Model 3387 was used for all other targets and participants in the CSP-486 study. There is a difference in length between the two models. The CSP-486 study is an ongoing multicenter study comparing the effects of six months best medical

therapy to DBS. When the patient receives surgery they are randomized for site to compare the efficacy of GPi vs. STN.

Generally the patient is brought back after about a week for a second procedure. During this procedure the pulse generator (Medtronic 7428 or 7426) is placed in the upper chest or stomach area depending on patient preference. Next the extensions (Medtronic 7482-66) were connected to the leads and the pulse generator.

Data Acquisition

Microelectrode recording was done using tungsten microelectrodes provided by Fred Haer (MER-4020 T) and Medtronic (MER-5000T, 9013S0831). The data was displayed on a monitor, auditory playback was provided through speakers, and recorded by using the Medtronic Leadpoint system. The waveforms were identified intraoperatively by a neurologist and neurosurgeon based on firing patterns, amplitudes, and frequencies. If the waveform was of interest it was saved for 30 seconds. The Leadpoint system also has a program to export the recorded data in a variety of file types including text, binary, and wav. The Leadpoint

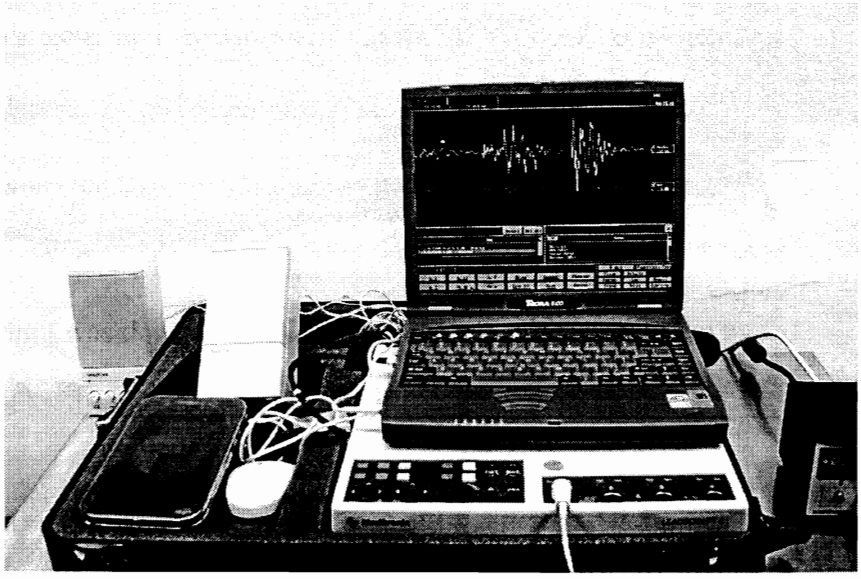

Figure 5: Leadpoint system

system acquired data at 24kHz with a 16-bit resolution. Figure 5 shows the Leadpoint system.

Once the data was collected, the files were then exported using, Leadpoint Export Utility a program also provided by Medtronic. The files were exported as binary and wav files. The binary files were exported with 32-bit word size. The wav files were used as an audio playback of the file. This allowed for further review and assurance that the data was appropriate.

Data Selection

There were 34 patients who had surgery with a target nucleus being the STN. Their MER files were examined for this study. Transcripts from the surgery were used to determine which files might be of interest. The extracted wav file was played to determine if the content of the file was of interest; and if so, if there were any portions of the file that needed to be excluded. Reasons for excluding part of the file were generally due to interference during the recording. Of the patients' whose files were considered 8 had MER files that contained ZI recording. The files examined had a minimum of 5 seconds of continuous recording in the ZI. The total number of files examined was 25.

Data Analysis

After the files were exported, the binary file was loaded into Plexon's offline sorting program. A threshold was set so that it was well above the noise level, between 6 and 9 standard deviations on the Peak Heights Histogram. The spikes were then sorted into units using the Valley-Seeking Method. This algorithm uses the inter-point distances in feature space to assign the waveforms to clusters. This works by calculating the

number of neighbors each point has in feature space. These points are defined when they are within a certain critical distance of the target point. The equation for critical distance is:

Dist = 0.25 * sigma * Parzen Mult

Sigma is the standard deviation of the distances of all points to the overall mean. Parzen Mult is a user-specified parameter named for its similarity to the Parzen density estimation kernel. (offline sorter users manual)

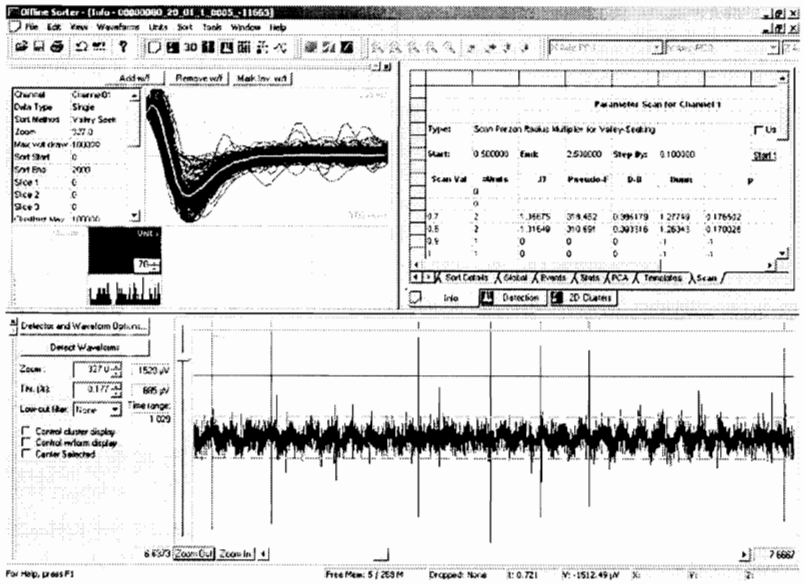

Figure 6: Offline sorter

In the case of the ZI, the recording was done on one single unit. Figure 6 is an illustration of the Plexon environment. The threshold is set and the waveforms are then sorted by meeting several criteria. First, if the spike does not cross the threshold it is not considered. Second, the waveforms are sorted by their shape using the Valley-Seeking Method

