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Quantification of striatal dopaminergic uptake in Parkinson's disease: a new multimodal method combining SPECT DaT and MPRAGE

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Thesis

QUANTIFICATION OF STRIATAL DOPAMINERGIC UPTAKE IN
PARKINSON’S DISEASE: A NEW MULTIMODAL METHOD COMBINING
SPECT DAT AND MPRAGE

by

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QUANTIFICATION OF STRIATAL DOPAMINERGIC UPTAKE IN PARKINSON’S DISEASE: A NEW MULTIMODAL METHOD COMBINING SPECT DAT AND MPRAGE

KARISHMA SMART

ABSTRACT

Parkinson’s disease (PD) is a neurodegenerative disease that causes degeneration of nigral dopaminergic terminals in the caudate and the putamen regions of the striatum in the basal ganglia. According to current practice, when an unequivocal clinical diagnosis of PD cannot be made, a single-photon emission computed tomography scan using the DaTscan radionuclide (SPECT DaT scan) is ordered. However, the assessment of SPECT DaT scans in the diagnosis of PD depends on the subjective judgment of a radiologist, which can pose problems for the accuracy of the diagnosis. Furthermore, as research studies generally do not quantify SPECT DaT scans when using them, their conclusions are not based on standardized data. The aim of this paper is to propose a method of quantification for SPECT DaT scans, to be employed in diagnostic and research environments. The methodology proposed in this thesis project will eventually be used for a much larger multimodal imaging project investigating the connectivity changes in the brain related to cognitive and affective symptoms in PD patients. Each of the 4 subjects in this project underwent a SPECT DaT scan and an MPRAGE scan (Magnetization Prepared
Rapid Gradient Echo), an anatomical MRI (magnetic resonance image). The SPECT DaT scans and the MPRAGEs were coregistered, and then a voxel-based quantification of the caudate and the putamen in the left and the right hemispheres was performed in every subject. First, the percentages of voxels with intensities exceeding various pericalcarine baselines were calculated. A pericalcarine baseline was used because the pericalcarine gyrus in the occipital lobe has been shown to have little to no dopaminergic activity, particularly on SPECT DaT scans. Next, asymmetry indices (AI) were calculated for two of the thresholds whereby the ratio of the percentage of voxels in the right to the left hemispheric region was taken. Wilcoxon Signed-Rank tests and bootstrapping analyses were performed on both the caudate and the putamen in all four subjects to determine the significance of any detected asymmetry. The quantification of the data and the AI values revealed asymmetries in the voxel intensities between the left and right hemispheres. This asymmetry was consistent with each subject’s side of physical symptom onset. According to the bootstrapping analyses, this asymmetry was significant in five of the eight comparisons. In summary, this methodology has potential to bring greater objectivity to the use of SPECT DaT scans in the diagnosis of PD and in research through its anatomically accurate, voxel-based quantification.
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<td>Parkinson’s Disease</td>
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<td>Progressive Supranuclear Palsy</td>
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INTRODUCTION

General Background of Parkinson’s Disease

Parkinson’s disease (PD) is a neurodegenerative disease in which dopaminergic neurons idiomatically start to dysfunction, leading to a wide variety of symptoms, ranging from loss of motor function to increased anxiety to dementia (1, 2). Currently, the majority of what is understood about PD deals with the motor aspect of the disease, and, until very recently, the diagnosis was entirely based on visible motor symptoms (2). Unfortunately, such a diagnostic method is less than ideal. It relies on the neurologist’s judgment, which often requires considerable experience, and the overlap of symptoms found in PD and other movement disorders, such as Essential Tremor (ET) and drug-induced Parkinsonism, lends itself to misdiagnosis and error (2, 3).

In 2011, the US Food and Drug Administration (FDA) approved the use of single-photon emission computed tomography (SPECT) scans to aid in distinguishing ET from PD (4). Since then, there has been increasing research into the efficacy of SPECT imaging in distinguishing PD from other Parkinsonian disorders, but the general method remains the same: a radiologist qualitatively determines the abnormality of the scan – based on asymmetrical dopaminergic uptake in the two striates – to confirm or deny a diagnosis of PD (5). Just as with the diagnostic method used by neurologists, the reading of SPECT scans is hardly an exact science and relies heavily on the radiologist’s subjective
judgment (2). Subtle asymmetry can easily be missed, which can prevent an accurate PD diagnosis from being made until later in the disease (2).

Although the current use of the SPECT scan to aid in the diagnosis of PD relies on motor symptoms already being present, a more accurate interpretation of these scans could help to identify PD in its early stages, when the motor symptoms are still too mild for a clear diagnosis. This could lead to starting treatment earlier in the disease, which has been shown to improve symptoms and slow the progression of PD. Quantifying the SPECT scans should allow for a less qualitative assessment of PD in a patient with a questionable diagnosis from a neurologist.

**History of Parkinson’s Disease**

Although Parkinson’s disease is by no means a recent disease – sources describe elements of the disease as early as the 12th century BC (6) – it was not until the early 19th century that the disease was first reported in a scientific capacity. At that time, there were a number of published accounts of PD-like symptoms, which inspired Dr. James Parkinson in 1817 to write about six cases in his own experience that involved symptoms consistent with PD (1). He titled this seminal paper “An Essay on the Shaking Palsy”, and it is widely considered the first comprehensive description of the disease. Indeed, for such a small sample size, only two subjects of which actually entered Dr. Parkinson’s practice, the essay is impressively accurate (1). The disease was known as *paralysis*
agitans (shaking palsy) until the late 19\textsuperscript{th} century when Dr. Jean-Martin Charcot, considered the “father of modern neurology” (1), moved to change the name to Parkinson’s disease.

\textbf{Medical/Biological Background of Parkinson’s Disease}

\textit{Neurological Basis of PD}

Classically, PD is understood as being caused by dysfunction and degeneration of dopamine (DA) neurons in the substantia nigra of the basal ganglia, which in turn affects their connections to various other parts of the brain, causing the wide range of symptoms seen in PD (1, 2). The degenerating neurons accumulate Lewy bodies (LBs); the presence of these LBs in the basal ganglia upon autopsy is the only current method of unequivocal diagnosis (1, 2). LBs are pathological cytoplasmic aggregates of alpha-synuclein that disrupt cell function surrounded by a coating of ubiquitin protein, which tags proteins for destruction (7).

The basal ganglia, of which there is one in each hemisphere of the brain, is a collection of subcortical nuclei and comprises of five main structures: the globus pallidus, the subthalamic nucleus, the nucleus accumbens, the substantia nigra, and the striatum (which is further subdivided into the caudate and the putamen) (1, 7). All the structures are involved in some way in the motor system, but they are also associated with a number of non-motor functions that can be affected in PD patients (7). For example, the nucleus accumbens is influential in
reward and motivation, particularly in regards to the motor system, and serves to increase the likelihood of a particular behavior through the expectation of reward (7). Parkinsonian dysfunction in the nucleus accumbens and the striatum is believed to be related to the increased impulsivity and onset of impulse control disorders seen in PD patients (7, 8). The globus pallidus and the substantia nigra help to modulate movement control by inhibiting unwanted movements, particularly in the face, through their tonic inhibition of the thalamus (8). The subthalamic nucleus also helps to suppress movement sequences through its excitatory inputs to the globus pallidus and the substantia nigra (7). Not surprisingly, therefore, the two prime targets for deep brain stimulation implant surgery (DBS) are the subthalamic nucleus and the globus pallidus (3). In DBS surgery, a remote-controlled stimulation device is implanted in one of these two areas and helps to artificially modulate unwanted movements, often greatly improving PD symptoms (3).

The striatum, the last of the aforementioned basal ganglia structures, receives cortical input and relays it to other areas of the basal ganglia (7). In the case of movement, the current understanding is that input from the motor cortex initiating a movement is relayed to striatal subregions, which then activate the particular striatopallidal pathway necessary for the desired motion; the pallidal inhibition of thalamic neurons is thus released (7). The rest of the tonically inhibitory pallidothalamic pathways are unaffected by the striatal output, and, in fact, the subthalamic nucleus often simultaneously activates its excitatory
pathways to the globus pallidus and substantia nigra to ensure suppression of all other pathways save for the one striatally activated (7). It is believed that non-motor pathways that involve the basal ganglia follow a similar model in that they receive cortical inputs and relay them back to relevant cortical targets (7). For example, the caudate is considered to be a part of the oculomotor, dorsolateral, and ventral/orbital cortico-striatal loops, whereas the putamen is involved in the sensorimotor circuit (9).

The basal ganglia contain both excitatory and inhibitory circuits (1, 7). One of the primary ways in which the nigral dopamine neurons dysfunction in PD is a decline in DA production and release, which upsets the careful balance of excitation and inhibition, leading to the variety of symptoms seen in PD patients (1). Over time, the dysfunction of existing nigral neurons turns into degeneration of those neurons (1). Thus, although medications have been shown to improve symptoms and, in some cases, maybe slow the progression of the disease, they eventually lose significant efficacy as PD progresses (1). This neuronal degeneration in the basal ganglia is not unnatural – around 5% of neurons die every decade after the age of 20. Thus, although some neuronal loss is expected with aging (7, 8), the advanced rate at which it occurs in patients with PD is pathological (1, 7). In fact, patients generally do not start to show Parkinsonian symptoms until 50% of the substantia nigra’s dopaminergic neurons have degenerated (7), corresponding to about an 80% loss of striatal dopamine (8).
Motor Symptoms

Today, the four cardinal symptoms associated with PD are bradykinesia, rigidity, resting tremor, and postural instability (2, 3). Often, a PD diagnosis is made if at least two of the first three symptoms (bradykinesia, rigidity, and resting tremor) are present (3), though the most common diagnostic criteria for PD, the UK Brain Bank criteria, requires that one of these two symptoms be bradykinesia. As postural instability is commonly seen in the elderly population and often does not appear until the later stages of PD, many clinicians do not consider postural instability when assessing a PD diagnosis (3). Another common feature of the disease is its asymmetric presentation of tremor, rigidity, and bradykinesia. Other common motor symptoms include gait disturbances, hypophonia (decreased vocal volume), hypomimia (diminished facial expression), and micrographia (small, cramped handwriting) (3).

Bradykinesia technically refers to the slowing of movement seen in PD patients, but the term can also be used to describe more generally bradykinesia, hypokinesia (smaller movements), or akinesia (lack of movement) (3). It has been shown to be closely correlated with loss of dopamine in the putamen and shows the greatest improvement with dopaminergic therapy. Interestingly, patients often also exhibit *kinesia paradoxical* reactions, where the typical bradykinesia temporarily dissipates in response to stress, such as being able to run out of a burning building (3). This supports the theory that PD motor
symptoms are due to compromised access to regulatory motor programs rather than deficits in the motor cortex or its connections to peripheral motor circuits (3).

Rigidity is increased muscle stiffness and is due to increased muscle tone unrelated to movement. For example, when a patient relaxes a joint, which would normally allow for easy passive movement by the examiner, the joint remains stiff and difficult to rotate. Muscle rigidity also commonly exhibits a positive response to dopaminergic therapy (3).

