EXPECTATION, THE PLACEBO EFFECT AND PARKINSON'S DISEASE: AN INVESTIGATION USING HIGH RESOLUTION POSITRON EMISSION TOMOGRAPHY

by

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Abstract

The placebo effect represents a fascinating example of how cognition can influence the physiology of the brain and body. The expectation of therapeutic benefit elicited by a placebo given in the guise of active medication has been proposed to be a form of reward expectation, and is associated with activation of brain reward circuitry. Prominent placebo effects occur in Parkinson's disease (PD), where the expectation of symptom improvement stimulates dopamine release in the striatum. In the work described in this dissertation, positron emission tomography with [^{11}C] raclopride was used to investigate the relationship between the strength of expectation of benefit and the degree of dopamine release in PD, and how this relationship corresponds to current models of dopamine function in reward. Chapter 3 describes a pilot study conducted in patients who had undergone subthalamic nucleus deep-brain stimulation (STN-DBS) in which we examined how awareness of stimulator status (ON or OFF) affected synaptic dopamine levels compared to when subjects were blind. No difference was detected between conditions; however, it proved to be difficult to maintain blinding due to the profound effects of STN-DBS. Chapter 4 describes the development of the methodology for the analysis of high-resolution PET data, in which we utilized the combined efforts of neuroscience and imaging physics to optimize the analysis of [^{11}C] raclopride PET data. In Chapter 5, I describe the use of verbal instructions to manipulate patients' expectations in order to investigate how the likelihood of receiving levodopa influenced dopamine release when the patients were in fact given placebo. Placebo-induced dopamine release was differentially modulated by expectation in the dorsal and ventral striatum: dopamine release in the putamen was related monotonically to expected reward value, whereas dopamine released in the ventral striatum reflected the uncertainty of benefit or the salience of the expectation. The placebo effect in PD therefore involves at least two related but separate mechanisms: the expectation of benefit itself, which is scaled to reflect the value of the

drug to the patient and is mediated by nigrostriatal dopamine, and the uncertainty or salience of benefit that is mediated by mesolimbic dopamine.

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DEDICATION

To my father, who, on our many walks in the woods as I was growing up, taught me about birds and trees and the reckless delight of learning about all living things.

Chapter I: Comprehensive literature review of the placebo effect

1.1 Introduction

Since the first medical practices and healing rituals were performed in ancient civilizations, the ability of the mind to influence the healing of the body has been recognized across many cultures. Even Galen himself, the father of evidence-based medicine wrote, "He cures most in whom are most confident" (Shapiro and Shapiro, 1997). Modern medicine has termed this the placebo effect. A placebo is defined as any therapy, or a component of any therapy, given in the guise of active medication or treatment but that is without specific activity for the condition being treated. Thus, the placebo effect is any effect attributable to the placebo (i.e. sugar pill, sham injection, sham surgery, etc.), but not to its pharmacodynamic properties (adapted from Wolf, 1959). As early as 1811, Hooper's Medical Dictionary defined a placebo as "an epithet given to any medicine adapted more to please than to benefit the patient" (Brody, 1980). Ironically, scientific investigation, in the realization of this phenomenon, needed to account for the placebo effect in the interpretation of experimental results, and thus it was largely considered to be a nuisance obscuring the true effects of the active treatment. However, with the growing amount of research available from clinical trials, the ability of placebos to produce therapeutic benefit in patients who suffer from various medical conditions has proven to be real and effective. It is now accepted that a prominent placebo effect may be present in pain disorders, depression, and Parkinson's disease (Enserink, 1999;Freeman et al., 1999;Turner, 1994) among other medical conditions, and advances in neuroimaging have enabled researchers to probe the neuropsychological and biochemical underpinnings of the placebo effect.

1.2 Investigating the Placebo Effect

1.2.1 Introduction

Investigating the placebo effect is logistically and ethically challenging. Deliberate deception must often be used, as the subject cannot be aware that they are receiving a placebo in

order to produce a placebo effect. As a result, the vast majority of placebo effects are still detected in double-blind, placebo-controlled trials aimed at testing new therapies, rather than explicitly probing the placebo effect itself, although research in this area is growing. The results of such studies have led to a shift in how the placebo effect is conceptualized; traditionally, the focus of investigation was on the inert physical agent itself (e.g. starch capsules), however the meaning of the term "placebo" has now broadened to include the entire psychosocial context that surrounds the patient in which the sham treatment is delivered. It is now understood that placebo effects may be driven by many different environmental factors that influence a patient's expectations, desires, emotions, and motivations (Price et al., 2008). As a result, the placebo effect is notoriously variable as the interaction of these factors is highly personalized to each individual, and it is this variability that makes it difficult to replicate results and derive scientific consensus regarding potential underlying mechanisms (see Section 1.2.4). In addition, true placebo effects can be confounded by the presence of other phenomena, including regression to the mean, spontaneous remission, compliance with demand, and perecptual bias. Some of the factors that must be considered when conducting placebo effect studies include the study design, the subjectivity of outcome measures, the presence of placebo responders and non-responders in the experimental sample, and various personal psychological factors of the subjects.

1.2.2 Importance of study design

Whereas traditional clinical experiments are designed to detect a physiological effect that can unequivocally be attributed to an intervention, research aimed at understanding the placebo effect itself requires a different approach. The likelihood of eliciting a placebo effect is highly dependent on the design of the trial, as well as the type of intervention, as the magnitude of the placebo effect is related to type of placebo administered; the greater the potency of treatment, the greater the placebo effect (de Craen et al., 2000). For example, placebo surgery seems to be more effective than a placebo pill (Brody, 1980;Kaptchuk et al., 2000;Shapiro and Shapiro,

1997), and as a study for the efficacy of arthroscopic knee surgery suggested, may produce the same outcome as the actual surgical procedure (Moseley et al., 2002). Double-blind placebo-controlled trials are the gold standard in medical research, and any therapeutic effect over and above that seen in the placebo group is attributed to the intervention. Many investigators have assumed that such a study design—a placebo group and an untreated group—should be ideal for detecting and quantifying the placebo effect (Ernst and Resch, 1995;Hrobjartsson and Gotzsche, 2001;Kaptchuk, 1998). Paradoxically however, with this study design and adequate informed consent, neither of the two groups will expect any benefit from the experiment and consequently, no full placebo intervention can be evaluated. The real placebo power in this scenario is lost since a patient with no expectation of clinical benefit is not likely to manifest a placebo effect. Another approach is the three-group study (Rosenthal, 1985), in which patients are randomly assigned to either an active drug group, a placebo group, or an untreated or natural history group in order to control for the placebo effect. However, in this study design, the patients' expectation of benefit may be too low, because a fully informed patient may realize that there is only a one in three chance of getting some benefit. It is therefore unsurprising that many studies with these designs have failed to demonstrate a placebo effect (Hrobjartsson and Gotzsche, 2001). In a meta-analysis of clinical trials involving two or three groups, it was concluded that with the exception of pain disorders, placebos offered no beneficial clinical effects (Hrobjartsson and Gotzsche, 2001). In fact, it has been shown that placebo effect percentages are lowest in double-blind studies, higher in single-blind studies, and highest in uncontrolled reports of a treatment believed to be effective but subsequently shown to be ineffective or a placebo (Shapiro and Shapiro, 1997). This observation shows that the simple act of being exposed to a placebo is not necessarily sufficient to provide clinical benefit to the patient, and the importance of the study design in producing placebo effects (Fuente-Fernandez et al., 2002).