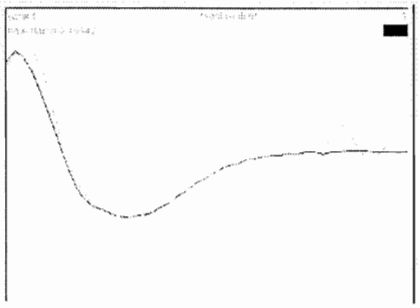

Figure 7: Best fit spike

described earlier. Figure 7 shows the best-fit spike and the shape to which it was matched for a particular file. The time stamp for when each spike crossed the threshold was manually recorded. The mouse curser was placed on these indicators to record the time stamp for each waveform. Figure 8 shows the threshold has been set for a particular file, once the sorting has taken place the waveforms that match the template are marked with an indicating mark above the waveform where the threshold is crossed. The arrow shows the threshold and the circle shows the time stamp.

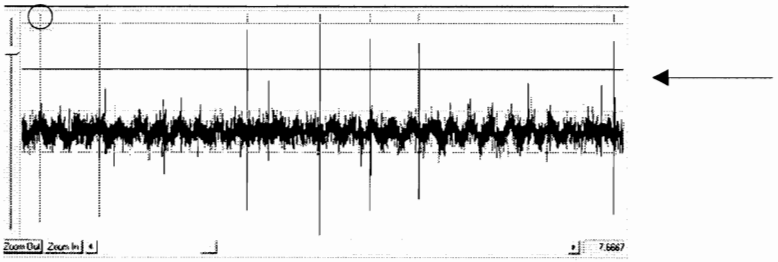

Figure 8: Threshold and time stamp for sorted file

An Excel file was created and to record the file information. This was done so that the data could be analyzed. The first worksheet contained the file information:

- File name
- Total recording time
- Sampling frequency
- Depth where the recording took place
- Time segments chosen for analysis
- The number of spikes which met the sorting criteria
- The threshold used in the offline sorter

- The time stamps of the first and last spikes

Several calculations were then made: the time elapsed time for the segments chosen for analysis, the spikes per second, and the time elapsed between the first and last spike time stamps. The spikes per second were calculated for two different criteria. First, it was calculated using the number of spikes and the time segments chosen for analysis. This gave a broad overview of the entire file. Next, it was calculated using the number of spikes and the time elapsed between the time stamps for the first and final spikes. This narrowed the scope further and provided a more concise view of the neuronal activity. The averages, standard deviations, minimums, and maximums were calculated for each file's:

- Depths
- Time segments
- Both calculations of the spikes per second
- The threshold value used in offline sorter
- The time elapsed between the first and final spikes.

The second worksheet examined the data from each file. Each file was looked at individually. The time stamps for each spike were recorded in this worksheet. These time stamps were used to calculate the time between spikes. The inverse of that value was calculated to give the firing frequency between the two spikes.

The third worksheet was used to compare the data from all the ZI files. The time stamps, time between each file, and the firing frequency from all the files were placed

into three continuous columns. The number of occurrences for each time interval was calculated.

A similar time sheet was created for a file containing STN spike time stamps. This was done so that a comparison between the ZI and STN could be done.

Analytical Results

Demographics

Of the eight patients whose MER files were used for this study six were male and two were female. Their average age was 59.75 years, with ages ranging from 47-84. The depth was measured in distance traveled with 20mm being the target distance at the bottom of the STN. The average depth of the recordings was 15.3mm with a standard deviation of 2.67. The average time selected for analysis was 17.41 seconds. The minimum time was 5 seconds and the maximum time was 30 seconds. The average number of spikes per second, using the first and last spike time stamps to calculate the elapsed time, had an average of 10.01 spikes/second.

ZI Analysis

The time from when one spike crossed the threshold until the next was calculated for 2660 spikes. This created 2635 elapsed time results. A histogram was created with these values to observe the most frequently occurring times. This histogram is shown in Figure 9.