The most well known feature of PD is resting tremor. Unlike forms of tremor seen in other conditions, resting tremor, as the name implies, is observed when the affected part of the body is at rest and disappears when the body part is being used (3). This is contrary, for example, to Essential Tremor, a benign movement disorder, where the tremor only appears when the body part is active. Commonly, in PD, the tremor starts in an upper extremity, and the classic example is a “pill rolling” tremor in the hand, where the thumb and index fingers rolls back and forth against each other as if rolling a pill between them (3). Tremor is less closely correlated with striatal dopamine loss, so it is less responsive to dopaminergic therapy than either bradykinesia or rigidity (3).

Although postural instability is not used to diagnose PD, it is a common late-stage symptom that contributes to patients’ risk of dangerous falls (3). To test for this symptom, the examiner stands behind the patients and tugs them backward by the shoulders to pull them off balance; a healthy person can regain balance in one backward step, whereas PD patients require multiple steps to
catch themselves (3). The Unified Parkinson’s Disease Rating Scale (UPDRS), for example, defines three or more backwards steps as pathological (10). In severe cases, the patient may not be able to move their feet back at all to catch themselves (3).

There are other symptoms that, although not evidence of PD in themselves, the presence of which can corroborate a PD diagnosis. One type of symptom that can support a diagnosis of PD is gait disturbance, which can manifest in a number of ways (3). Many patients begin to shuffle, often dragging the leg on the affected side of the body, and maintain a flexed posture, which is seen as a perpetual bending over forward. Others can exhibit difficulties initiating gait movements or changing gait directions (3). A poorly understood feature is gait freezing, where the patient inexplicably freezes in the middle of walking, often when changing direction or crossing a threshold, such as passing through a doorway. A second type of corroborating symptom is the decreased amplitude of certain aspects of motor function (3). One example is the hypomimia that some patients experience, where their emotional range is intact, but their ability to express their emotions facially (due to hypokinesia of facial musculature) has been compromised (3, 11). Other examples include the micrographia not uncommonly exhibited by patients in late-stage PD (3) and severe cases of hypophonia, in which patients may be unable to speak louder than a whisper (3).
Non-Motor Symptoms: Psychiatric and Autonomic

Aside from the characteristic motor symptoms, patients with PD can experience a number of non-motor symptoms as well. These can be of a neuropsychiatric or autonomic nature (3). Often, these symptoms are more disabling for patients than their motor ones; unfortunately, most of these symptoms can be attributed to other diseases, and so their association with PD is still gaining recognition (3).

The neuropsychiatric symptoms can include depression, anxiety, apathy, dementia, and impulsivity. Depression is the most common psychiatric symptom and often precedes the onset of the PD diagnosis. The profile of depression in PD patients is also different than in patients suffering from primary depression, such as fewer feelings of guilt and self-reproach (3, 12). Apathy, as well as social withdrawal and anhedonia (loss of interest in previously enjoyed activities), are also more common in patients with PD-related depression (3, 13). Anxiety, which is nearly as common as depression and is distinct from the stress derived from being diagnosed with PD, can present in up to 40% of patients (3, 14). Dementia is quickly becoming recognized as a part of PD, though it tends to present much later in the disease’s progression (15). Interestingly, Dr. James Parkinson confidently described PD with the “intellects being uninjured” (1); however, it is now known that patients with PD are six times more likely to develop dementia than their age-matched peers (3). Finally, impulsivity has been documented as more common in patients with PD than in controls. This
lack of impulse control can appear as problems with gambling, shopping, eating, and changes in sexual behavior (3). Impulse control disorders, when they are a symptom of PD, are believed to be a result of mesolimbic dopaminergic system dysfunction arising from the dopaminergic deficiency caused by the disease (3); however, paradoxically, they can also be caused by dopaminergic therapy for the pharmacological treatment for PD (3). (Please refer to the sections Medication Therapy and Side Effects of Medication Therapy for further explanation.)

Patients with PD can also experience sleep disturbances due to their disease. The most common sleep disorder associated with PD is REM behavior disorder, in which patients act out their dreams, often violently, which can disturb both their own and their bed partner’s sleep (3). Although REM behavior disorder is not a symptom of PD, it precedes PD in nearly 40% of cases (3).

The autonomic symptoms can include pain, dysphagia (difficulty swallowing), and urinary and olfactory dysfunction (3). A meta-analysis suggested that dysphagia may be present in up to one third of PD patients. In addition to impeding the swallowing of medication tablets, dysphagia has been linked to an increase in risk of pneumonia, which is a main cause of death in PD patients (16). In PD, urinary dysfunction usually presents as incontinence. This is believed to be due to the loss of striatal dopamine, particularly in the putamen, in basal ganglia circuits that normally inhibit micturition (emptying the bladder) (7). Finally, pre-motor olfactory dysfunction (i.e. presenting before the onset of motor symptoms) most often presents as hypoosmia – loss of the sense of smell
– presumably due to the degrading connections between the olfactory bulb and the substantia nigra (3).

**Medication Therapy**

Currently, the most common and the oldest pharmacological treatment of PD is levodopa, or L-dopa. Approved by the FDA in 1967, its continued efficacy affirms its wide consideration as a “miracle drug” (1). As PD is primarily a disease of dopaminergic degeneration, L-dopa was developed quite logically being that dopa is the natural precursor to dopamine in the body. With the idea that exogenous dopamine could mitigate the effects of PD dopamine loss, L-dopa was developed as dopa can cross the blood-brain barrier while dopamine cannot (1). L-dopa is usually given as a levodopa-carbidopa combination (called Sinemet) in which the carbidopa acts to prevent peripheral transformation of dopa into dopamine. The addition of carbidopa maximizes the amount of dopa that actually crosses the blood-brain barrier as well as prevents side effects of increased peripheral dopamine, such as nausea, vomiting, and hypotension (1). Once in the brain, L-dopa is taken up pre-synaptically by DA neurons where intracellular enzymes convert it into DA to be released (1). However, L-dopa does not cure PD, and eventually the diseases progresses, albeit more slowly, to a point where there are not enough functioning neurons in which L-dopa can be stored and converted to dopamine (1).
In an effort to minimize L-dopa dosage to extend its efficacy, Sinemet is often given in combination with a DA agonist. DA agonists bind predominantly to D2 receptors in the basal ganglia to stimulate the post-synaptic receptors in the absence of naturally produced DA (1). Despite being generally less well-tolerated, DA agonists do have certain advantages over L-dopa: as DA agonists act post-synaptically, they continue to be a viable pharmacological therapy after L-dopa’s efficacy diminishes due to the paucity of DA neurons (1).

_Side Effects of Medication Therapy_

Another complication of PD is that the medication therapy can cause its own side effects that mirror PD symptoms. A common example is that impulse control disorders can be due to PD or dopaminergic therapy (3). In a healthy individual, endogenous DA binds equally to D1 and D2 receptors; however, the DA agonists currently available have a much greater affinity for D2 receptors. Additionally, the disproportionately greater stimulation of D2 receptors occurs phasically, peaking each time the medication is taken (1). This effectively mimics the phasic DA release associated with the expectation of reward without the subsequent signal suppression that normally engages when the anticipated reward is not received. This dysregulation of the reward system by DA agonists is responsible for the impulse control disorders caused by dopaminergic therapy (1).
Dopaminergic therapy can also produce side effects that would not have otherwise been seen in the natural progression of PD. A common side effect that patients often have to contend with is the dyskinesia that L-dopa can introduce (1). Dyskinesia is involuntary, abnormal movements, often seen as writhing or wiggling movements in the limbs and face (3). It is believed that this side effect is due to hypersensitivity of dopamine receptors arising from dopamine levels that are disproportionately high compared to the dwindling numbers of basal ganglia neurons (1).

**Background of Diagnostic Methods**

*UK Brain Bank Criteria*

Currently, PD must be clinically diagnosed, most commonly by the UK Brain Bank criteria, which were delineated by the UK Parkinson's Disease Society (3). According to these criteria, a PD diagnosis requires the presence of bradykinesia in concurrence with rigidity, rest tremor, postural instability, or any combination thereof as well as overall disease progression (though the rate can vary widely) (3). In addition, there is very often unilateral onset and subsequent persistent asymmetry of symptoms. Patients with PD also usually show a 70-100% positive response to L-dopa treatment at first, and, in fact, a lack of response to L-dopa can be considered suggestive of a non-PD Parkinsonism (3). This is because a lack of response to L-dopa probably indicates that basal
ganglia dopaminergic neuron dysfunction is not involved in the etiology of the patient’s symptoms.

*Issues with Clinical Diagnosis Methods*

Although a clinical diagnosis by a movement disorder specialist is considered the “gold standard” (1), one factor that can compromise its efficacy is that the diagnostic process is fairly qualitative and therefore subject to human error. Another undermining factor is that, in practice, many diagnoses of PD are actually made by general neurologists who do not have the same specific expertise as movement disorder specialists. These two factors contribute to the 25% misdiagnosis rate of PD (3, 5). Misdiagnoses in PD come in two flavors: patients with another Parkinsonian disorder incorrectly diagnosed as PD and patients with PD incorrectly diagnosed with another Parkinsonian disorder.

Parkinsonian disorders are often grouped into two categories based on whether the symptoms are due to an extrapyramidal neuron dysfunction in the basal ganglia (3). Basal ganglia function is preserved in the non-neurodegenerative Parkinsonian disorders, which include Essential Tremor (ET), drug-induced Parkinsonism, and vascular Parkinsonism (3). ET is a benign movement disorder involving a tremor that only appears during motion. Drug-induced Parkinsonism is usually caused by prolonged exposure to antidopaminergic drugs. Vascular Parkinsonism presents with PD-like symptoms that are due to lesions or ischemic damage localized in the basal ganglia (3).
Atypical Parkinsonism, which includes multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD), is also due to basal ganglia dysfunction (3). There are certain symptoms that if present may suggest an alternate diagnosis. For example, postural instability and falls tend to appear late in PD but present early in PSP (3). Another example is persistent asymmetry. PD’s initially unilateral presentation ultimately becomes bilateral, whereas CBD's marked asymmetry lasts much longer (3). Although these kinds of symptoms may theoretically facilitate the differential diagnosis of Parkinsonian syndromes, they can take time to present. This often delays both a correct diagnosis and the initiation of appropriate treatment (3). Additionally, presentations of each disease vary widely. To further complicate the matter, Parkinsonian symptoms can deviate from those expected of their syndromes and can sometimes present similarly to other Parkinsonian diseases (3, 5). Indeed, it might be that only by observing the intrinsic underlying brain changes can an unequivocal diagnosis be determined.