It is clear that the patient's knowledge about whether or not he or she may be receiving a placebo during the study impacts the manifestation of a placebo response. Thus, the placebo effect may be greater in patients who have not been informed that they might receive a placebo during the study (Kaptchuk, 2001). So from a technical point of view, the best way to detect a placebo effect might be deliberately not to inform the patients that they may be receiving an inactive treatment, but this approach would clearly be unethical in most circumstances. However, this approach has been explored in Benedetti's "overt versus covert" study design, in which the subject is given the intervention without their knowledge, often under an unknown time sequence of drug administration. This enables the investigators to separate out the nonspecific effects of the treatment (Figure 1.1).

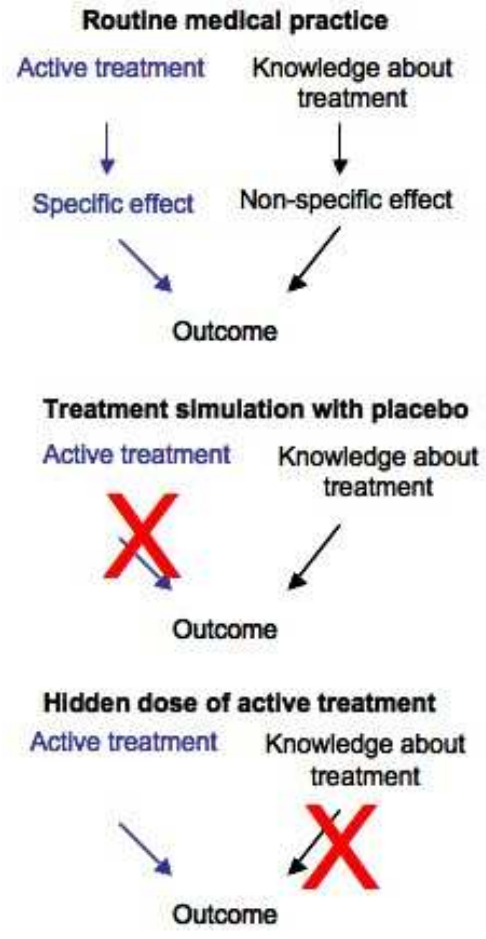


Figure 1.1 Every treatment in clinical practice has a specific and a non-specific effect. The non-specific effect comes from the knowledge that a treatment is being given. The effectiveness of

the active treatment can be assessed either by eliminating its specific effect (placebo study) or by eliminating the non-specific effects (hidden treatment). Adapted from Colloca et al., 2004.

This scenario represents a radically different approach to the analysis of placebo effects, as the placebo effect is assessed without a placebo group (Amanzio et al., 2001;Price, 2001). In this experimental approach, the placebo component is eliminated and the specific effects of the treatment are maintained. In order to eliminate the placebo component, patients must not be aware that a treatment is being given, for example, a painkiller is delivered via a hidden injection but the subject is not told when the drug is being administered. The patients give informed consent for the administration of a medical procedure but they do not know when it will be given. For example, the patient is in a bed with an intravenous line attached to a preprogrammed infusion machine and the drug can be delivered at the first, fourth, or tenth hour without the patient's knowledge. If the drug is really effective, symptom reduction should be temporally correlated with drug administration. The difference between outcomes on hidden treatment and on open treatment is the placebo component.

1.2.3 Subjectivity and placebo responders

In addition to careful study design, another challenge common in placebo effect research is the subjectivity of the measurement scales that are used to detect and quantify placebo responses. The vast majority of placebo effect research has been conducted in the field of pain, where placebo analgesia is quantified by the subjective reduction in pain ratings. Studies in depression have equally subjective outcome measures, as participants are asked to rate the improvement in their mood on a visual analog scale. In contrast, Parkinson's disease is a disorder in which the response to treatment can be assessed directly by the examiner, and this direct measurability might allow a better evaluation of the placebo effect by clinicians (Fuente-Fernandez et al., 2002). This being said, it is equally important to emphasize that the clinical scales used for measuring motor function are subjective themselves. Also, patients may be less prone to report

clinical changes than the clinicians are to observe them (Freed et al., 2001), adding another dimension of subjectivity.

It is well known that there are placebo responders and non-responders, yet there do not appear to be any consistent personality or genetic traits that predict who will fall into which category. Retrospective analysis of neuroimaging studies containing a placebo group can be useful in detecting any unique brain changes in subjects who positively respond to the placebo. One study conducted in patients suffering from major depression –a condition with a notoriously strong placebo effect – found that placebo responders had lower pretreatment frontocentral concordance as measured by electroencephalography (EEG), as well as faster cognitive processing time and lower reporting of late insomnia (Leuchter et al., 2004). In a study investigating the brain metabolic response to fluoxetine and placebo in depressed subjects, both placebo and drug responders were found to display a unique signature of ventral striatal and orbitofrontal cortical metabolic increases that predicted a positive clinical response (i.e. remission in depressive symptoms) (Mayberg et al., 2002). Further complicating the issue is the observation that an individual may respond to a particular placebo at a given time, yet fail to maintain a placebo effect on subsequent exposures to the same placebo, or respond to a different placebo.

1.2.4 Psychological and environmental factors

As noted above, patients with no expectation of benefit are not likely to manifest a placebo effect. However there are several additional factors complicating the issue, on the sides of the experimenter or physician, the subject or patient, and the treatment milieu itself. Such 'unspecific' treatment effects include predisposing factors such as training, empathy, suggestions, expectancies, worries and concerns, previous illness experience and a history of successful or failed therapy, and health behaviours; it also includes mechanisms based on their interaction, for example time, duration and intensity of patient-doctor communication. Other factors to consider

are the patient's knowledge about his or her disease, their desires and motivations, as well as their suggestibility, attitude and degree of optimism or pessimism. Such factors are difficult to assess in standard trials, and likely contribute to the high variability seen in placebo effects.