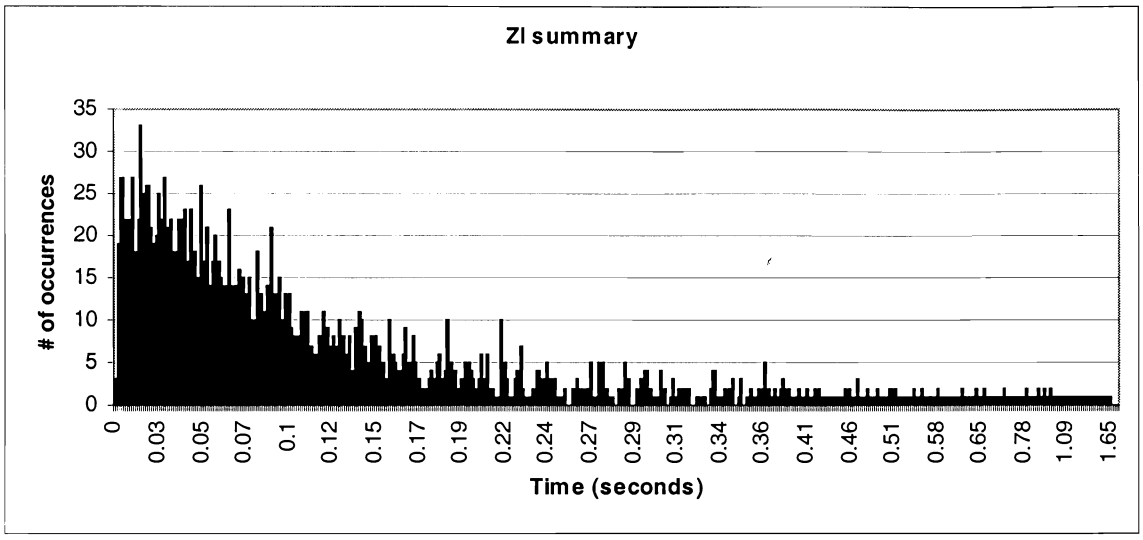

Figure 9: Summary of all ZI data

The inverse of the elapsed time was used to find the firing frequency. A histogram was also created for this set of values. This histogram shows the most frequently occurring firing frequencies within the ZI are 66.67 Hz, 200 Hz, 90.91 Hz and 34.48 Hz. The average frequency was 6.65 Hz. Figure 10 shows the frequency histogram.

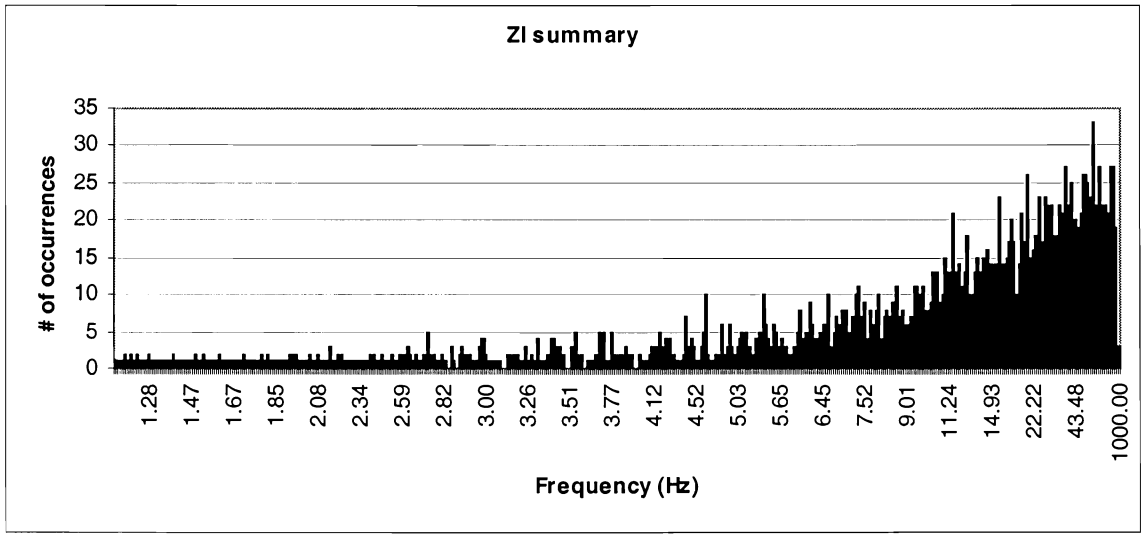

Figure 10: ZI firing frequencies

Since most of the lower frequencies aren't of interest since they occur only one or two times; 90% of the data was examined. This contained the highest frequencies. The results are shown in Figure 11. The average firing frequency was 11.57 Hz. The percentage of firing frequencies equal to or over 40 Hz was 21%.

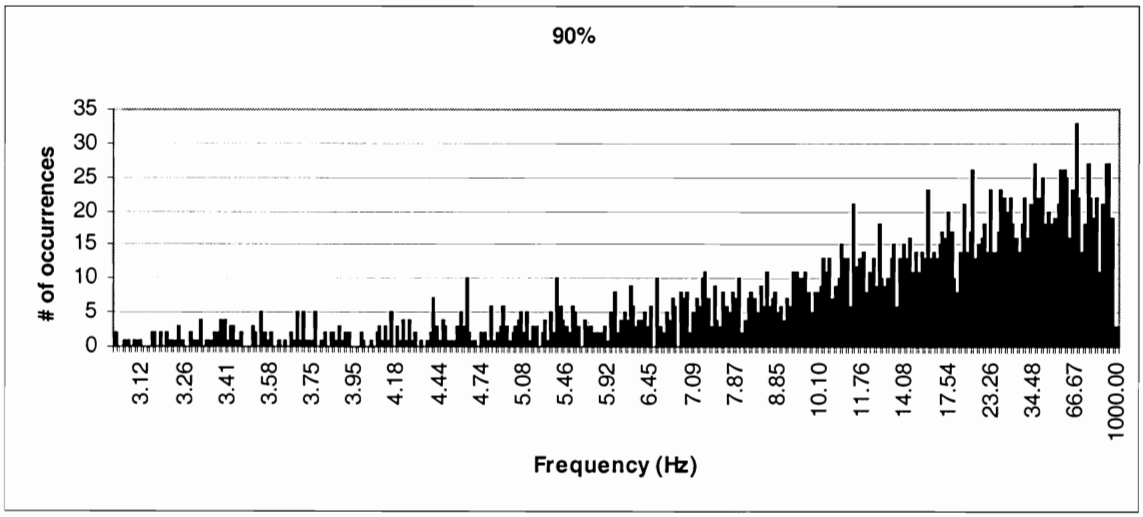

Figure 11: 90% of ZI data

Next, a closer look was taken at what the firing frequencies occurred between spikes that fired within 0.1 seconds, or 10Hz of one another. The 25 files were examined for occurrences of 3 or more spikes with a time between them of less than 0.1 seconds. There was one less elapsed time value for each group of 3 or more spikes, so for 3 spikes there would be 2 time values, for 4 spikes – 3 time values, etc. This data was also formatted into a histogram to show the most frequent occurring firing frequencies within the ZI for spike trains. Figure 12 shows the frequencies present in the spike trains that were analyzed.