**Unified Parkinson’s Disease Rating Scale**

The UPDRS is used as a clinical rating scale to assess the severity of a patient’s PD. The most recent version has six parts that address the various domains of Parkinsonian symptoms (3). The original UPDRS included only Parts I-IV: Parts I, II, and III deal with, respectively, neuropsychological symptoms, symptoms affecting daily life such as excessive salivation and micrographia, and
motor symptoms, and Part IV addresses complications of pharmacologic therapy (3). Part V is a modified Hoehn and Yahr staging scale, which identifies the patient’s stage of PD, and Part VI is the Schwab and England Activities of Daily Living Scale, which estimates the patient’s percentage of independence in daily living activities. Both scales are concerned principally with motor function (3).

**MRI Scans**

*General Information*

Magnetic resonance imaging (MRI) is a form of imaging that uses strong magnetic fields, rather than the nuclear radiation of single-photon emission computed tomography (SPECT) and positron emission tomography (PET) scans, to produce 3D representations of the brain (18). The patient is placed inside a large magnetic coil that forms a large uniform magnetic field. This magnetic field is oscillated, applying the resultant energy to the area of interest, which is absorbed by the hydrogen atoms in water molecules (18). These excited protons emit radio waves that are detected by the scanner and used to determine the proton’s location (18). The excited protons decay back to their equilibrium state – their energy level prior to the magnetic field induction – at different rates according to characteristics of the particular tissue they comprise. These variations produce a contrast between areas in the image that allows for differentiation of distinct tissues (18).
An important feature of neuroimaging, particularly in MRIs, is the segmentation of the image into voxels. Just as pixels are uniform 2D areas that make up a larger 2D image, such are voxels for a 3D space. The use of voxels in neuroanalysis allows for more standardization in image interpretation.

**MPRAGE**

Magnetization Prepared Rapid Gradient Echo (MPRAGE) is an MRI technique that rapidly acquires T1-weighted 3D images of the brain using magnetization pulse sequences (19). These inversion pulses increase the signal intensity, producing an image with high resolution and clear contrast. Thus, MPRAGE is well-suited to image neuroanatomy unlike functional scans such as SPECT (19).

**Limitations to Use of MPRAGE in PD Imaging**

Despite the widespread applicability that MPRAGE has enjoyed, its suitability in imaging PD has not been convincingly demonstrated (1, 5). Generally, MPRAGE has not shown any structural changes in PD, and any visible atrophy has not been specific enough to identify PD (1). However, other neurodegenerative diseases have a characteristic pattern of structural atrophy, and so MPRAGE has been employed to differentiate other Parkinsonian disorders from PD (1).
SPECT Scans

General Information

SPECT scanning is a form of nuclear medicine tomographic imaging used to produce 3D functional representations of the brain. A radionuclide is injected into the patient, which is then used to produce the image (3). Radionuclides are gamma-emitting radioisotopes, usually with a specific ligand attached, which release a single gamma photon that is detected by the scanner (5). The scanner acquires multiple 2D images across the entire brain from different angles, and then a computer applies a tomographic reconstruction algorithm to the 2D images to produce a 3D representation of the brain (5).

SPECT DaT scans have been approved by the FDA for the differentiation of PD and ET, and they are used widely in PD research (4). The DaT refers to the DAT protein that the scan’s radionuclide targets. DAT is a pre-synaptic transporter on dopaminergic neurons in the caudate and putamen that re-uptakes DA from the synapse to be recycled (5, 7). The radionuclide used diagnostically is $^{123}$I-Ioflupane, marketed as DaTSCAN. DaTSCAN contains a radioligand that binds to the dopamine transporter (DAT) (5). When the DaTSCAN radioligand binds to DAT, it releases a gamma photon. As PD progresses, there are fewer dopaminergic neurons with DAT, and so the scan detects fewer gamma photons (1). Therefore, indirectly the scan detects the presence of DAT and by association the integrity of nigrostriatal dopamine pathways (1). There are radionuclides that bind to the post-synaptic D2 receptor
and measure function based on the number of receptors, which is up- or down-regulated according to dopaminergic activity. However, the number of pre-synaptic transporters provides a more accurate estimation of relevant dopaminergic function, as it is the pre-synaptic neurons’ diminishing dopamine production that forms the etiological basis of PD (1).

Advantages

The inclusion of SPECT DaT scanning in the study and diagnosis of PD brings a number of advantages. Many studies have shown the high sensitivity and specificity of DaTSCAN in the differentiation of PD from other Parkinsonian disorders, particularly those without extrapyramidal degeneration (2, 3). There is strong evidence that the availability of DAT as measured with DaTSCAN reflects striatal dopamine levels (5, 20). Furthermore, these DaTSCAN measures also correlate with disease severity (3). At the moment, PD can only definitively be diagnosed upon autopsy (though, in practice, a “gold standard” diagnosis is treated as close to definitive) because the accumulation of LBs responsible for PD is not visible without brain dissection (1). Although SPECT DaT cannot detect LBs, it allows for disease assessment on a much deeper level etiologically than a purely clinical exam through the visualization of underlying dopaminergic brain changes (1).

As there is often ambiguity from which disease a patient’s Parkinsonian symptoms stem, a SPECT DaT scan can reveal the extent of striatal dopamine
loss, providing valuable information for a diagnosis (2, 5). Studies looking at the
SPECT DaT scans of patients with PD and age-matched healthy controls have
found clear differences in striatal dopamine loss, as have studies comparing PD
patients with those suffering from non-extrapyramidal Parkinsonian disorders (2,
5). In fact, SPECT DaT has been shown to have 95% specificity and sensitivity
in differentiating PD from ET (2, 3). Limited research into SPECT DaT imaging of
atypical Parkinsonian disorders has suggested different patterns of striatal
dopamine loss in MSA, PSP, and PD. Further investigation could reveal
identifying patterns of degeneration to better differentiate within this group of
idiopathic Parkinsonian diseases (1, 2).

Limitations

Despite the potential SPECT DaT has for improving diagnostic ability and
expanding our understanding of PD, it has two important limitations that must be
considered when addressing its use: poor resolution and subjective assessment.
The first limitation is the SPECT scan's poor resolution. Unlike PET scans,
whose radionuclides release two gamma photons for superior localization, the
single gamma photon of SPECT scans severely reduces the resolution (1, 2).
Outside of the vaguely defined striatal areas illuminated in the scan, SPECT DaT
provides negligible anatomical detail, which poses a serious problem for their
interpretation (5).
Some studies have attempted to mitigate the problem of SPECT DaT’s poor resolution by coregistering the image to a scan with much greater resolution. Seibyl et al. developed a methodology whereby the SPECT DaT scan of each PD patient was coregistered to a composite MPRAGE image made up of the anatomical scans from four healthy controls in a previous study (21). A number of studies have since employed this methodology in their work (20), but it is seriously flawed in that the composite MPRAGE and the SPECT DaT came from different people. Thus, any differences in anatomy between the two scans were ignored. Importantly, if the anatomical location of the composite image’s striatum is not exactly the same as in the patient, then any results regarding striatal activation in the SPECT DaT scan are compromised.

The second limitation in the current diagnostic use of SPECT DaT scans is the general lack of quantification (2). When a SPECT DaT scan is read clinically, the radiologist qualitatively judges the asymmetry of activation in the striatal areas, usually without any anatomical localization or a healthy scan for comparison (3). Indeed, there is very little consensus of what constitutes “abnormal” enough to merit a PD diagnosis. Just as the manifestation of PD symptoms varies widely across the patient population, so too does, in all probability, the extent of degeneration that elicits the onset of PD (1). In some clinical settings, the signal detected in highly activated striatal areas, less activated striatal areas, and extra-striatal areas are compared to determine the
extent of dopamine loss. However, this methodology is not standardized, and
the comparison remains fairly qualitative (5).

Some studies have begun to incorporate basic quantification into their
methodology but so far only for research purposes. Marek et al. simply used the
average striatal uptake of dopamine (based on the binding of the radionuclide) as
a quantitative measure (20). Seibyl et al. calculated a striatal binding ratio
(SBR), the ratio of the average striatal uptake to the average uptake in the
occipital lobe, an extra-striatal region with typically very little to no DAT activity
(21). Ravina et al. built on the Seibyl et al. method and reported the percent
change in SBR between different time period and between PD patients and
controls (22). However, all three of these methods average dopaminergic uptake
over large areas, which masks any subregional differences.

Proposed Improvements

Though the lack of quantification and the poor resolution are currently
important concerns regarding the use of SPECT DaT, they are certainly issues
that can be improved upon. A higher resolution image would allow for more
precise localization of activated areas, and a more quantitative assessment of
the images would increase the accuracy and relevance of the scan’s
dopaminergic measures. Once these concerns are addressed and their effects
mitigated, SPECT DaT scans have a very promising future in deepening our
understanding of PD and improving diagnostic accuracy. This project aims to
address just these two issues. The SPECT DaT scans were coregistered to the subject's own MPRAGE to improve the scan's resolution while increasing confidence in the accuracy of anatomical location. The quantification is voxel-based and does not average across the entire region. Thus, it should reveal any significant subregional variation that may be lost with such averaging.
The Current Project

The context for this thesis is a larger project (Development of a New Multimodal Imaging Technique Involving Striato-Frontal Structural, Functional, and Dopaminergic Connectivity to Study the Progression of Cognitive Decline in Parkinson’s Disease), which aims to use the quantification method developed in this thesis in conjunction with diffusion tensor imaging (DTI) white matter tractography, functional connectivity MRI (fcMRI) seed-based analysis, and neuropsychological testing to study the brain changes in structural, functional, and dopaminergic connectivity between the striatum and cortical areas that relate to cognitive and affective symptoms in patients with PD. DTI white matter tractography uses directional information about water diffusion in brain tissue to delineate the structural white matter tracts in the brain. The fcMRI seed-based analysis identifies brain areas that are functionally connected to a pre-specified seed region based on the concurrence of similar blood-oxygen level changes.

The goals of this larger project are twofold. The first is to develop a new methodology combining multimodal imaging and neuropsychological assessments with which to improve future study of cognition and mood in patients with Parkinson’s disease. The second is to build upon the current understanding of the etiology of cognitive and affective changes seen in PD with more comprehensively integrated neurological and behavioral investigation. The hope is that, eventually, this method and the results therein can be used towards development of predictive abilities for various cognitive and affective symptoms,
such as dementia and depression, in PD patients. As a result, patients
diagnosed with PD may benefit from earlier interventions and possibly even
preventative measures for their predicted symptoms.

This thesis project, however, is more clinically directed. The aim of this
thesis project is to develop a method with which to quantify diagnostic SPECT
DaT scans. The hope is that this method can be used to increase the objectivity
of PD diagnosis through neuroimaging.
METHODS

Overview

The four participants whose data contributed to this thesis were patients suffering from Parkinson’s disease. The overall purpose of this study was to develop and present a method for the quantification of SPECT scans, which are used in the differential diagnosis of Parkinson’s disease. For each subject, an MPRAGE MRI scan and a SPECT DaT scan were obtained. Using the FreeSurfer image processing and analysis software (publicly available at http://surfer.nmr.mgh.harvard.edu), the MPRAGE and the SPECT scans were coregistered. Labels were drawn delineating the caudate and the putamen within the striatum of each hemisphere. The total number of voxels and the number of voxels exceeding particular intensity thresholds in each label were quantified using various statistical analyses. Please refer to the Quantification section of the Methods.