1.3 Mechanisms underlying the placebo effect

Two alternate theories have developed with respect to the underlying psychological mechanisms of the placebo effect. The expectation theory proposes that the patient's expectation of improvement drives the placebo effect. According to this view, placebo administration triggers the cognitive expectation of improvement that drives the downstream physiological placebo response. Alternatively, the conditioning theory states that the placebo effect is essentially a classically conditioned response. In this case, the unconditioned stimulus (US) is the active substance and the unconditioned response (UR) is the pharmacological effect in the body. The conditioned stimulus (CS) could be the syringe, pill casing, or therapeutic setting in which the US is administered, and through repeated pairings (anywhere from the medical treatments people experience in their lives, to experimentally-created experiences), a conditioned response (CR), or placebo effect, is created. Thus, according to this "stimulus substitution model," a person comes to associate the physical appearance of a drug or treatment with their physiological reaction to the drug (Kirsch, 1997). Importantly, this implies that the placebo effect can occur subconsciously. Original investigations into the placebo effect yielded models that supported one or the other of these theories (Evans, 1985;Wickramasekera, 1985;Ader, 1997;Kirsch, 1997), however the distinction is somewhat artificial, in that conditioning is a mechanism by which one can create or enhance expectations, and particularly in sentient animals, it is the expectation itself, rather than the final physiological response that may be conditioned. Furthermore, some conditioning procedures produce the opposite effect of placebos implying the existence of compensatory mechanisms. For example, the UR to morphine is a decrease in pain sensitivity, but the CR to a stimulus that has been paired with

morphine is hyperalgesia. Similarly, the UR to tranquilizers is a decrease in activity and arousal, but the CR to stimuli which have been paired with tranquilizers causes increased activity in laboratory animals. However, placebo morphine produces analgesia, and placebo tranquilizers produce sedation, therefore the placebo effect is likely more complex than a simple conditioned response. It is therefore likely that both expectation and conditioning contribute to placebo effects in different degrees, and act synergistically. Benedetti and colleagues investigated the separate contributions of expectation and conditioning in placebo analgesia, Parkinson's disease and hormone secretion (Benedetti et al., 2003b). Verbally-induced expectations of analgesia/ hyperalgesia and motor improvement/worsening completely removed the effects of a conditioning procedure in the first two classes of patients, whereas verbally-induced expectations had no effect on hormone secretion. Their findings suggest that expectation and conditioning play different roles in different circumstances in the mechanism of the placebo effect: when conscious perception is involved, expectation is sufficient to override a conditioned placebo effect and the strongest placebo effects occur when conditioning and expectation interact synergistically (Benedetti et al., 2003b).

1.4 The placebo effect in Parkinson's disease

1.4.1 Results from clinical trials

Substantial placebo effects occur in PD, which are for the most part detected in placebo-controlled trials aimed at testing new pharmacological, surgical or physical therapies. For example, in a double-blind trial of pergolide, significant improvement with respect to baseline was seen in both the pergolide-treated group (30% after 24 weeks) and the placebo group (23% after 24 weeks) (Diamond et al., 1985). In the large clinical trial of Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP), 21% of patients demonstrated a blinded investigator-determined 'objective' improvement in motor function during placebo therapy over six-months (Goetz et al., 2002). Goetz and colleagues reported that 14% of the patients enrolled

in a six-month, randomized, placebo-controlled clinical trial of ropinirole monotherapy achieved a 50% improvement in motor function while on placebo treatment (Goetz et al., 2000). In this particular study all domains of parkinsonism were subject to the placebo effect, but bradykinesia and rigidity—those features of PD which are best correlated to dopamine function—tended to be more susceptible than tremor, gait or balance. Finally, in a meta-review, Shetty and colleagues demonstrated that 12 of 36 articles reported a 9-59% improvement in PD patient motor symptoms following placebo treatment (Shetty et al., 1999).

The importance of including a placebo group when investigating the efficacy of surgical procedures for treating PD has been emphasized (Freeman et al., 1999) but remains a source of controversy (Macklin, 1999;Weijer, 2002;London and Kadane, 2002). In a recent study on the effect of intrastriatal implantation of fetal porcine ventral mesencephalic tissue to treat PD (Watts et al., 2001), the degree of motor performance improvement at 18 months was substantial, but was the same in the sham group. In one multi-centre, randomized, double-blind, sham surgery-controlled study of human fetal transplantation for PD (Olanow et al., 2003), there was no significant clinical benefit of the transplant compared to sham surgery, even though pilot studies performed using identical technique had demonstrated substantial benefit (Hauser et al., 1999). Indeed, in another study of human fetal transplantation, both subjective and objective (blinded examiner) outcomes were better predicted by which treatment the patient thought s/he was assigned to rather than the actual treatment assignment (Freed et al., 2001;McRae et al., 2004). As previously mentioned, several factors could explain the differences in the magnitude of the placebo response between different trials. Variations in the information given to the patients, differences in group characteristics and/or the surgical procedures could contribute to a range of placebo responses. Naturally, ethical issues and consideration of the risks and benefits inherent in the conduct of the study will dictate whether or not a placebo treatment group is feasible.

Clinical results such as these have provided the impetus for experiments that aim to study the placebo effect itself. For example, Mercado and colleagues (2006) demonstrated that patients with subthalamic nucleus deep-brain stimulation (STN-DBS) as treatment for PD had a greater degree of improvement in their motor performance as measured by the Unified Parkinson's Disease Rating Scale (UPDRS) when they thought that their stimulators were turned on, and performed even worse when they thought that their stimulators were off, compared to the conditions in which they were blind to stimulator function (Mercado et al., 2006). Benedetti and colleagues used standard clinical measures to demonstrate that sham STN-DBS can improve bradykinesia (Pollo et al., 2002;Benedetti et al., 2003b), and also that saline given in the guise of apomorphine can reduce rigidity in patients conditioned to the effects of the active medication (Benedetti et al., 2004). However, it is equally important to emphasize that the clinical scales used for measuring motor function are subjective themselves.

1.4.2 Results from neuroimaging studies

Neuroimaging has been critical in establishing a clear physiological basis for the placebo effect in PD, and placebo studies represent an example of how imaging can enable significant strides to be made into areas where most previous investigation had depended on conjecture based on clinical observation. Using [^{11}C] raclopride (RAC) PET, de la Fuente-Fernandez and colleagues (2001) demonstrated that a placebo injection could induce the release of endogenous dopamine (DA) in the striatum of PD patients (de la Fuente-Fernandez et al., 2001b). In this study, patients underwent four PET scans and were aware that they would be receiving an injection of active drug (the DA receptor agonist apomorphine) for three of the scans and a placebo for one, but they were not told the scan order. The investigators found a substantial DA release in response to placebo, corresponding to a change of 200% or more in extracellular DA concentration and comparable to the response to amphetamine in subjects with an intact DA

system (Figure 1.2). Furthermore, the DA release in the motor areas of the striatum was greater in those patients who reported clinical improvement (i.e. placebo responders).

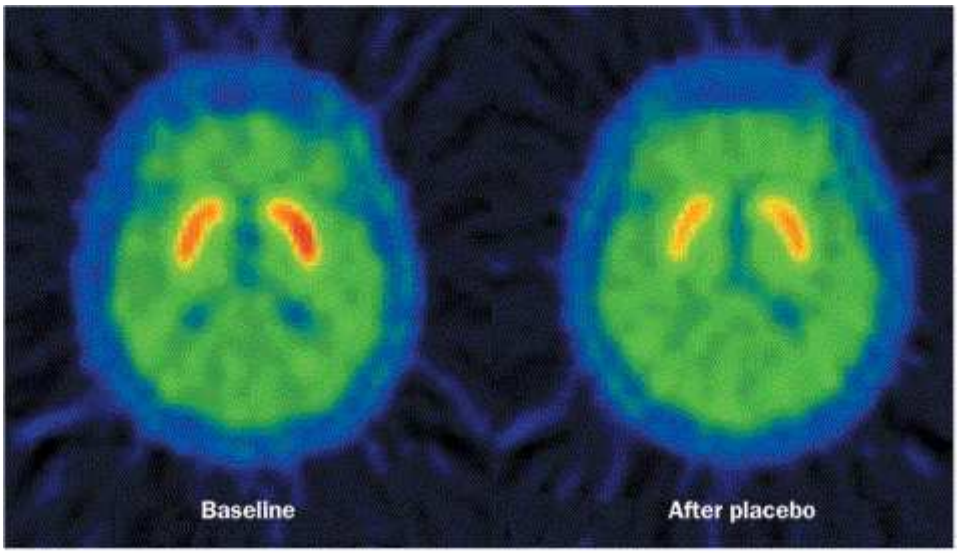


Figure 1.2 [^{11}C] Raclopride PET scans of a patient with Parkinson's disease at baseline (left) and following injection of saline (placebo, right). The observed decrease in RAC binding in the striatum in response to placebo, as evidenced by a decrease in signal, indicates tracer displacement by endogenous dopamine.