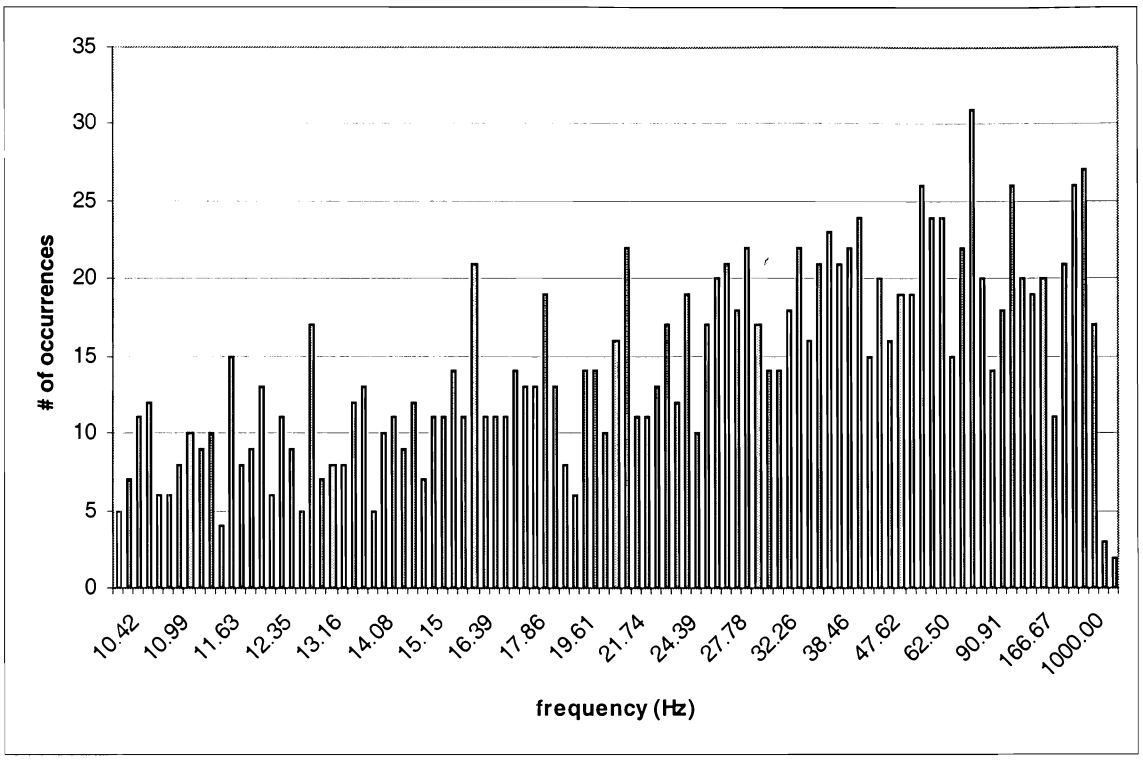

Figure 12: ZI spike train data

STN Analysis

To validate the findings of the ZI recordings a similar procedure was done with a recording from the STN. This would further elaborate the differences between the two nuclei, which are very apparent during surgery. Recording in the STN generally has input from many cells due to the density and concentrated activity of the STN. Figure 13 shows the Interspike Intervals for the entire file. The most frequency occurring frequencies were 167 Hz, 100Hz, and 200Hz, each occurring more than 20 times. The average firing frequency was 22.6Hz. This value is slightly below the range of frequencies expected.