Patient Recruitment

All four subjects were patients of Dr. Raymon Durso at the Boston VA Movement Disorders Clinic. He briefly discussed the project with his Parkinson’s disease patients who had undergone a SPECT scan, and, if they indicated interest, he forwarded their contact information to the lab. The participant was then recruited over the phone for the Cognition and Parkinson’s Project, but their
data was also used for this thesis project. When each patient arrived for his first scheduled visit of the project, formal consent for his participation was acquired, in which the details of the study and his involvement were explained to him. The Institutional Review Board of the VA Boston Healthcare System in Jamaica Plain approved access to medical records of patients who provided consent; this included medical histories and brain scans acquired for clinical purposes. Although the patients returned for other visits, only the visits involving the consenting process and the MRI scan were relevant to this thesis. The MRI scan was part of the same visit as that of the consent acquisition – the first visit – for all but one of the four subjects. The SPECT DaT scan, on the other hand, had been ordered previously by Dr. Durso for clinical purposes. In conjunction with the acquisition of formal consent, an MRI safety screening was also done to ensure the patients were eligible to undergo an MRI scan. For one patient, an X-ray was required for MRI clearance, which was also ordered by Dr. Durso.

**Patient Information**

The four patients were primarily recruited for the Cognition and Parkinson’s Project, which is looking at religiosity and cognition in patients with PD. Every patient had a subject number for the Cognition and Parkinson’s Project; however, in the interest of increased protection of patient confidentiality, they have each been given a number from 1 through 4 for this thesis project.
In addition to the MPRAGE and the SPECT DaT scan acquired for each subject, three pieces of information regarding the subject’s disease were collected. The Hoehn & Yahr stage of each subject was collected from Dr. Durso, the neurologist and PD expert associated with the lab. The patient was asked for the number of years since his PD diagnosis and the side of onset on which his disease initially presented. Each patient’s Hoehn & Yahr stage and the number of years since initial diagnosis of PD were used to assess disease severity.

**SPECT Imaging**

SPECT DaT scans were used to determine the extent of dopaminergic loss in the striatal subregions of interest. Each patient had undergone a SPECT scan out of clinical necessity to confirm a Parkinson’s disease diagnosis. Dr. Durso referred all four patients after obtaining their SPECT scan and confirming their diagnosis. Part of the consent the patients provided was to allow access to their SPECT scans.

SPECT scan images were acquired using a Philips Forte gamma camera at the Boston VA Medical Center in West Roxbury, MA. Head motion was restricted with a head strap for minimal blurring due to motion. The SPECT scan used the DaTscan radionuclide (I-123 Iofluopane), which binds to presynaptic dopamine transporters, primarily in the striatum. The patient was injected with a thyroid blocker approximately 5 hours prior and with the radionuclide
approximately 4 hours prior to the scanning session. As DaTscan contains iodine, the thyroid blocker is required to protect hyperactivation of the thyroid from the radionuclide. Each image was 128x128 pixels and took about 30 seconds; the scanner takes 128 images total over the course of an hour.

**MRI**

All MR images were acquired using a Siemens MAGNETOM Trio 3-Tesla system with a 12-channel phased-array and body-coil transmission at the VA Medical Center in Jamaica Plain, Boston, MA. Head motion, which can blur the images, was restricted using a thick foam pillow and adjustable padded clamps to restrain the head. Earplugs were utilized to attenuate scanner noise and a mirror was attached to the headcoil to allow the subject to see outside the bore of the scanner. Participant pulse and heart rate were monitored during imaging with a pulse oximeter.

**MPRAGE**

MPRAGE was one of the scans obtained during MRI scanning sessions. Two 3D anatomical images were acquired for each patient with resolution of 256x256 mm and field-of-view of 256x256 mm. The scans had a repetition time (TR) of 2530 ms, an inversion time (TI) of 1100 ms, and an echo time (TE) of 3.32 ms. Each scan was comprised of 176 1-mm slices with 0.5 mm gaps and a flip angle of 7 degrees.
**Image Pre-Processing**

MPRAGE was used to accurately visualize and identify the regions being explored. The pre-processing of the MPRAGE scans, known as cortical reconstruction or recon-ing, was done with the FreeSurfer software.

The entirely automated FreeSurfer recon process involves tessellation of the grey-white matter boundary (23) and surface deformation following intensity gradients to optimally place the grey/white and grey/cerebrospinal fluid (CSF) borders at the location where the greatest shift in intensity defines the transition to the other tissue class (24). The non-brain tissue is removed (25), and magnetic field gradient non-linearity (25) and topology (23) are corrected for. A white matter segmentation and a cortical parcellation are produced based on probabilistic models of tissue MR parameters and of known anatomical locations (26; 27).

The white matter segmentation was used to help identify the location of the striatal activation, or lack thereof, in the SPECT scans. A radiologist at the Boston VA Jamaica Plain campus was consulted about the automatic segmentation. He corroborated the accuracy of the automated procedure in one subject and provided direction on how to verify the accuracy of the other three scans so that the correct areas in each subject were investigated.
Coregistration

Although coregistration of MRI and SPECT has been done previously (28), the novel method proposed in this project involves the coregistration of a SPECT scan to the subject’s own MPRAGE. The coregistration of images was done using BBRegister, a tool within FreeSurfer that uses a boundary-based cost function to align two sufficiently different images from the same subject (29). BBRegister automatically creates a registration matrix that essentially warps one image into the space of another so that they can be analyzed together (29). Coregistering the SPECT DaT to the subject’s own MPRAGE permitted us to accurately locate anatomy as well as create a common space for these otherwise very different scans. After each automated registration with BBRegister, the results were checked with FreeView, FreeSurfer’s viewing tool.

Neuroanatomical Labeling

The labels used in the quantification were drawn in the FreeSurfer tool Freeview, using its drawing tool. The automatic segmentation (aseg) volume created in the recon-all process served as a guide by identifying the areas of interest based on the MPRAGE. The labels were drawn on the SPECT DaT volume by using the drawing tool to highlight relevant voxels. The label files contained the 3D (x,y,z) coordinates and intensity values of each voxel. In each subject, six labels (three in each hemisphere) were drawn: the caudate, the putamen, and the pericalcarine gyrus. The striatum in each hemisphere was
represented in the SPECT DaT image as red or yellow to indicate the high intensity of these highly innervated dopaminergic areas. However, the aseg volume offered much greater accuracy in anatomical localization. This increased the confidence that the intensity values being studied were indeed striatal. The pericalcarine gyrus, which is a cortical area in the occipital lobe (22), served as a dopaminergic baseline in the quantification; as there is little to no dopaminergic activity in the pericalcarine gyrus (22), the intensities of its voxels were low due to the lack of bound radionuclide.

**Quantification**

The voxel-based quantification of the SPECT scans was done using the intensity information contained as output in the label files. First, general information was calculated for each region. The total number of voxels in each region was reported. The range and the range size were calculated for the striatal intensities in each region.

Next, the intensities of the voxels in each of the pericalcarine gyri were averaged to produce a mean value for the baseline. The number of voxels and the percentage of voxels in each of the caudate and the putamen that exceeded various thresholds were calculated. These thresholds were the pericalcarine baseline and twice, triple, quadruple, five times, six times, and seven times that baseline. This gave a more detailed idea of the extent to which each region
exceeds the pericalcarine baseline. Using the total number of voxels in each region, the proportion of voxels that exceeded each level was reported.

Finally, an asymmetry index was calculated for the two striatal regions whereby the ratio was taken of the proportion of voxels in the left hemisphere to right hemisphere that exceeded three and four times the pericalcarine baseline. These two thresholds were chosen because they are the highest levels above the baseline for which there were asymmetry index values (the ratio of the right hemisphere region intensity values to the left), respectively, for both regions in every subject and for both regions in at least 2 subjects. These ratios were used for three purposes. The first was to show that the asymmetry in the regions in each subject corresponded to the side of onset on which the patient’s disease initially presented. The second was to show that the patients with asymmetry indices much greater than or less than 1 (i.e. a greater difference in intensity between the two hemispheres) had SPECT scans that appeared more asymmetrical in activation. The third was to show that the region – caudate or putamen – with the smaller asymmetry index, indicating a greater unilateral deactivation, corresponded to greater degeneration of dopaminergic terminals from entering nigral DA neurons in that region as seen on the patient’s SPECT scan. Here, the caudate and the putamen in each hemisphere were marked according to the MPRAGE by arrows in order to indicate the hemisphere with the greatest dopaminergic loss.
Statistics

The significance of the asymmetry revealed in the quantification was determined by Wilcoxon signed-rank tests, in which the region in the right hemisphere was compared to the same region in the left hemisphere. The Wilcoxon signed-rank test is a non-parametric hypothesis test used to test the significance of the difference between two related samples based on a comparison of their population mean ranks. In this case, the pairwise comparison involved subtracting the first voxel’s intensity in the right hemisphere label file from the first voxel’s intensity in the left hemisphere label file and then repeating this procedure for all the rest of the data pairs. In other words, the absolute value difference in intensity for each voxel pair was calculated. These differences were ranked and then signed; a negative sign indicated that the left hemispheric voxel had a greater intensity, and a positive sign indicated that the right hemispheric voxel had a greater intensity. The mean rank was calculated separately for positively signed and negatively signed ranks. Therefore, for all eight tests, a larger mean positive rank indicated greater voxel intensities in the right hemispheric region, and a larger mean negative rank indicated greater voxel intensities in the left hemispheric region. A paired difference test was deemed appropriate because the two samples in each test were not independent: the two regions came from the same brain at the same time using the same measure. A non-parametric test was required because the distribution of voxel intensities in each region was not assumed to be normal. In fact, the more-affected
hemisphere’s regions were hypothesized to have voxel intensity distributions skewed towards lower values. The Wilcoxon signed-rank tests were performed in SPSS [Version 22.0 (Released 2013); IBM Corp., Armonk, NY, USA] (30). For each of the eight tests performed, the mean rank of each region, the critical value, and the p-value were reported.

As Wilcoxon signed-rank tests are paired difference hypothesis tests, they can only be run on complete pairs. SPSS automatically truncated all the data that exceeds the lowest common number of complete pairs between samples. Thus, all incomplete pairs in the larger of the two regions were excluded from the analysis. This exclusion posed a potential problem, as the Wilcoxon tests were not run on the complete data set. The possibility exists that these excluded voxels, had they been included, would have changed the outcome of the hypothesis test. To account for this issue, a bootstrapping analysis was performed on all eight contrast pairs within the four subjects. The bootstrapping analysis was performed in MATLAB [Version 7.11.0.548 (R2010b) 64-bit (win64); The MathWorks, Inc., Natick, MA, USA] (31), in which a difference median and the associated confidence intervals around this median were calculated based on 300,000 iterations of randomly selected samples, which matched the sample size used in the Wilcoxon tests. If the random difference median of zero, which indicates the null hypothesis, fell inside the confidence interval around the difference median, then there was no significant difference between the samples. This meant that any significant difference that SPSS found was due to the data
being fortuitously truncated rather than to true significance. If SPSS did not find a significant difference, then such a result would confirm this lack of significance. However, if this random median of zero fell outside the confidence interval for the calculated difference median, then there was a significant difference between the two samples. This meant that any significant difference that SPSS uncovered was indeed legitimate. If SPSS did not reveal any significant difference, but the bootstrap did, then the data truncated by SPSS must have contributed significantly to the difference median.
RESULTS

Patient Information

Each patient was asked for the time since his initial diagnosis of PD and the side of onset on which his disease first presented. His Hoehn & Yahr stage in his “on” state was determined by his neurologist, Dr. Raymon Durso, the PD expert in our lab. A patient is considered to be in an “on” state when his PD medications are exerting an effect on his motor symptoms.