In a recent related study, Strafella and colleagues (2006) also used RAC PET to demonstrate striatal DA release in response to sham repetitive transcranial magnetic stimulation (rTMS) in PD patients. In this study, patients underwent two PET scans, one baseline scan in which no rTMS was used, and a placebo scan where they were told that they had a 50/50 chance of receiving either real rTMS or sham rTMS, but in all cases received the sham treatment. They found that the decrease in RAC binding was greater in the putamen contralateral to the more symptomatically affected side (Strafella et al., 2006). Although the patients who perceived clinical benefit had a slightly higher amount of DA release in the dorsal and ventral striatum, the difference failed to reach statistical significance. Taken together, these results indicate that the biochemical basis for the placebo response in PD is to replace the depleted DA in striatal areas that are responsible for motor symptoms. These results are corroborated by an electrophysiology study performed in PD patients undergoing STN-DBS surgery in which it was shown that a

placebo (saline injection) evoked changes in neuronal firing in the subthalamic nucleus (STN) in placebo responders. The neurons displayed a decrease in mean discharge frequency and a shift from bursting to non-bursting activity in response to placebo, which was correlated with a reduction in upper limb rigidity (Benedetti et al., 2004). The authors speculated that the change in STN neuron firing was a downstream effect of placebo-induced DA release in the striatum.

1.4.3 The reward expectation hypothesis of the placebo effect

What remains unclear is how a biochemical placebo effect is produced in the first place. Through prior experience with a particular treatment, expectations are generated about the resulting physical response to that treatment, in what Kirsch has termed 'response expectancy', which is proposed to be central in producing the physiological placebo effect (Kirsch, 1997). This may be particularly true in the case of PD, which is a chronic illness requiring several doses of medication per day, where patients frequently experience their doses taking effect and wearing off. These expectations become central in producing the physiological placebo effect, such as striatal DA release in PD patients and endogenous opioid release in placebo analgesia. Improvement in mobility in PD, mood in depression, and relief from pain can all be profoundly rewarding in their own right for an individual suffering from any of these conditions, while improvement in mobility or mood might not be seen as such in a healthy individual with normal function. In such cases, the placebo can be considered a rewarding stimulus, and would thus be capable of activating reward circuitry in the brain. This concept is in keeping with neuroimaging studies indicating that reward circuitry is activated in humans in response to "cognitive" rewards, including money (Thut et al., 1997), beautiful faces (Aharon et al., 2001), sports cars (Erk et al., 2002), pleasant music (Blood and Zatorre, 2001), humor (Mobbs et al., 2003), and romantic love (Aron et al., 2005).

In support of this hypothesis, placebos have been shown to stimulate reward pathways in the brain, and the ventral striatum in particular (de la Fuente-Fernandez et al., 2002;Scott et al.,

2008). In our previous study, the patients expected apomorphine for 3 out of 4 scans, thus they knew that their chance of receiving active drug was 75% for each scan. Although all patients in the study showed biochemical placebo responses, only half of the patients reported placebo-induced motor improvement. Those patients also released larger amounts of DA in the motor striatum, suggesting a relationship between the amount of striatal DA release and perceived clinical benefit. However, this relationship was not seen in the ventral striatum, where all patients displayed increased DA release regardless of whether they felt any improvement as a result of placebo administration (Figure 1.3) (de la Fuente-Fernandez et al., 2002).

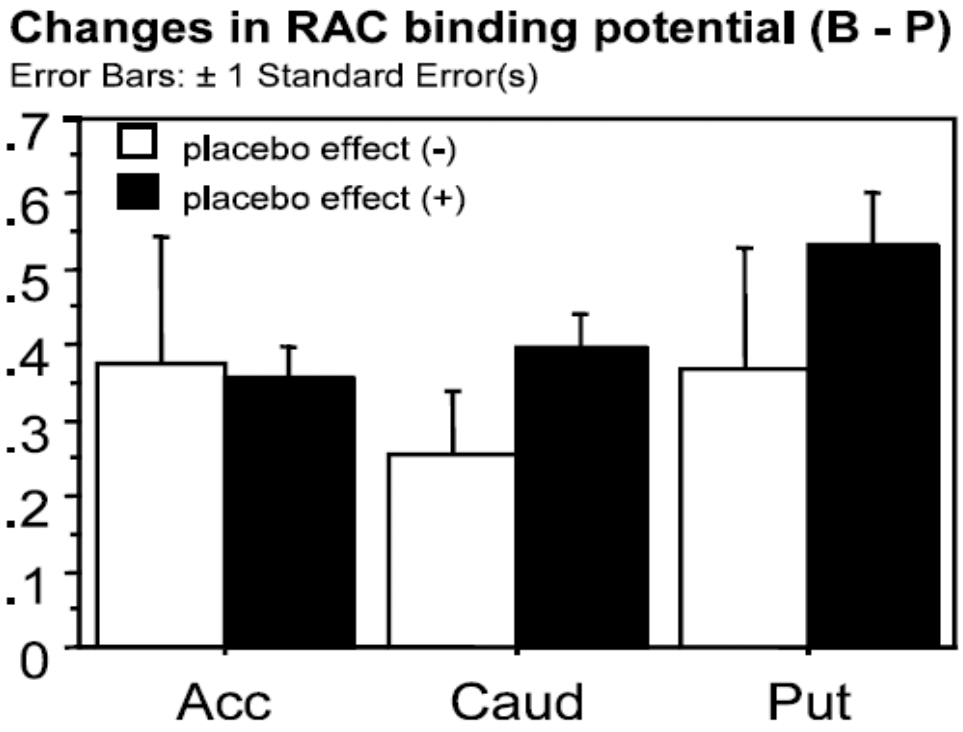


Figure 1.3 Placebo-induced changes in RAC binding potential in the ventral (nucleus accumbens, Acc) and dorsal (caudate nucleus, Caud; putamen, Put) striatum of 6 PD patients. The changes represent the difference in RAC BP between baseline (B) and post-placebo (P) values (i.e. B–P). In the Acc, there were no differences in placebo-induced RAC BP changes between patients who perceived a clinical benefit after placebo injection (solid bars, n=3) and those who did not (open bars, n=3) ($p=0.23$). In contrast, both in the caudate nucleus and putamen, this biochemical placebo effect was greater in patients who reported placebo-induced clinical benefit than in those without (caudate, $p < 0.05$, and putamen, $p < 0.01$).