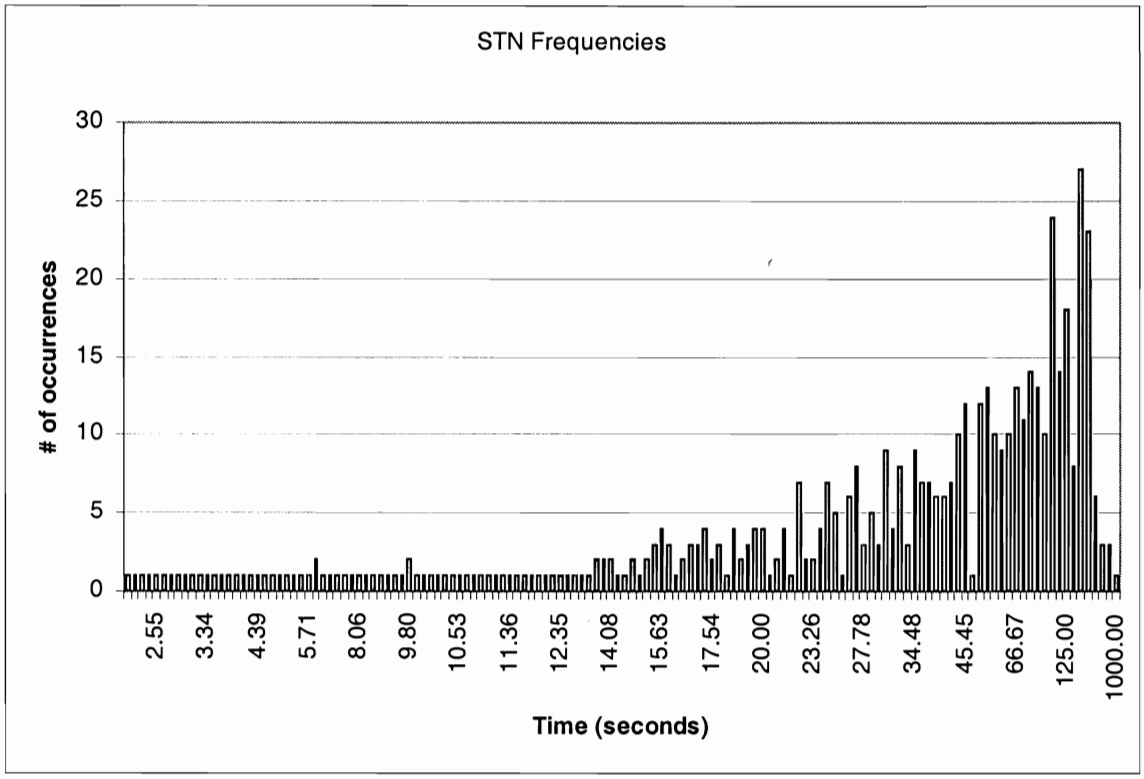

Figure 13: STN data

Again 90% of the file was examined to look at data points with more occurrences. Figure 14 shows the result of narrowing the data. When this selection is examined the average firing frequency is 37.7 Hz.

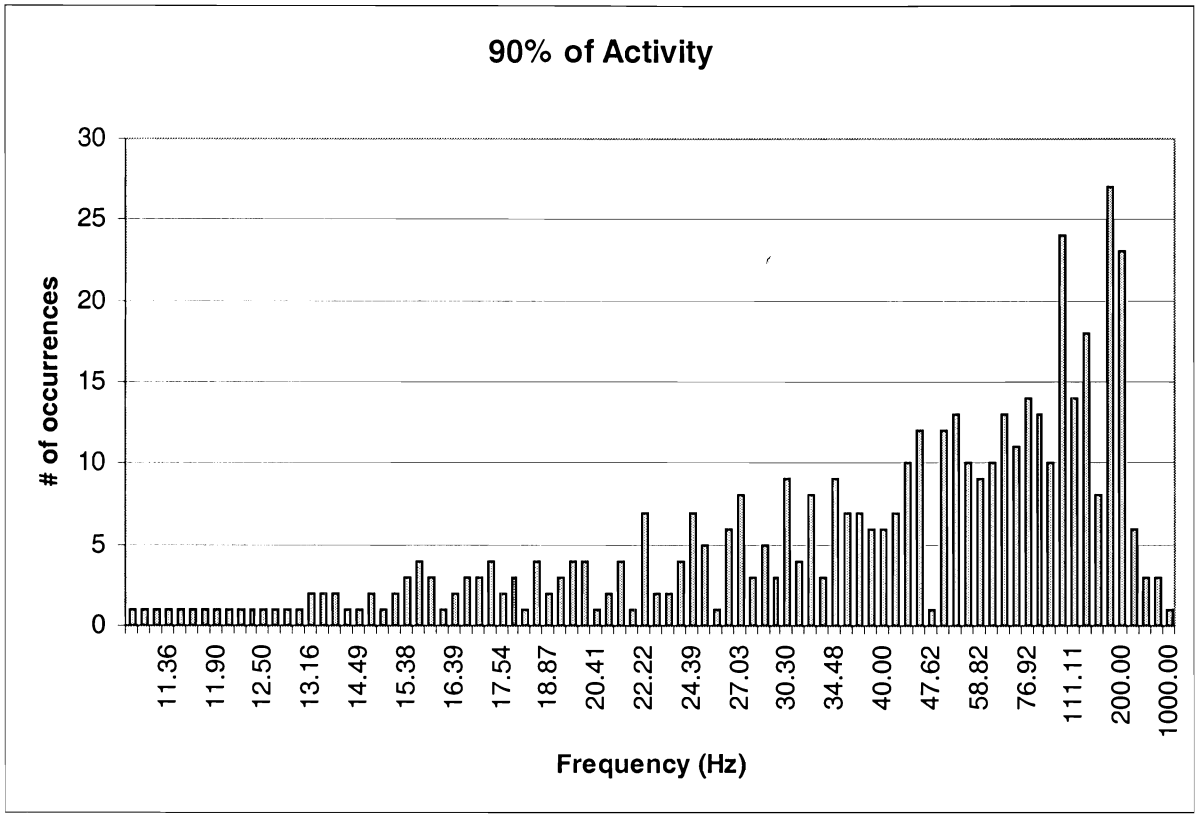

Figure 14: 90% of STN data

Next spikes were examined for occurrences of 3 or more spikes with a time between them of less than 0.1 seconds in the same manner as the files from the ZI. Figure 15 shows a summary of this data.