Table 1. General Information about Each Subject.

<table>
<thead>
<tr>
<th></th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Side of Onset (of Motor Symptoms)</td>
<td>Right</td>
<td>Right</td>
<td>Left*</td>
<td>Right</td>
</tr>
<tr>
<td>Hoehn &amp; Yahr Stage</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Years Since Diagnosis</td>
<td>7</td>
<td>2</td>
<td>16</td>
<td>4</td>
</tr>
</tbody>
</table>

*Subject 3’s side of disease onset has been debated. The patient was initially diagnosed with left-body onset PD. However, he had motor symptoms presenting on both sides of his body throughout his medical history. After he was referred to the Neurology service at the Boston VA Hospital in Jamaica Plain (to the care of Dr. Durso), he underwent a SPECT scan. The radiologist read the SPECT scan as revealing left putamen deactivation to indicate a right-body onset of PD. The left-body onset was chosen for this project because it was the initial side of onset noted in the patient’s medical history. This helped to maintain consistency, as the other three subjects’ side of onset listed was the initial side of onset clinically determined.
Quantification

*General Information About Each Region*

After each subject’s scans were coregistered, the caudate and putamen in each hemisphere were quantified. Although the range of intensities, range size, and the total number of voxels differed in each subject, they were similar between each hemisphere’s caudate and putamen within every subject.
Table 2. General Information about Each Scan’s Caudate and Putamen Regions.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Left Caudate</th>
<th>Left Putamen</th>
<th>Right Caudate</th>
<th>Right Putamen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>3429</td>
<td>5059</td>
<td>3149</td>
<td>5393</td>
</tr>
<tr>
<td></td>
<td>Range Size: 622</td>
<td>Range Size: 629</td>
<td>Range Size: 697</td>
<td>Range Size: 625</td>
</tr>
<tr>
<td>Total</td>
<td>4464</td>
<td>6074</td>
<td>3925</td>
<td>6144</td>
</tr>
<tr>
<td></td>
<td>Range Size: 211</td>
<td>Range Size: 259</td>
<td>Range Size: 230</td>
<td>Range Size: 278</td>
</tr>
<tr>
<td>Total</td>
<td>3788</td>
<td>5614</td>
<td>4453</td>
<td>5411</td>
</tr>
<tr>
<td></td>
<td>Range Size: 704</td>
<td>Range Size: 583</td>
<td>Range Size: 545</td>
<td>Range Size: 443</td>
</tr>
<tr>
<td>Total</td>
<td>3341</td>
<td>4488</td>
<td>3571</td>
<td>3829</td>
</tr>
</tbody>
</table>

The range is the range of intensity values over an entire region. The range size is the size of this range, and it was calculated by subtracting the lowest intensity value in a single region from the highest intensity value in the same region. The total is the total number of voxels in a particular region.
Pericalcarine Thresholds

The pericalcarine baseline in each hemisphere was determined by calculating the mean intensity of the pericalcarine gyrus on the SPECT scan. The percent of voxels whose intensities exceed two through seven times the pericalcarine baseline was reported in each region for each subject. The percentage exceeding the pericalcarine baseline was included for reference. The percentages here were based on the total number of voxels that exceed the particular threshold.

Table 3. Percent of Voxels that Exceed Five Pericalcarine Baseline Thresholds in the Caudate and Putamen Regions in Subject 1.

<table>
<thead>
<tr>
<th>Threshold</th>
<th>Left Caudate</th>
<th>Left Putamen</th>
<th>Right Caudate</th>
<th>Right Putamen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pericalcarine Baseline</td>
<td>95.77</td>
<td>100</td>
<td>94.57</td>
<td>100</td>
</tr>
<tr>
<td>2x Baseline</td>
<td>75.27</td>
<td>93.81</td>
<td>81.96</td>
<td>98.39</td>
</tr>
<tr>
<td>3x Baseline</td>
<td>12.57</td>
<td>55.68</td>
<td>40.43</td>
<td>79.29</td>
</tr>
<tr>
<td>4x Baseline</td>
<td>0</td>
<td>2.85</td>
<td>13.59</td>
<td>31.58</td>
</tr>
<tr>
<td>5x Baseline</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.19</td>
</tr>
<tr>
<td>6x Baseline</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7x Baseline</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The mean pericalcarine intensity, which served as the pericalcarine baseline, is 236.22 for the left hemisphere and 204.30 for the right hemisphere.
Table 4. Percent of Voxels that Exceed Five Pericalcarine Baseline Thresholds in the Caudate and the Putamen Regions in Subject 2.

<table>
<thead>
<tr>
<th></th>
<th>Left Caudate</th>
<th>Left Putamen</th>
<th>Right Caudate</th>
<th>Right Putamen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pericalcarine Baseline</td>
<td>98.61</td>
<td>99.87</td>
<td>99.03</td>
<td>100</td>
</tr>
<tr>
<td>2x Baseline</td>
<td>79.75</td>
<td>89.04</td>
<td>88.10</td>
<td>94.39</td>
</tr>
<tr>
<td>3x Baseline</td>
<td>66.02</td>
<td>72.98</td>
<td>76.76</td>
<td>58.69</td>
</tr>
<tr>
<td>4x Baseline</td>
<td>45.18</td>
<td>46.87</td>
<td>65.96</td>
<td>30.26</td>
</tr>
<tr>
<td>5x Baseline</td>
<td>22.29</td>
<td>22.42</td>
<td>52.23</td>
<td>16.15</td>
</tr>
<tr>
<td>6x Baseline</td>
<td>0.11</td>
<td>2.24</td>
<td>22.14</td>
<td>6.32</td>
</tr>
<tr>
<td>7x Baseline</td>
<td>0</td>
<td>0</td>
<td>7.52</td>
<td>2.33</td>
</tr>
</tbody>
</table>

The mean pericalcarine intensity, which served as the pericalcarine baseline, is 119.64 for the left hemisphere and 96.41 for the right hemisphere.
Table 5. Percent of Voxels that Exceed Five Pericalcarine Baseline Thresholds in the Caudate and Putamen Regions in Subject 3.

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Left Caudate</th>
<th>Left Putamen</th>
<th>Right Caudate</th>
<th>Right Putamen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pericalcarine Baseline</td>
<td>99.23</td>
<td>99.96</td>
<td>99.64</td>
<td>98.50</td>
</tr>
<tr>
<td>2x Baseline</td>
<td>71.09</td>
<td>85.04</td>
<td>57.44</td>
<td>77.05</td>
</tr>
<tr>
<td>3x Baseline</td>
<td>27.01</td>
<td>56.36</td>
<td>24.34</td>
<td>45.09</td>
</tr>
<tr>
<td>4x Baseline</td>
<td>6.81</td>
<td>36.02</td>
<td>11.18</td>
<td>26.83</td>
</tr>
<tr>
<td>5x Baseline</td>
<td>1.35</td>
<td>15.32</td>
<td>1.66</td>
<td>9.41</td>
</tr>
<tr>
<td>6x Baseline</td>
<td>0</td>
<td>1.53</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7x Baseline</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The mean pericalcarine intensity, which served as the pericalcarine baseline, is 45.49 for the left hemisphere and 53.21 for the right hemisphere.
Table 6. Percent of Voxels that Exceed Five Pericalcarine Baseline Thresholds in the Caudate and Putamen Regions in Subject 4.

<table>
<thead>
<tr>
<th></th>
<th>Left Caudate</th>
<th>Left Putamen</th>
<th>Right Caudate</th>
<th>Right Putamen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pericalcarine Baseline</td>
<td>90.75</td>
<td>99.98</td>
<td>94.23</td>
<td>99.32</td>
</tr>
<tr>
<td>2x Baseline</td>
<td>29.30</td>
<td>28.72</td>
<td>58.08</td>
<td>75.06</td>
</tr>
<tr>
<td>3x Baseline</td>
<td>5.54</td>
<td>1.05</td>
<td>12.43</td>
<td>6.53</td>
</tr>
<tr>
<td>4x Baseline</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5x Baseline</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6x Baseline</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7x Baseline</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The mean pericalcarine intensity, which served as the pericalcarine baseline, is 174.94 for the left hemisphere and 211.45 for the right hemisphere.

**Asymmetry Indices**

The asymmetry index (AI) was calculated for each region as a ratio of the proportion of voxels in the right hemisphere to the proportion of voxels in the left hemisphere whose intensities exceed either three or four times the pericalcarine baselines. For example, the AI of the caudate would be $AI = \frac{\text{no. of right hemisphere caudate voxels}}{\text{no. of left hemisphere caudate voxels}}$.

There are no AI data for either the caudate or the putamen in Subject 4 for the higher threshold, as there were no voxels in either region whose intensity exceeded 4 or 5 times the pericalcarine baseline. There are no AI data for the caudate in Subject 1 because there were no voxels in the left caudate whose...
intensity exceeded quadruple the pericalcarine baseline, though there were such voxels in the right caudate.

Table 7. Asymmetry Indices for the Proportions of Voxels that Exceed Triple and Quadruple the Pericalcarine Baseline in the Caudate and Putamen Regions in Subjects 1-4.

<table>
<thead>
<tr>
<th></th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>3x Pericalcarine Baseline</td>
<td>Caudate</td>
<td>3.22</td>
<td>1.16</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Putamen</td>
<td>1.42</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>4x Pericalcarine Baseline</td>
<td>Caudate</td>
<td>N/A</td>
<td>1.46</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>Putamen</td>
<td>11.08</td>
<td>0.65</td>
<td>0.74</td>
</tr>
</tbody>
</table>

AI values that are greater than 1 indicate that there were more voxels in the right hemisphere region than in the left. AI values that are less than 1 indicate that there were more voxels in the left hemisphere region than in the right.