Compared to the dorsal striatum, which is involved in voluntary movement, the ventral striatum is classically associated with motivation (Mogenson and Phillips, 1976;Berridge and Robinson, 1998;Ikemoto and Panksepp, 1999), goal-directed behaviour (Mogenson et al., 1980), and reward anticipation (Apicella et al., 1992;Fiorillo et al., 2003;Schultz et al., 1997;Schultz, 1998;Schultz et al., 1992). The investigators concluded that the DA released in the ventral striatum was associated with the patients' expectation of improvement in their symptoms, which could in turn be considered a form of reward. Thus, DA release in the ventral striatum can be seen as necessary but not sufficient for the placebo effect to occur. This is in keeping with other studies, in which ventral striatal DA release is better correlated with 'drug wanting' than the perceived subjective effects of the drug (Leyton et al., 2002;Evans et al., 2006). In depressed subjects, glucose metabolic increases were seen in the ventral striatum and orbitofrontal cortex following one week of fluoxetine or placebo treatment, prior to the onset of any antidepressant effect, which was interpreted to be a result of the expectation of improvement early in the trial (Mayberg et al., 2002;Benedetti et al., 2005). Related to this, placebo-induced increases in NAC metabolic activity have additionally been shown in healthy subjects and in cocaine abusers when a psychostimulant was expected instead (Volkow et al., 2003;Volkow et al., 2006). In pain, it has recently been shown that in addition to endogenous opioid release, DA release in the NAC is associated with the anticipated and subjectively perceived effectiveness of the placebo, as well as reductions in pain ratings (Scott et al., 2008). Furthermore, stronger placebo analgesic responses were associated with greater reward responsivity. Thus, converging evidence points to an important, global role of reward expectation and the associated brain structures – namely mesolimbic DA neurons and the ventral striatum – in the mechanism of the placebo effect (Figure 1.4). We have proposed that this "permissive component" interacts with a prefrontal cortical-driven component capable of activating disease-specific physiological changes, likely

mediated by brain structures involved in monitoring the emotional and physical internal state of the individual, such as the anterior cingulate and medial prefrontal cortices.

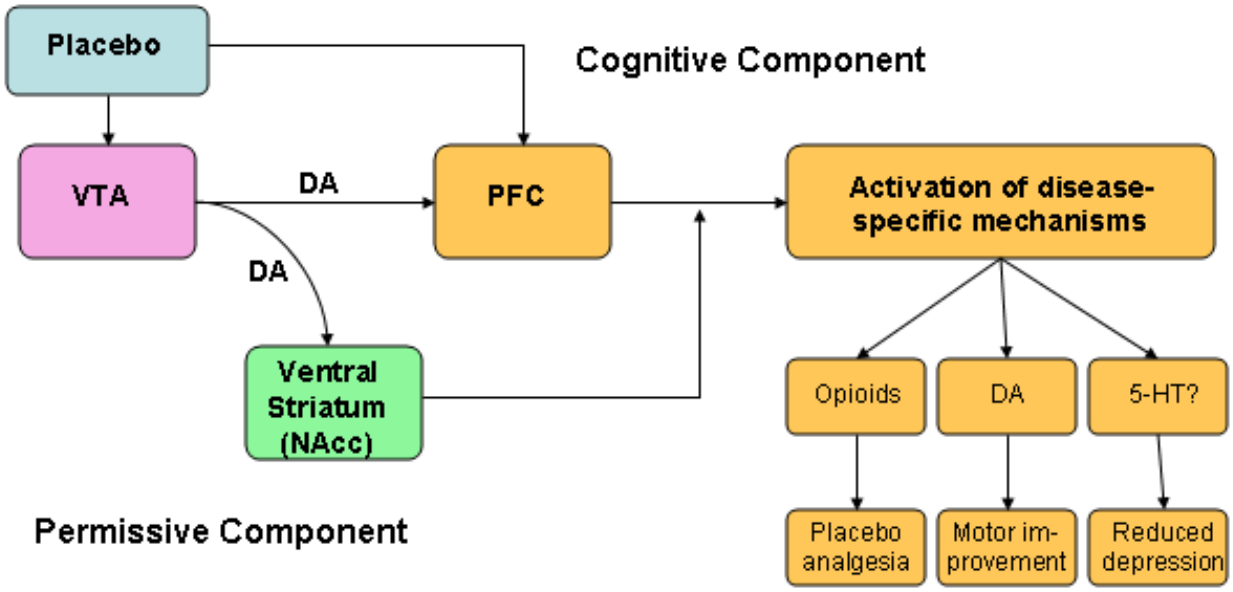


Figure 1.4 Theoretical schematic of the role of the reward circuitry in the placebo effect. DA-producing neurons arising in the ventral midbrain (ventral tegmental area, VTA) that send projections to the ventral striatum, are activated by placebo-induced expectation of clinical benefit, which is a form of expectation of reward. This represents the permissive component of the placebo response (green). Reward-activated mesocortical DA projections to the prefrontal cortex (PFC) modulate higher cognitive processing, which in turn activate downstream disease-specific mechanisms that mediate the different placebo responses, including a reduction in pain (placebo analgesia), improvement in motor performance in PD, and mood improvement. This pathway represents the cognitive component of the placebo effect (orange). The permissive component is common to all placebo effects, whereas the cognitive component is specific to the disease or condition of the patient. In this case, 'placebo' refers to the actual placebo itself as well as the environmental context in which the placebo is administered, which may also produce anticipatory effects due to conditioning.

1.5 Imaging the placebo effect in other conditions

1.5.1 Pain

Prominent placebo effects occur in many disorders other than PD. Much of the relevant research has occurred in the field of pain, where the investigator can recruit healthy subjects and induce various types of experimental pain. The first neurochemical evidence for the mechanism

of the placebo effect was published in 1978, when it was shown that placebo analgesia could be blocked by naloxone, indicating that it was mediated by endogenous opioids (Levine et al., 1978). Since then, several studies have further implicated endogenous opioids in the mechanism of placebo analgesia (Gracely RH et al., 1983;Levine JD and Gordon NC, 1984;Benedetti, 1996;Amanzio and Benedetti, 1999). Zubieta and colleagues (2005) used displacement of the μ-opioid receptor agonist PET tracer [^{11}C] carfentanil to indirectly demonstrate endogenous opioid release during placebo analgesia. The placebo (saline injection) was administered with the expectation of analgesia during a pain challenge, and found endogenous opioid release in the rostral ACC, dorsolateral prefrontal cortex (DLPFC), anterior insula, and the nucleus accumbens (NAC). In the high placebo responders, increased opioid transmission in the NAC was positively correlated with the subjective change in pain intensity ratings and reductions in the negative affective ratings experienced during the pain challenge. In the DLPFC, μ-opioid system activation was negatively correlated with the magnitude of the expected analgesic effect of the placebo rated before placebo administration, suggesting that a reduction in opioid inhibitory control in this region has a permissive effect on the engagement of other pain control regions, such as the insula, ACC, thalamus and/or midbrain (Zubieta et al., 2005). These results substantiate those of an fMRI study that separated the neural activations underlying pain anticipation and experience (Wager et al., 2004). The investigators used a well-established expectancy-manipulation paradigm (Voudouris et al., 1989;Montgomery and Kirsch, 1997;Price et al., 1999) to enhance belief in the placebo by surreptitiously decreasing the level of thermal pain when a topical placebo cream was applied on the forearm. Placebo treatment substantially decreased the subjects' reported pain and also the pain-related activity in the insula, contralateral thalamus, and ACC. During the expectation of analgesia, increased BOLD signal was observed in the DLPFC, orbitofrontal cortex (OFC) and ACC, as well as in the periaqueductal grey area (PAG) of the brainstem, and the PAG increases were positively correlated with DLPFC and OFC