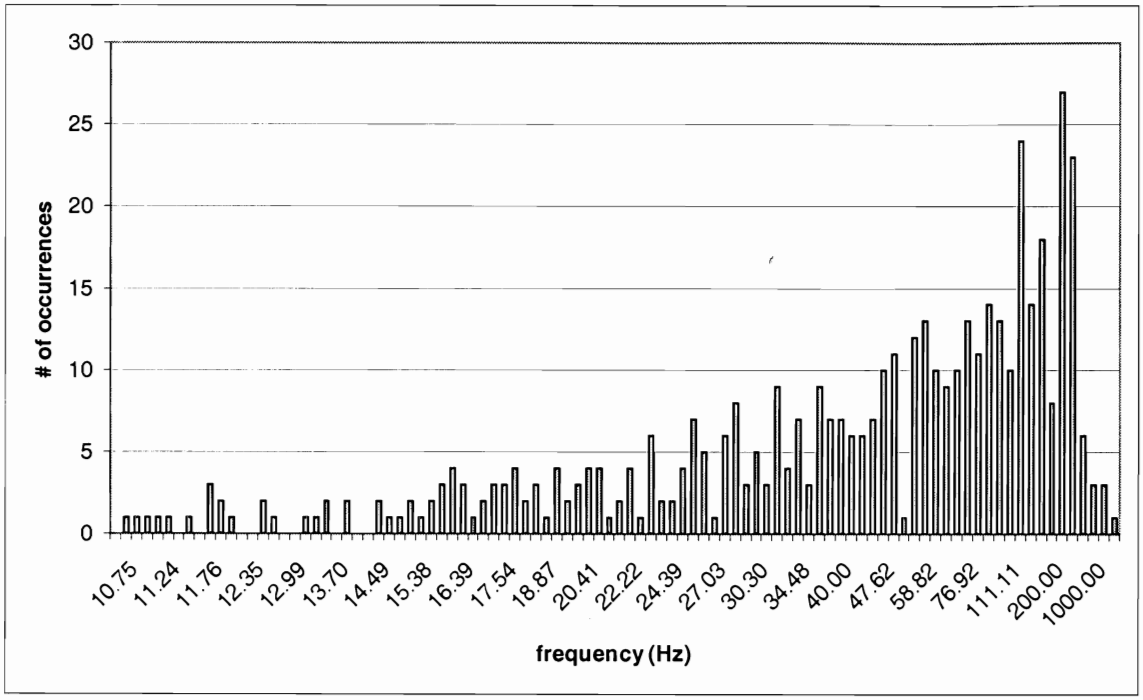

Figure 15: STN spike train data

ZI vs. STN

A direct comparison of the two nuclei further elaborates the differences in the firing frequencies between the two nuclei. Figure 16 shows the comparison of one ZI file and STN file.

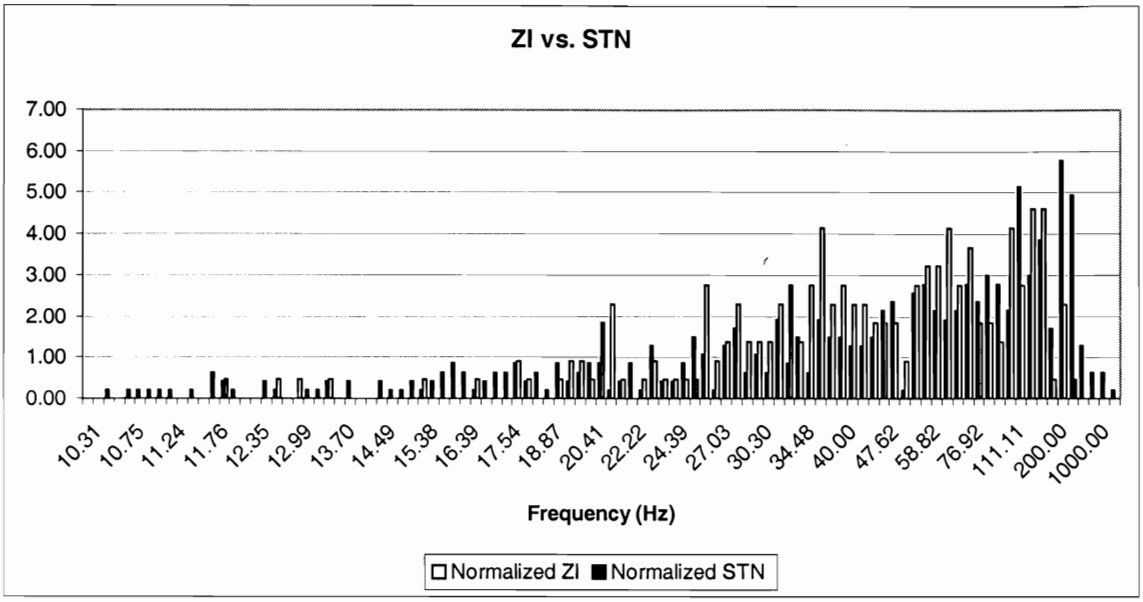

Figure 16: Comparison of the ZI and the STN

Closer examination shows the differences in the high frequencies, which are represented with the two normalized recordings. Figure 17 shows the differences in higher frequencies between the ZI and the STN.