Scans

The following images are single-slice axial views of a 3D image of each subject’s SPECT scan coregistered to his own MPRAGE. The colors of the SPECT DaT represent the scan’s intensity, reflecting the amount of dopaminergic uptake: blue and green indicate, respectively, negligible and low-level activity, whereas yellow and red indicate, respectively, high and extremely high levels of dopamine activity in the striatum. The SPECT DaT also revealed the asymmetric dopaminergic activity of PD. Three of the four subjects had right-onset PD, which originates in the left hemisphere, and the fourth subject had left-
onset PD originating in the right hemisphere. All of this corresponded to the asymmetry seen in the quantification of the data. The scans also reflected the localization of the dopaminergic loss – in other words, whether the caudate or putamen contributed more to the asymmetric deactivation – which was also supported by the quantification data. All four scans are presented in the radiological convention whereby the left hemisphere is on the right side of the scan and vice versa.
Figure 1. Subject 1 – SPECT DaT Coregistered to MPRAGE.

The white arrows are pointing to the left and right caudate. The gold arrows are pointing to the left and right putamen. The scan is in radiological view, so the left hemisphere is on the right side of the scan, and vice versa.
Figure 2. Subject 2 – SPECT DaT Coregistered to MPRAGE.

The white arrows are pointing to the left and right caudate. The gold arrows are pointing to the left and right putamen. The scan is in radiological view, so the left hemisphere is on the right side of the scan, and vice versa.
The white arrows are pointing to the left and right caudate. The gold arrows are pointing to the left and right putamen. The scan is in radiological view, so the left hemisphere is on the right side of the scan, and vice versa.
Figure 4. Subject 4 – SPECT DaT Coregistered to MPRAGE.

The white arrows are pointing to the left and right caudate. The gold arrows are pointing to the left and right putamen. The scan is in radiological view, so the left hemisphere is on the right side of the scan, and vice versa.
Statistics

A Wilcoxon Signed-Rank test was performed on the intensity data derived from the caudate and the putamen of each subject’s brain. For each of the eight tests, the mean rank for the positive ranks and for the negative ranks was reported, as well as the critical Z-value, the p-value, and the result of the hypothesis test. The results of the related bootstrapping analyses are also provided in the form of a histogram.

Subject 1

In the caudate, the mean rank was significantly higher for the positive ranks, meaning that the left caudate had significantly higher voxel intensities. In the putamen, the mean rank was significantly higher for the negative ranks, meaning that the right putamen had significantly higher voxel intensities.

Table 8. Analysis Between Hemispheres of Subject 1 Using Wilcoxon Signed-Rank Test.

<table>
<thead>
<tr>
<th>Region Tested</th>
<th>Mean Rank of Negative Ranks</th>
<th>Mean Rank of Positive Ranks</th>
<th>Critical Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate</td>
<td>1520.05</td>
<td>1631.37</td>
<td>$Z = -3.712$</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td>Putamen</td>
<td>2638.29</td>
<td>2399.69</td>
<td>$Z = -6.770$</td>
<td>$p &lt; 0.001$</td>
</tr>
</tbody>
</table>

According to the bootstrapping analysis, the left caudate had a significantly greater median as the null hypothesis median fell outside the left-
side of the confidence interval around the difference median. The right putamen had a significantly higher median than the left putamen as the null hypothesis median fell outside the right-side confidence interval around the difference median. Thus, the bootstrapping analysis and the Wilcoxon test were in agreement regarding both the caudate and the putamen.
Figure 5. Bootstrapping Analysis of the Difference Median of the Left Versus Right Caudate in Subject 1.

The green H0 median is on the zero value of the x-axis, which is at the far left of the graph.
Figure 6. Bootstrapping Analysis of the Difference Median of the Left Versus Right Putamen in Subject 1.

The green H0 median is on the zero value of the x-axis, which is at the far right of the graph.
Subject 2

In the caudate, the mean rank was significantly higher for the positive ranks, meaning that the left caudate had significantly higher voxel intensities. In the putamen, the mean rank was also significantly higher for the positive ranks, meaning that the left putamen had significantly higher voxel intensities.

Table 9. Analysis Between Hemispheres of Subject 2 Using Wilcoxon Signed-Rank Test.

<table>
<thead>
<tr>
<th>Region Tested</th>
<th>Mean Rank of Negative Ranks</th>
<th>Mean Rank of Positive Ranks</th>
<th>Critical Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate</td>
<td>1844.36</td>
<td>2061.86</td>
<td>$Z = -7.916$</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td>Putamen</td>
<td>2236.47</td>
<td>3458.33</td>
<td>$Z = -33.26$</td>
<td>$p &lt; 0.001$</td>
</tr>
</tbody>
</table>

According to the bootstrapping analysis, there was no significant difference between the left and right caudate as the null hypothesis median fell within the confidence interval around the difference median. The left putamen had a significantly higher median than the right putamen as the null hypothesis median fell outside the left-side confidence interval around the difference median. Due to the large scale of the graph, the null hypothesis median was not shown in order to more clearly show that the confidence intervals and the difference median all fall on the 151-mark. Thus, the bootstrapping analysis and the Wilcoxon test were in agreement regarding the putamen. However, for the
caudate, the bootstrapping analysis did not find the significant difference that the Wilcoxon test identified.
Figure 7. Bootstrapping Analysis of the Difference Median of the Left Versus Right Caudate in Subject 2.

The H0 cannot be rejected as the right-sided limit of the confidence interval falls onto the same zero value as the H0 median. To allow for visibility of both lines, the red confidence interval limit is shown as a dotted line superimposed upon the green H0 median.
Figure 8. Bootstrapping Analysis of the Difference Median of the Left Versus Right Putamen in Subject 2.

The difference median in this data set was so consistent, that only one value was calculated for every iteration. The confidence intervals are shown as red dotted lines superimposed upon the yellow sample median. The H0 median is not shown, as the scale required to present both the H0 median and the sample median in one image is too large to adequately visualize the results. The H0 median is always on the zero value of the x-axis.
Subject 3

In the caudate, the mean rank was significantly higher for the negative ranks, meaning that the right caudate had significantly higher voxel intensities. In the putamen, the mean rank was significantly higher for the negative ranks, meaning that the right putamen had significantly higher voxel intensities.

Table 10. Analysis Between Hemispheres of Subject 3 Using Wilcoxon Signed-Rank Test.

<table>
<thead>
<tr>
<th>Region Tested</th>
<th>Mean Rank of Negative Ranks</th>
<th>Mean Rank of Positive Ranks</th>
<th>Critical Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate</td>
<td>2052.80</td>
<td>1572.46</td>
<td>Z = –23.01</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Putamen</td>
<td>2743.82</td>
<td>2608.56</td>
<td>Z = –2.831</td>
<td>p = 0.005</td>
</tr>
</tbody>
</table>

According to the bootstrapping analysis, the right caudate had a significantly higher median than the left caudate as the null hypothesis median fell outside the right-side confidence interval around the difference median. The left putamen had a significantly higher median than the right putamen as the null hypothesis median fell to the left side of the confidence interval around the difference median. Thus, the bootstrapping analysis and the Wilcoxon test were in agreement regarding the caudate. However, for the putamen, the bootstrapping analysis and the Wilcoxon test found different hemispheres to have significantly greater voxel intensities.
Figure 9. Bootstrapping Analysis of the Difference Median of the Left Versus Right Caudate in Subject 3.

The green H0 median is on the zero value of the x-axis, which is at the far right of the graph.
Figure 10. Bootstrapping Analysis of the Difference Median of the Left Versus Right Putamen in Subject 3.

The green H0 median is on the zero value of the x-axis, which is at the far left of the graph.
Subject 4

The Wilcoxon signed-rank test did not find a significant difference between the mean rank of the positive ranks and of the negative ranks, meaning that it did not find the left and right caudates to be significantly different. In the putamen, the mean rank was significantly higher for the negative ranks, meaning that the right putamen had significant higher voxel intensities.

Table 11. Analysis Between Hemispheres of Subject 4 Using Wilcoxon Signed-Rank Test.

<table>
<thead>
<tr>
<th>Region Tested</th>
<th>Mean Rank of Negative Ranks</th>
<th>Mean Rank of Positive Ranks</th>
<th>Critical Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate</td>
<td>1627.71</td>
<td>1688.43</td>
<td>Z= –0.020</td>
<td>p= 0.984</td>
</tr>
<tr>
<td>Putamen</td>
<td>1976.79</td>
<td>1857.42</td>
<td>Z= –2.921</td>
<td>p= 0.003</td>
</tr>
</tbody>
</table>

According to the bootstrapping analysis, the right caudate had a significantly higher median than the left caudate as the null hypothesis median fell outside the right-side confidence interval around the difference median. There was no significant difference between the left and right putamen as the null hypothesis median fell within the confidence interval around the difference median. Thus, the bootstrapping analysis and the Wilcoxon tests were not in agreement regarding either region. For the caudate, the Wilcoxon test appears to have missed the significant difference uncovered by the bootstrapping analysis. For the putamen, the bootstrapping analysis appears not to have seen
the significant difference that the Wilcoxon test identified.
Figure 11. Bootstrapping Analysis of the Difference Median of the Left Versus Right Caudate in Subject 4.

The green H0 median is on the zero value of the x-axis, which is at the far right of the graph.
Figure 12. Bootstrapping Analysis of the Difference Median of the Left Versus Right Putamen in Subject 4.
DISCUSSION

Overview

The neurological study of PD through imaging is still in its infancy, despite its long clinical history. When a clinical diagnosis of PD presents equivocally, the current form of diagnostic imaging is the SPECT DaT scan, which utilizes a radionuclide that binds to the dopamine transporter DAT in the striatum. The goal of this thesis project was to propose a way to improve the poor resolution and lack of quantitative measure in the use of the SPECT scans through the coregistration of MPRAGE scans. The hope is that this method can improve both the clinical diagnosis through imaging. Overall, the data appeared generally consistent with the patient information, suggesting that the methodology has great potential for true and meaningful efficacy.

The “more-affected hemisphere” was defined as the hemisphere contralateral to the side of the body on which the motor symptoms first presented, and the “less-affected hemisphere” was defined as the hemisphere ipsilateral to the side of motor symptom presentation. For all four subjects, the total number of voxels in each region was similar between hemispheres (Table 2). For each more-affected hemispheric region in each subject, the range size was either similar to or less than the corresponding less-affected hemisphere region (Table 2). This corresponded well with the side of onset of each patient’s disease, as it suggests that, despite the comparable sizes of the regions, the
intensities of the voxels in the more-affected hemisphere regions did not extend as high as in the less-affected hemisphere. 

Though the subjects' pericalcarine baselines varied greatly, they were similar between hemispheres within each subject (Tables 3-5). In general, there was a smaller proportion of voxels that exceeded each level above the pericalcarine baseline in the more-affected hemisphere than in the left-affected hemisphere for both the caudate and the putamen in all four subjects (Table 3-5).

**Subject 1**

In Subject 1, the proportion of voxels that exceeded each of the seven levels above the baseline was smaller in the left-hemisphere regions than in the right-hemisphere regions (Table 3). This difference was more clearly seen in the AI data. In both the caudate and the putamen, the AI was greater than 1 for both triple and quadruple the pericalcarine baseline (Table 7). The left striatum in the subject's scan was visibly less activated than the right striatum (Figure 1), which appropriately matched the left-hemisphere onset (right-body onset) of the subject's PD. The left caudate in Figure 1 showed very little red – the color intensity indicating the highest activation – and this reflected the low proportion of voxels with intensities above triple the pericalcarine baseline and the lack of voxels with intensities above quadruple the pericalcarine baseline in the left caudate as seen in Table 3. The left putamen in Figure 1 was less consistently red, i.e. less highly activated, than the right putamen; this matched the greater
The proportion of voxels that exceeded triple and quadruple the pericalcarine baseline in the right putamen (Table 3).