activation. Given that the PAG is an area strongly linked to the descending control of pain and the endogenous opioid system, these results suggest that opioid systems are engaged by prefrontal cortically-driven expectations of analgesia (Benedetti et al., 2005). These data echo results of a PET study measuring regional cerebral blood flow (rCBF) during thermal pain in which remifentanyl or placebo was given, and it was shown that both interventions increased rCBF in the OFC and ACC and that these increases covaried with rCBF increases in the brainstem (PAG, pons and medulla) (Petrovic et al., 2002). Finally, a recent study used an expectancy manipulation paradigm similar to that reported by Wager *et al.* (2004) to investigate the BOLD signal changes during heat pain before and after placebo acupuncture (Kong et al., 2006), and observed significant differences in the anterior insula, lateral PFC, rostral ACC, and the inferior parietal lobule. The authors also found a negative correlation between the activity in the lateral/orbital PFC, rostral ACC, cerebellum, pons, and right fusiform and parahippocampal gyri, and the corresponding difference in subjective pain ratings, indicating that the stronger the placebo analgesia (i.e. lower pain ratings), the greater the activity in these brain areas (Kong et al., 2006). That these results contrast those of Wager et al. (2004), who found placebo-induced BOLD signal reductions in the thalamus, insula and ACC during pain, highlights the important issue of the intrinsic variability of the placebo response; although both studies used similar expectation-enhancement procedures, they used different placebos (cream vs. acupuncture), different methodologies, and the subjects were given vastly differing instructions in different environments and thus had different expectations. In light of these findings, it reasonable to postulate that there is a spectrum of placebo analgesic effects which engage endogenous opioid (Benedetti, 1996;Benedetti et al., 1999;Benedetti et al., 2003b) and also non-opioid (Gracely RH et al., 1983;Amanzio and Benedetti, 1999;Colloca and Benedetti, 2005) systems in varying degrees. However, as described, certain prefrontal cortical structures are involved consistently across placebo analgesia studies, including the superior medial PFC, midrostral dorsal anterior

cingulate, and the dorsolateral, ventrolateral and orbitofrontal cortices. Interestingly, these areas are frequently implicated in studies examining the voluntary regulation of affective responses (Benedetti et al., 2005).

1.5.2 Depression

Clinical trials of antidepressants have shown particularly strong placebo effects (Walsh et al., 2002), which can in some cases be indistinguishable from those of the active drug (Mayberg et al., 2002). Indeed, Kirsch and Sapirstein concluded from their meta-analysis of 19 trials of antidepressants that about 75% of the effectiveness of these drugs results from the placebo effect (Kirsch and Sapierstein, 1998). A recent, highly publicized meta-analysis also reported that the overall effect of new-generation antidepressant medications – including all but one of the most prescribed selective serotonin reuptake inhibitors – was indistinguishable from placebo, and below the recommended criteria for clinical significance with the exception of trials involving the most extremely depressed patients (Kirsch et al., 2008). Interestingly, this pattern was due to a decrease in the response to placebo rather than an increase in the response to medication.

Detecting true placebo responses in depression is complicated by the natural waxing and waning of symptoms in some patients, the difficulties in measuring improvement using rating scales, and the unavoidable confound of selecting patients who have had multiple different treatments and thus bring expectations and learning with them into the study (e.g. they know that antidepressant medications require more than three weeks to take therapeutic effect) (Benedetti et al., 2005). Despite these and other variables, some studies have successfully mapped out the placebo response in depressed patients. Mayberg and colleagues (2002) conducted an FDG-PET study that examined the brain regional glucose metabolism in response to fluoxetine or placebo treatment in a group of depressed men, where scans were acquired at baseline, one and six weeks following treatment. The PET data showed an overlap between the areas of metabolic change in the fluoxetine and placebo groups at six weeks, although the fluoxetine group had additional

areas not seen in the placebo group (Mayberg et al., 2000;Mayberg et al., 2002). This metabolic pattern was completely different in patients who received cognitive behavioural therapy, indicating that the physiological placebo response closely matches the active drug response that it is designed to simulate (and also that cognitive behavioural therapy is not simply a placebo). As discussed, this may also be the case in placebo analgesia (Petrovic et al., 2002) and in PD (de la Fuente-Fernandez et al., 2001b). However, Leuchter and colleagues (2002) used quantitative electroencephalography (EEG) to demonstrate that although depressed medication responders and placebo responders were virtually indistinguishable clinically, the placebo responders had changes in prefrontal cordance that were not seen in medication responders or in non-responders (to either medication or placebo). This suggests that the placebo response may depend on altered prefrontal activity early in therapy and that the placebo response was not functionally equivalent to the drug response (Leuchter et al., 2002). Thus, it remains unclear if placebo-derived improvements in depression share a common mechanism with the therapeutic effect of active treatment.

The effects of expectation of clinical improvement in depression have also been examined. In the study by Mayberg and colleagues (2002), at one week, before any clinical antidepressant effect was seen, both the fluoxetine and placebo groups demonstrated ventral striatal and OFC glucose metabolic changes, which were not seen in those patients who were ultimately drug non-responders. Since none of the patients in either group demonstrated any signs of clinical improvement at this time, the investigators interpreted these results as the expectation component of the subsequent antidepressant response (Benedetti et al., 2005). These data are supported by a recent EEG study conducted in depressed subjects which demonstrated that a positive clinical outcome appeared to be predicted in part by decreases in prefrontal EEG cordance that occurred during the first week of the clinical trial during the placebo lead-in phase, in the absence of drug (venlafaxine or fluoxetine) treatment (Hunter et al.,

2006). Although it is not possible to identify the specific brain areas involved, the authors suggested that early neurophysiological changes in prefrontal brain areas represent nonspecific changes that occur in response to the treatment environment, such as interactions with study personnel, pill administration, and structured assessments, shaping the expectations of the patient which have the capacity to influence the treatment outcome.