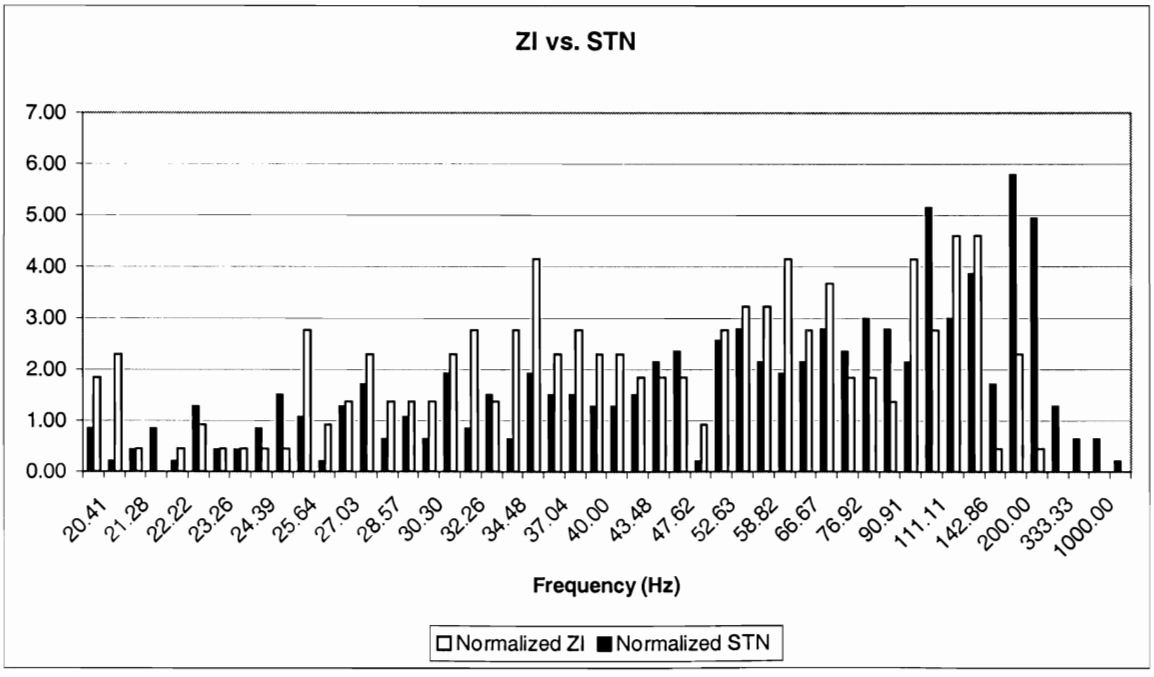

Figure 17: Comparison of the high frequency content of the ZI and STN

Conclusions

Objective

The objective of this study has been to characterize the ZI using its firing frequency. With more accurate characterization surgical personnel could more readily recognize the ZI during Deep Brain Stimulation procedures. This would give a more complete picture to the personnel within the operating room. The more information obtained during MER; the more complete picture is obtained from the current track, and better planning can be done for future tracks. Firing frequencies, along with other observations, are used to differentiate one nucleus from another during MER. Firing frequency is generally determined by looking at the Interspike Intervals of recordings done within the nucleus.

ZI Results

To meet the stated objective, intraoperative ZI recordings were examined. The time intervals between 2660 spikes were calculated. This yielded 2635 ISIs, which were examined.

The ISIs gives the firing frequencies through a simple mathematical operation. When the ZI files were examined it showed that the ZI has some high frequency peaks. The ZI, however, does continue to have activity into the lower frequency ranges. The files were further examined to look at an instance where spikes were tonically firing, that

is without pauses between occurrences. A pause was defined to be any elapsed time greater than or equal to 0.1 seconds. This examination still showed a broad range of firing frequencies.

Variations in the firing frequencies could be due to several things. The simplest explanation is that these recordings were done on eight different individuals. However, since multiple recordings were taken from most of these patients it is unlikely to explain the wide range of ISIs observed. There are two other possible reasons for such variation.

First, the effects of Parkinson's Disease on the ZI is unknown. The changes in activity due to Parkinson's Disease of the nuclei sending information to the ZI could cause a change in firing frequency. A study could be done to see if there is any correlation between disease progression and the firing frequencies.

Secondly, the region of the ZI where the recordings were made could also account for the variations. The ZI is made up of a heterogeneous collection of cells, which seem to have between 2-6 zones. Recording in different zones may result in changes in firing frequency. Verification of the location in the ZI where the recording was made may further explain the variation in firing frequencies.

Literature Cited

Literature Cited

American Parkinson Disease Association Inc. www.apdaparkinson.org March 10, 2005.

Benazzouz, A., Gao, D.M., Ni, Z.G., Piallat, B., Bouali-Benazzouz R., Benabid, A.L. Effect of High-Frequency Stimulation of the Subthalamic Nucleus on the Neuronal Activities of the Substantia Nigra Pars Reticulata and the Ventrolateral Nucleus of the Thalamus in the Rat. *Neuroscience* 99: 28-295, 2000.

Benazzouz, A., Tai, C.H., Meissner, W., Biolac, B., Bezard, E., Gross, C. High-Frequency Stimulation of both Zona Incerta and Subthalamic Nucleus Induces a Similar Normalization of Basal Ganglia Metabolic Activity in Experimental Parkinsonism. *The FASEB Journal* 18: 528-529, 2004.

Benedetti F., Colloca L., Lanotte M., Bergamasco B., Torre E., Lopiano L. Autonomic and Emotional Responses to Open and Hidden Stimulation of the Human Subthalamic Region. *Brain Research Bulletin* 63: 203-211, 2004.

Deuschl G., Raethjen, J., Baron, R., Lindemann, M., Wilms, H., Krack, P. The Pathophysiology of Parkinsonian Tremor: A Review. *Journal of Neurology* 247S5: V/33-V/48, 2000.

Dostrovsky, J.O. and Lozano, A.M. Mechanisms of Deep Brain Stimulation. *Movement Disorders* 17S3: S63-S68, 2002.

Faist, M., Kurz, D., Berger, W., Maurer, C., Pollak, P., Lucking, C.H. Effect of Bilateral Subthalmic Nucleus Stimulation on Gait in Parkinson's Disease. *Brain* 124: 1590-1600, 2001.

Falkenberg, J.H., McNames, J., Burchiel, K.J. Statistical Methods of Analysis and Visualization of Extracellular Microelectrode Recordings. EMBC : 2515-2518, 2003.

Habler, C., Alesch, F., Mazal,P.R., Pilz, P., Jellinger, K., Pinter, M.M., Hainfellner, J.A., Budka, H. No Tissue Damage by Chronic Deep Brain Stimulation in Parkinson's Disease. *Annals of Neurology* 48: 372-376, 2000.

Henderson, J.M., Pell, M., O'Sullivan, D.J., McCusker, E.A., Fung, V.S.C., Hedges, P., Halliday, G.M. Postmortem Analysis of Bilateral Subthalamic Electrode Implants in Parkinson's Disease. Movement Disorders 17: 133-137, 2002.

James, M.D., MacKenzie, F.J., Tuohy-Jones, P.A., Wilson, C.A. Dopaminergic Neurons in the Zona Incerta Exert a Stimulatory Control on Gonadotrophin Release via D1 Dopamine Receptors. *Neuroendocrinology* 45: 348-355, 1987.

Kaneoke, Y. and Vitek, J.L. Burst and Oscillation as Disparate Neuronal Properties. *Journal of Neuroscience Methods* 68: 211-233, 1996.