The Wilcoxon Signed-Rank test found that the mean rank in Subject 1 was significantly higher for the positive ranks of the caudate and significantly higher for the negative ranks of the putamen, meaning that the left caudate and the right putamen had significantly higher voxel intensities than their respective counterparts (Table 8). The bootstrapping analysis found the left caudate to have significantly greater values than the right caudate as the null hypothesis median fell to the left of the confidence interval around the difference median (Figure 5). This result supported the conclusion of the associated Wilcoxon test. On the other hand, the bootstrapping analysis found that the right putamen had significantly greater voxel intensities than the left putamen as the null hypothesis difference mean fell well to the right of the sample difference mean's confidence interval (Figure 6). This result was in agreement with the conclusion of the Wilcoxon test. The result of the latter bootstrapping analysis was also consistent with the quantification of the data in Table 3, the AI values in Table 7, and the visible asymmetry in Figure 1.

The data collected on Subject 1’s caudate (Table 3, Table 7, and Figure 1) were consistent with the subject’s right-body, left-hemisphere onset of PD (Table 1). The statistical analyses on the caudate were not supportive; however, this may suggest that the patient’s greater right caudate dopaminergic loss was simply not implicated in the right-body onset of motor symptoms, hence its
inconsistency with the side of onset. Dopaminergic loss in the caudate is not generally associated with motor symptoms in PD (9). All the data collected on this subject’s putamen as well as the associated statistical analyses points to greater loss of dopaminergic activation in the left hemisphere corresponding to right-body disease onset, and this was consistent with the patient’s described side of onset as seen in Table 1.

**Subject 2**

In Subject 2, the proportion of voxels whose intensities exceeded each of the four levels above the pericalcarine baseline was smaller in the left caudate than in the right caudate (Table 4). There was a greater proportion of voxels whose intensities exceed the baseline and twice the baseline in the right putamen than in the left putamen; however, interestingly, the proportion of voxels whose intensities exceeded triple, quadruple, and quintuple the pericalcarine baseline was slightly greater in the left putamen than in the right (Table 4). Accordingly, the AI values for both thresholds were greater than 1 for the caudate and were less than 1 for the putamen (Table 7). These data may appear somewhat ambiguous, and Figure 2, at first glance, mirrors this. Upon closer look, though, the left-hemisphere-onset (right-body onset) nature of Subject 2’s disease becomes clear. In the right putamen, the top 2% of voxel intensities exceeded 7 times the baseline, but, in the left putamen, the top 2% only exceeded 6 times the baseline (Table 4). These higher intensity voxels present
in the right putamen have already degenerated in the left putamen. In the scan, the left putamen tail was no longer visible, and the left caudate was not as consistently highly activated — indicated by less red coloring — than the right caudate (Figure 2).

The Wilcoxon Signed-Rank test in Subject 2 found that the mean rank was significantly higher for the positive ranks in both the caudate and the putamen, meaning that the left hemisphere regions of the striatum had significantly higher voxel intensities (Table 9). The bootstrapping analysis found no significant difference between the left and right caudate as the null hypothesis median fell within the confidence interval around the median (Figure 7). This suggests that the conclusion of the Wilcoxon test was simply due to the truncation of data rather than a true difference. However, the bootstrapping analysis found that the left putamen had significantly higher intensity values as the null hypothesis fell to the left of the confidence interval around the difference median (Figure 8). This supports the Wilcoxon test’s conclusion as well as the AI values for the putamen of this subject (Table 7). The bootstrapping and the Wilcoxon test results were not consistent with the left-hemisphere, right-body disease onset of this subject (Table 1).

If the bootstrapping analysis is accurate in finding that the right putamen has lower voxel intensities than the left putamen, then, unfortunately, this leaves the incongruence between the right-body disease onset and the greater dopaminergic loss in the right putamen unexplained. The accuracy of the
bootstrapping analysis was double-checked because the consistency of the
difference median is unusual. There may be some inherent characteristic of the
voxel intensities or of the patient’s brain that can account for this apparent
discrepancy, but that is out of the scope of this project. The bootstrapping
analysis did not find a significant difference between the intensity values of the
left and right caudate. Therefore, the left caudate may still have lower intensity
values (Table 4, Table 7), but the difference does not hold statistical significance.
Possibly, this difference will hold statistical significance later in the disease
progression.

Subject 3

In Subject 3, the proportion of voxels whose intensities exceeded each of
the seven thresholds above the pericalcarine baseline, as seen in Table 5, is
smaller in the right putamen than in the left putamen for all levels above the
baseline. This is consistent with the patient’s left-body disease onset. The right
caudate has a smaller proportion of voxels with intensities that exceeded each
threshold until 4 times the baseline (Table 5). At this point, there was
inexplicably a greater proportion of voxels with intensities that exceeded this
threshold. However, at the next threshold, the proportion in the right caudate
was only marginally larger (Table 5). Indeed, except for the caudate AI at the
quadruple baseline threshold, the AI values were all less than 1, as was
expected for a right-hemisphere disease onset (left-body onset) (Table 7). In the
scan in Figure 3, more of the right caudate’s voxels were indicated as highly activated by the red coloring, though the left and right caudate were still roughly the same size. This corresponds to the data in Table 5: the proportion of voxels was greater in the left caudate whose intensities exceeded twice the pericalcarine baseline but greater in the right caudate for quadruple the baseline, and the proportions were similar for the other thresholds. The left-body onset was more clearly explained when contrasting the putamen between hemispheres. Although the left putamen in Figure 3 was not very highly activated, the right putamen was not present at all on the scan. This corresponded to the AI values in Table 7, which were less than 1 for both thresholds.

The Wilcoxon Signed-Rank test found that the mean rank in Subject 3 for the negative ranks was significantly higher for both striatal regions, meaning that the right caudate and the right putamen had significantly higher voxel intensities than their respective counterparts (Table 10). The bootstrapping analysis, on the other hand, found that the right caudate and the left putamen had significantly greater voxel intensities than the left caudate and the right putamen. For the caudate, the null hypothesis difference median of zero fell to the right of the confidence interval around the sample difference median (Figure 9). Thus, for the caudate, the bootstrapping analysis and the Wilcoxon test were in agreement. For the putamen, the null hypothesis difference median of zero fell to the left of the confidence interval around the difference median (Figure 10).
This oppugns the conclusion of the Wilcoxon test and suggests that its contrary result was due to the truncation of data that would have otherwise exerted a meaningful influence on the sample comparison.

Although the bootstrapping analysis of the putamen was not in agreement with the Wilcoxon test conclusion, it was actually more consistent with the quantification of the data (Table 5), the AI values (Table 7), the SPECT scan (Figure 3), and the patient’s left-body (right-hemisphere) onset of PD. Neither the bootstrapping analysis nor the Wilcoxon test of the subject’s caudate produced results consistent with the rest of the quantification data (Table 5), but this may be attributed to the lack of statistical significance of the quantification data. Although neither statistical test offered conclusions consistent with the patient’s side of disease onset (Table 1), the caudate is not usually considered to be implicated in the motor symptoms of PD (9).

Subject 4

In Subject 4, the proportion of voxels whose intensities exceeded each of the seven thresholds above the pericalcarine baseline was greater in the right hemisphere for both regions (Table 6), as was expected for this patient’s right-body disease onset (left-hemisphere onset). There were no voxels in any region that exceeded quadruple the pericalcarine baseline, and, as such, there were no AI values for this threshold, but, for the lower threshold, both the AI values were, accordingly, greater than 1 (Table 7). In the subject’s scan, the left striatum was
visibly less activated than the right striatum, as evidenced by the clearly fewer number of red- and orange-colored voxels in the left hemisphere (Figure 4), which corresponded well with the right-body onset of the patient’s PD. The AI value for the putamen was much greater than that of the caudate in this subject (Table 7), and, accordingly, the difference in activation between the left and right putamen regions appeared greater on the scan in Figure 4 than did the difference in activation between the left and right caudate. In fact, while there was still a small portion of the left caudate being highly activated, there was very little left putamen visible at all.

The Wilcoxon Signed-Rank test in Subject 4 found that the mean rank for the putamen was significantly higher for the negative ranks, meaning that the right putamen had significantly higher voxel intensities (Table 11). The right caudate was not significantly different from the left caudate according to the Wilcoxon test (Table 11). Interestingly, the bootstrapping analysis did not support either of these conclusions. For the putamen comparison, the null hypothesis difference median fell within the confidence interval of the sample difference median (Figure 12). This suggests that the significant difference uncovered by the Wilcoxon test may have been a false positive arising from the fortuitous truncation of data. However, it is possible that as the patient’s disease progresses, this difference will become statistically significant. Regarding the caudate, the null hypothesis difference median fell to the right of the confidence interval around the difference median (Figure 11). This means that the right
caudate voxel intensities were significantly higher than the left caudate voxel intensities. The truncation of data most likely caused the Wilcoxon test to instead find no significant difference between the regions.

Despite the clear asymmetry in the proportions calculated for both regions (Table 6), in the AI values in Table 7, and in Figure 4, neither the Wilcoxon test of the caudate data nor the bootstrapping analysis of the putamen supported the perceived greater left-hemisphere loss of dopaminergic activation. The subject’s PD is already at Hoehn & Yahr Stage 2, so the lack of significant difference between the left and right putamen seen in the bootstrapping analysis could be due to his disease having progressed far enough that the less-affected hemisphere’s dopaminergic loss has begun to catch up to the right hemisphere’s.

**Further Interpretation**

Each subject’s data can be further interpreted in the light of the patient’s clinical history, which was reported in Table 1. Subject 1, for example, has had a PD diagnosis for 7 years and is already at Hoehn & Yahr Stage 3 of his disease, while Subject 2 was only diagnosed 2 years ago and is at Hoehn & Yahr Stage 1. Subject 2 had voxel intensities up to 6 times the pericalcarine in all four regions of his scan (Table 4), while the highest threshold above which Subject 1 has voxel intensities in every region is 3 times his pericalcarine baseline (Table 3). This reflects nicely how these two subjects are at very different stages in their disease progression. Subject 4, on the other hand, has a much more slowly
progressing disease than Subject 1 as there were even smaller proportions of voxels whose intensities exceeded triple the pericalcarine baseline (Table 6) than is seen in Subject 1, and yet his disease is only at Hoehn & Yahr Stage 2. Subject 3, though, has the slowest disease progression of all four participants as his voxel intensities surpassed 5 times the pericalcarine baseline in all four regions (Table 5), and he is only at Hoehn & Yahr stage 1, though he was diagnosed with PD 16 years ago. Though these associations between disease severity and the quantification data are certainly intriguing, being able to show statistical significance would strengthen the validity of these relationships.