1.6 Conclusion

The placebo effect operates within the context of brain circuitry that enables semi-voluntary control over affective and physiological responses. Based on the evidence derived from PD, pain, and depression, it is clear that there is not one placebo effect but many, with different underlying mechanisms. However, they all have in common a component of expectation, which may well involve the DLPFC and dopaminergic activity in the ventral striatum. This could be considered a 'permissive' component, integrating motivational and reward-expectation circuitry enabling the belief that there will be improvement in one's symptoms (Lidstone et al., 2005). This state of expectation, driven by prefrontal cortical and limbic areas, may in turn trigger a downstream biochemical response specific to the condition in question; in the case of PD, DA release in the dorsal striatum, and in placebo analgesia, endogenous opioid release. Thus, 'permissive' refers to activation of the common element of reward expectation (or motivation) that is required to initiate the specific physiological component of the placebo effect. The degree of overlap between the mechanisms of placebo responses in different conditions is unknown, and it is likely that the placebo responses in most conditions involve the combined effects of many neurochemicals, including monoamines, opioids, serotonin and hormones. Indeed, a recent PET study demonstrated the combined actions of endogenous opioids and DA in the mechanism of placebo analgesia in healthy controls (Scott et al., 2008). It is interesting to note that in two of the studies mentioned here, the magnitude and location of the biochemical placebo effect correlated with symptomatic

improvement: 1) the DA release in the putamen of the dorsal striatum in the PD patients who perceived the most improvement in their motor functions (de la Fuente-Fernandez et al., 2001b), 2) the increased endogenous opioid release in the ACC, insula and NAC in subjects who experienced relatively less pain by measures of intensity, unpleasantness and affect (Zubieta et al., 2005). This suggests that the placebo effect goes where it is most needed, or serves a protective or adaptive function based on the environmental context. This could explain the high variability of the placebo response in that it is tailored to reflect the perceived needs of the individual, which differs greatly among subjects. Despite this heterogeneity, which can only be controlled to some extent in clinical trials, it is remarkable that based on the evidence to date, the neurochemical placebo effect appears to mirror the pharmacological effect which it is designed to mimic, as seen in Mayberg *et al.* (2002), Petrovic *et al.* (2002) and de la Fuente-Fernandez *et al.* (2001). This indicates a crucial role for the context, particularly the expectations generated by the environment, in the manifestation of placebo responses, whether it is a clinical trial or an experiment designed to study the placebo effect itself. As neuroimaging techniques continue to be refined and improved, researchers will continue to gain further insights into not only the mechanisms underlying the placebo effect, but also the larger fundamental processes of how environmental cues are integrated into the regulation of thoughts, emotions and physiological state for our behaviour and survival.

1.7 Research Objectives

The main purpose of this work was to further explore the reward hypothesis of the placebo effect using PD as a model. Specifically, the objective was to better understand the relationship between the expectation of therapeutic benefit and DA release in the striatum in PD patients. PD is a good model in which to study this aspect of the placebo effect for two reasons: first, both components of the placebo response (i.e. the permissive/reward-related and cognitive-

related) involve DA in the striatum, and can therefore be measured directly using RAC PET, and 2) the clinical placebo effect in PD (i.e. improvement in motor symptoms) can be measured objectively by a blinded examiner, in addition to any subjective measures reported by the patient. Thus, the placebo effect can be quantified at three levels in Parkinson's; biochemically (PET data), objectively (clinical scales), and subjectively (patient self-report).

In order to generate placebo effects in the patients, it was necessary to use deception to manipulate their expectations. This was achieved in two ways. For the first study (Chapter 3), an overt/covert paradigm was used in PD patients who had undergone surgery for subthalamic nucleus deep-brain stimulation (STN-DBS). This patient population is ideal for overt/covert treatment as it is theoretically possible to adjust the stimulators without the patient's knowledge, so a true placebo effect can be detected (and conversely, the true therapeutic effect can also be detected). In the second study (Chapter 5), expectations were manipulated using explicit verbal instructions. The subjects were told that they had a certain probability of being given active medication (levodopa) when in fact they were given placebo. This enabled the quantification of the synaptic DA released in response to specific strengths of expectation, as well as the mathematical characterization of the relationship in different striatal subregions i.e. monotonic (implying the stronger the belief, the greater the DA release), or inverted-U (implying that the greater the uncertainty, the greater the DA release).

Both of these experiments involved the use of RAC PET, and at the time this research was conducted, the research centre was implementing a new, ultra-high resolution (HRRT) PET scanner. Thus, the first study was conducted on the lower-resolution tomograph (ECAT), and the second study was conducted on the HRRT, which was in fact the first human study in the centre to use this new PET camera. Due to the substantial differences in the nature of the data collected the methodology that had previously been used for analyzing the ECAT images could not be directly applied to the HRRT data. Therefore, the second objective of this work was to

develop and implement the framework and methods for the analysis of the higher resolution PET data (Chapter 4). This required developing an in-depth understanding of the physical and computational aspects of PET data, and using neuroscience to guide the development and refinement of the imaging analysis methods.

CHAPTER II General introduction: dopamine, reward and Parkinson's disease

2.1 Basal Ganglia

2.1.1 Introduction

The basal ganglia are a highly interconnected group of subcortical forebrain nuclei that include the striatum (caudate and putamen), globus pallidus (internal and external segments), the subthalamic nucleus, and the substantia nigra (pars compacta and pars reticulata) (Parent, 1990). These nuclei work in concert with the cortex to organize and execute goal-directed behaviours requiring the integration of motor, cognitive and limbic circuits. Cortical inputs first converge in the striatum, then are subsequently processed through the series of remaining nuclei via distinct pathways and projected back to the cortex via the thalamus. The basal ganglia are best known for their role in voluntary motor control, forming the 'extrapyramidal' motor system (in contrast to the descending corticospinal or 'pyramidal,' system). In addition to the control of movement, the basal ganglia are involved in several other aspects of goal-directed behaviour, namely the processes that lead to the initiation of movement, including the elements that drive actions such as motivation, emotions and cognition (Haber and Gdowski, 2004). Ventral areas of the basal ganglia play a key role in reward and reinforcement and the development of addictive behaviours and habit formation. More central basal ganglia regions are involved in cognitive functions, such as procedural learning and working memory. Finally, the dorsolateral areas of the striatum are involved in motor control. Given their involvement in these various domains, it follows that several diseases affecting mental health, such as schizophrenia, obsessive compulsive disorder, and addiction, as well as diseases that affect motor function, such as Parkinson's disease and Huntington's are associated with basal ganglia pathology.