Kolmac C.I. and Mitrofanis J. Distribution of Various Neurochemicals within Zona Incerta : an Immunocytochemical and Histochemical Study. *Anatomy of Embryology* 199: 265-280, 1999.

Krack, P., Poepping, M., Weinert, D., Schrader, B., Deuschl, G. Thalamic, Pallidal, or Subthalamic Surgery for Parkinson's Disease? *Journal of Neurology* 247S2: II/122-II/134, 2000.

Legendy, C.R. and Salcman M. Bursts and Recurrences of Bursts in the Spike Trains of Spontaneously Acive Striate Cortex Neurons. *Journal of Neurophysiology* 53: 92-939, 1985.

Limousin-Dowsey, P., Pollak, P., Van Blercom, N., Krack, P., Benazzouz, A., Benabid, A.L. Thalamic, Subthalamic Nucleus and Internal Pallidum Stimulation in Parkinson's Disease. *Journal of Neurology* 246S2: II/42-II/45, 1999.

Lozano, A.M. Deep Brain Stimulation for Parkinson's Disease. *Parkinsonism & Related Disorders* 7: 199-203, 2001.

Maurice, N., Thierry, A.M., Glowinski, J., Deniau, J.M. Spontaneous and Evoked Activity of Substantia Nigra Pars Reticulata Neurons during High-Frequency Stimulation of the Subthalamic Nucleus. *The Journal of Neuroscience* 30: 9929-9936, 2003.

McIntyre, C.C., Mori, S., Sherman, D.L., Thakor, N.V., and Vitek, J.L. Electric Field and Stimulating Influence Generated by Deep Brain Stimulation of the Subthalamic Nucleus. *Clinical Neurophysiology* 115: 589-959, 2004.

McIntyre, C.C., and Thakor, N.V. Uncovering the Mechanisms of Deep Brain Stimulation for Parkinson's Disease through Functional Imaging, Neuronal Recording, and Neural Modeling. *Critical Review in Biomedical Engineering* 30: 249-281, 2002.

Mitrofanis, J. Some Certainty for the "Zone of Uncertainty"? Exploring the Function of the Zona Incerta. *Neuroscience* 130: 1-15, 2005.

Mitrofanis, J. and Mikuletic L. Organization of the Cortical Projection to the Zona Incerta of the Thalamus. *Journal of Computational Neurology* 412: 173-185, 1999.

Mobin, F., DeSalles, A.A.F., Behnke, E.J., Frysinger, R. Correlation between MRI-Based Stereotactic Thalamic Deep Brain Stimulation Electróde Placement, Macroelectrode Stimulation and Clinical Response to Tremor Control. *Movement Disorders* 72: 225-323, 1999.

Miyai, I., Fujimoto, Y., Yamamoto H., Ueda Y., Saito T., Nozaki, S., Kang, J. Long-Term Effect of Body Weight-Supported Treadmill Training in Parkinson's Disease: A Randomized Controlled Trial. *Archives of Physical Medicine Rehabilitation* 83: 1370-1373, 2002.

Miyai, I., Fujimoto, Y., Ueda, Y., Yamamoto H., Nozaki, S., Saito, T. Treadmill Training with Body Weight Support: Its Effect on Parkinson's Disease. *Archives of Physical Medicine Rehabilitation* 81: 849-852, 2000.

Nandi, D., Aziz, T.Z., Liu, X., Stein, J.F. Brainstem Motor Loops in the Control of Movement. *Movement Disorders* 17S3: S22-S27, 2002.

Ossowska, K., Karcz-Kubicha, M., Wardas, J., Krezolek, A., Wolfarth, S. Zona Incerta-Lateral Hypothalamus as an Output Structure for Impulses Involved in Neuroleptic Drug-Induced Catalepsy. *Naunyn Schmiedebergs Archives of Pharmacology* 347: 415-420, 1993.

Parkisnon's Disease Foundation. www.pdf.org March 10, 2005.

Perier, C., Tremblay, L., Feger J., Hirsch, E.C. Behavioral Consequences of Bicuculline Injection in the Subthalamic Nucleus and the Zona Incerta in Rat. *The Journal of Neuroscience* 19: 8711-8719, 2002.

Perier, C., Vila, M., Feger, J., Agid, Y., Hirsch, E.C. Functional Activity of Zona Incerta Neurons Is Altered after Nigrostriatal Denervation in Hemiparkinsonian Rats. *Experimental Neurology* 162: 215-224, 2000.

Pohl, M., Rockstroh, G., Ruckriem, S., Mrass, G., Mehrholz, J. Immediate Effects of Speed-Dependent Treadmill Training on Gait Parameters in Early Parkinson's Disease. *Archives of Physical Medicine Rehabilitation* 84: 1760-1766, 2003.

Schaltenbrand, Georges and Wharen, Waldemar. Atlas for Stereotaxy of the Human Brain. Thieme: New York 1998.

Tonelli, L. and Chiaraviglio, E. Dopaminergic Neurons in the Zona Incerta Modulates Ingestive Behaviour in Rats. *Physiologic Behavior* 58: 725-729, 1995.

VITA

Heather Joyce Green

A. Personal History

Citizenship: United States of America

B. Education History

Virginia Commonwealth University, Richmond, Virginia
Major: Biomedical Engineering
Degree: Masters of Science
Year: 2005
Thesis: Characterization of the Zona Incerta

Louisiana Tech University, Ruston, Louisiana
Major: Biomedical Engineering
Degree: Bachelors of Science
Year: 2001

C. Professional Positions

May 2003 – August 2005
Medical Technologist, Hunter Holmes McGuire VA Medical Center, Richmond, Virginia
Part of surgical team performing Deep Brain Stimulation.
Performed microelectrode recording.