Hopefully, larger studies in the future can statistically confirm that longitudinal quantification data of patient’s SPECT DaT scans matches their clinical disease progression.

**A Presentation of the Method’s Utility**

Subject 3 offers an opportunity to present an important potential utility for this method. As noted in Table 1, this patient was initially diagnosed with left-body onset of PD; however, the side of onset was later contested. He had motor symptoms presenting on both sides of the body, which oppugned the diagnosis of PD. A SPECT scan was ordered to establish the accuracy of a PD diagnosis and to shed light on the side of disease onset. When the scan was clinically assessed, the radiologist concluded that there was decreased activation in the left putamen, implying right-body disease onset. For the sake of continuity, the
side of onset listed for this subject in this project (left-body onset; see Table 1) was the one initially reported by the patient, just as was the case for the other three participants. Determining which hemisphere has greater dopaminergic loss (particularly in the putamen) would help to illuminate whether the initial diagnostician’s left-body onset diagnosis or the radiologist’s suggestion of right-body onset is more accurate.

There is evidence that the patient’s right putamen exhibited greater dopaminergic loss than the left putamen, corresponding to a left-body disease onset. First of all, most of the percentages of voxels with intensities exceeding pericalcarine thresholds supported greater dopaminergic loss in the right hemisphere. For every level above the pericalcarine baseline, the percentage of voxels whose intensities exceeded three times the pericalcarine baseline was greater in the left putamen than in the right putamen (Table 5). (The percentage of voxels with intensities exceeding two and three times the pericalcarine baseline was higher in the left caudate (Table 5).) Additionally, the majority of the AI values supported greater dopaminergic loss in the right hemisphere. The AI values for three times the pericalcarine baseline were less than 1 for both the caudate and the putamen (Table 7). This indicated greater proportions of voxels with intensities exceeding the thresholds in the left hemispheric regions. The AI value for four times the pericalcarine baseline in the putamen was less than 1 as well (Table 7), also suggesting a greater proportion of voxels with intensities exceeding the threshold in the left hemisphere. Finally, the left putamen, though
not very highly activated, could still be seen in the scan (Figure 3), whereas the right putamen was not visible at all. This greater right-hemisphere loss of dopaminergic terminals seen in much of the data is consistent with the initial determination of left-body onset of PD. Furthermore, the bootstrapping analysis showed that the right putamen had lower voxel intensities, corresponding to the greater right-hemisphere dopaminergic loss seen in left-body disease onset.

On the other hand, there is also evidence supporting a right-body onset PD diagnosis. Although the quantification of the data does not seem to endorse the right-body onset as much as the left-body onset, the statistical testing offers a different result. The Wilcoxon tests on the caudate and the putamen both reported reduced intensities of voxels in the left hemisphere, and the latter corresponds to right-body disease onset. Moreover, the radiologist who read the patient’s scan evaluated the scan as having greater right-hemispheric activation to support a right-body onset of PD. Interestingly, this is in fairly direct contradiction to the quantification of the putamen’s data.

The present understanding of PD is far from perfect, though advances in neuroimaging have precipitated further insights into the neurology of the disease. Although there is much still to be explained about the etiology and progression of PD, the current consensus is that the putamen is more heavily involved in the motor aspects of the disease, while the caudate is more implicated in the cognitive and affective symptoms (9). This dichotomous understanding of the role of the striatum in PD is poised to change with new information from
neuroimaging. However, if the respective propensities of the caudate and the putamen remain true, then they may offer an explanation of this subject’s data. The quantification of the data as well as the scan and the statistical testing suggest greater dopaminergic loss in the right putamen. If the putamen is indeed more associated with motor symptoms, and the right putamen reveals greater dopaminergic loss, then this corroborates the initial diagnosis of left-body onset PD. If the caudate is less implicated in motor dysfunction than the putamen, then the greater decrease in dopaminergic activation exhibited by the right caudate than the left caudate may not have necessarily produced the motor symptoms that instigated the clinical diagnosis of PD. In this case, the initial diagnostician was correct in his observation of left-body onset, and the radiologist was mistaken in subjectively concluding greater left putamen decrease in activation to confirm right-body onset of PD. Thus, the quantification of the scan elucidated the nature of the patient’s asymmetrical striatal dopaminergic loss where a qualitative judgment of asymmetrical activation in the scan could not.

**Potential for Improvement**

This project has a number of limitations that, for the most part, can and should be remedied in future studies validating this methodology. An important consideration is the partial volume effect (PVE). The PVE accounts for the errors that arise from the inherently poor spatial resolution of the SPECT scan. The low spatial resolution blurs the image such that areas of true activation are spread
out into neighboring voxels (32). This decreases the intensities of the center of activation while increasing the intensity of the surrounding voxels, thereby detracting from the accuracy of the location and intensity of dopaminergic uptake (32). Partial volume correction (PVC) corrects the errors due to the PVE. Running the intensity values through a PVC algorithm, for which there are software packages available written specifically for SPECT scans, would increase confidence in the accuracy of the subsequent quantification data (33). It is important to note that the statistical results which did not support the quantification of the data or which did not support the side of onset described by the patient may in fact affirm the patient’s side of disease onset once they have been partial-volume corrected. This methodology also needs to be validated on a larger scale. A study with many more than the four subjects being used in this project would permit the use of more robust statistical testing. In addition, validating this method in long-term settings would support its use in longitudinal investigations. Unfortunately, an unavoidable drawback of this method is that it can currently only be performed on subjects who have no contraindications to MRI or SPECT scanning.

Looking Forward

Validation of this methodology with larger studies and the use of robust statistical testing will advance its use in a number of different environments. As mentioned earlier, longitudinal use may help to more quantitatively measure the
changes in dopaminergic activation in the striatum in order to investigate clinical disease progression in more detail. This methodology can lend its quantitative nature to the standardization of PD diagnosis via SPECT imaging as it can tease out asymmetry and deactivation information that may not be otherwise apparent through solely visually inspecting SPECT scans. This capability may lead both to more accurate differential diagnoses of the various extrapyramidal neurodegenerative diseases such as MSA and PSP and to earlier identification of PD and subsequently initiation of treatment or preventative measures. Finally, the method will bring quantification of SPECT data to research studies utilizing SPECT scans to study all aspects of PD. For instance, the quantification can be performed with even smaller striatal subregions to give an increasingly more detailed picture of the dopaminergic changes occurring due to the disease. The lab plans to use this SPECT quantification methodology in conjunction with other imaging modalities, including fMRI and DTI, to study changes in cognition and affect in patients with PD. The ability to quantify SPECT scans will be an important advantage both to diagnosticians and to researchers involved with PD.

**Conclusion**

Between the four subjects, there were sixteen regions being quantified – the left and right caudate, and the left and right putamen. As the putamen is the region implicated in the motor symptoms of PD (9), only 8 of these quantified regions are relevant to the patient’s side of onset. The quantification of the data
supported the side of onset in all eight putamen regions. Sixteen statistical tests were run: a Wilcoxon signed-rank test and a bootstrapping analysis each for the caudate and the putamen in every subject. Although the Wilcoxon tests did not generally produce results consistent with the rest of the quantification data, the bootstrapping analyses were supportive of the quantification data in six of the eight analyses. The scans were clearly consistent with the quantification of the data and the side of disease onset in two subjects. In a third subject, the scan was reflective of the quantification of the data and of the side of onset once it was explored more deeply. In one subject, though, the scan was reflective of the side of onset once considered more carefully but not of the quantification data. Overall, the results of this project substantiate the side of symptom onset described by the patients and their clinicians. With the inclusion of PVC, the results will most likely be even more accurate. In summary, this thesis project has shown the great potential of this methodology.
# LIST OF JOURNAL ABBREVIATIONS

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<th>Journal Abbreviation</th>
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<td>Behav Brain Res</td>
<td>Behavioural Brain Research</td>
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<td>BMJ</td>
<td>British Medical Journal</td>
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<td>Cereb Cortex</td>
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<td>IEEE Trans Med Imaging</td>
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<td>Proc Natl Acad Sci USA</td>
<td>Proceedings of the National Academy of Sciences</td>
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EDUCATION

Boston University School of Medicine, Boston, MA
M.S., Medical Science
May 2015

Cornell University College of Arts and Sciences, Ithaca, NY
B.A., Biological Sciences
May 2013

The Lawrenceville School, Lawrenceville, NJ
Secondary School
May 2009

RESEARCH EXPERIENCE

Summer of 2008 and Summer of 2009
Interned in a laboratory in the Microbiology Department of the Weill Cornell Medical School research colon cancer under Francis Barany, PhD. Procedures included growing cell cultures and performing PCR experiments, among others.

Academic Year 2010-2011
Spent second undergraduate year at Cornell as a research assistant in a Social Psychology laboratory studying the effects of emotional induction on judgment under Tom Gilovich, PhD.

July 2014-Present
Full-time research work for my Master's thesis in the McNamara Lab, a BU Department of Neurology lab at the Boston VA Hospital in Jamaica Plain under
Patrick McNamara, PhD. I also worked with Dr. Edward Modestino, PhD, another member of the lab. For my Master’s thesis, I developed a method of SPECT scan quantification to increase the objectivity of Parkinson’s disease diagnosis. I also administer neuropsychological testing to participants and schedule patient visits and MRI scans for Dr. McNamara’s Cognition and Parkinson’s Project. I was responsible for writing and compiling an NIH R01 grant application that Dr. Modestino submitted February 2015. I have also attended the MGH-Martinos Center FreeSurfer course, which teaches how to use the FreeSurfer neuroimaging analysis software.

TEACHING EXPERIENCE

Cornell University
Teaching Assistant for Introductory Physics for 4 semesters (Fall 2010, Spring 2011, Fall 2011, Spring 2012).

Boston University
Teaching Assistant for Behavioral and Biological Aspects of Stress and Trauma (Fall 2014).

Teaching Assistant for Critical Reading and Analyses of the Medical Literature (Spring 2015).

Trainings
I attended the FreeSurfer training course at MGH-Martinos Center in Charleston, MA in October 2014. There, I formally learned how to use the various tools in the FreeSurfer software package to supplement the knowledge I acquired since starting my thesis work.

Internships
Nine-week internship during the summer of 2012 in the Morristown Medical Center Summer Student Fellowship, which involved being exposed to various aspects of hospital medicine. These areas included morning rounds, observing surgeries of all kinds, shadowing a pathologist, watching an autopsy, and much more.

Extracurricular Activities
- I was on Cornell Big Red Raas for 4 years as a senior member. Cornell Big Red Raas is a competitive team that performs an Indian folk dance called garba-raas from the Western state of Gujarat. The team performs on campus and competes against other collegiate teams across the country. I hope to pursue this activity again in the near future.
- I was a member of the Society for India, a cultural association at Cornell University that tries to spread awareness of and appreciation for Indian
culture. I participated in events and choreograph segments of my class dance in the annual Diya Jale Diwali celebration show.