2.1.2 Striatum

The striatum is the major input structure of the basal ganglia and derives its afferent input from three major sources: the cerebral cortex, the thalamus and the brainstem. Glutamatergic

projections from virtually all cortical areas converge onto striatal neurons, of which the major class are medium spiny projection neurons (MSNs) that use GABA as a neurotransmitter (Oorschot, 1996). The striatum also receives a heavy dopaminergic innervation from the midbrain, as well as serotonergic inputs from the dorsal raphe nucleus and noradrenergic innervation from the locus coeruleus (Blandini et al., 2000). Based on connectivity and histological and functional considerations, the anterior ventral-most extension of the striatum is referred to as the ventral striatum, and includes the nucleus accumbens (NAC), the medial and ventral portions of the caudate and putamen, and the striatal cells of the olfactory tubercle (Kunishio and Haber, 1994;Selemon and Goldman-Rakic, 1985). The dorsal striatum includes the remaining regions of the caudate and putamen. While the ventral and medial borders of the ventral striatum are relatively clear, its dorsolateral borders merge imperceptibly with the dorsal striatum proper, as the cellular composition is fairly homogeneous and can only be distinguished histochemically (Haber, 2003). The dendritic arbours of MSNs are among the most densely spined neurons in the brain, suggesting that they are specialized for integrating information from several different sources simultaneously. For example, the primate NAC receives afferent input from the hippocampal formation, the entorhinal, olfactory, anterior cingulate and orbitofrontal cortices, the insula, the thalamus, the amygdala, the substantia nigra, as well as intrinsic cholinergic input from aspiny neurons (Heimer et al., 1997). Cortical glutamatergic inputs terminate mainly on the heads of the spines, thalamic inputs terminate mainly on the shafts of spines, and dopaminergic inputs also terminate on the shafts so as to modulate cortical inputs (Haber and Gdowski, 2004) (Figure 2.1).

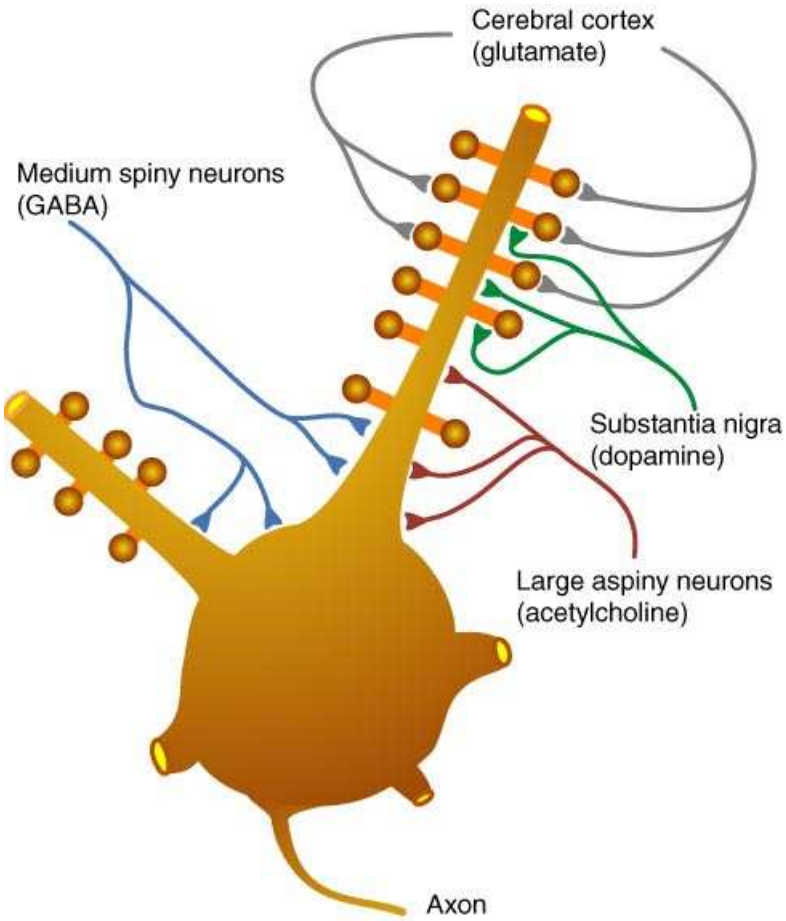


Figure 2.1 Cartoon of the pattern of termination of afferents on an MSN in the striatum. The soma and the proximal dendrites with their spines are shown. Midbrain DA afferents (green) from the substantia nigra form synapses on the necks of dendritic spines, placing them in a unique position to modulate glutamatergic cortical afferent input (grey). MSNs also receive intrinsic input from other MSNs (blue) and cholinergic interneurons (red). Adapted from Mink, 2003.

Striatal output is to the globus pallidus and the substantia nigra, pars reticulata (SNpr), which in turn project to the thalamus, which then projects back to the cortex, completing cortico-striatal-thalamo-cortical loops. The functional topography of the frontal cortex is preserved within these cortical-basal ganglia loops, creating a series of circuits which are functionally distinct, yet operate in parallel to control the various aspects of goal-directed behaviour (Alexander et al., 1986) (Figure 2.2). These parallel loops contain reciprocal connections with functionally similar brain regions to maintain these functional networks, but are also connected by axon collaterals at each level and thereby communicate with regions associated with different

cortical-basal ganglia circuits. Such a parallel, integrated organization enables the transfer of information between circuits (for example, limbic, to cognitive, to motor) so that the organism can continuously update and modify goal-directed behaviours (Haber, 2003).

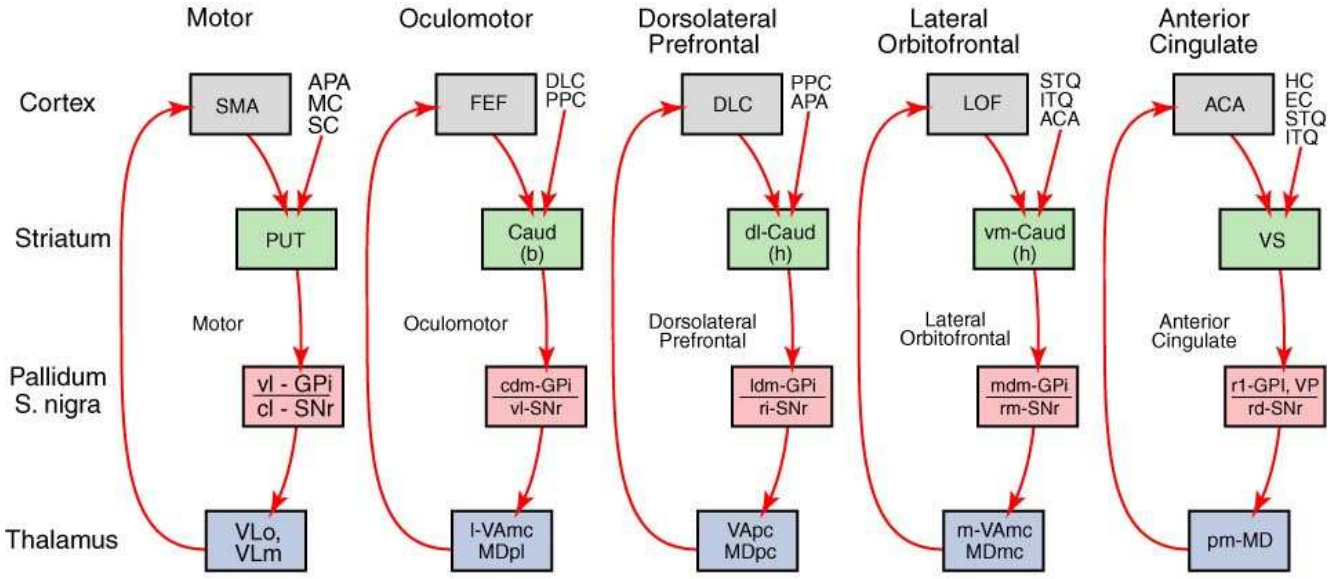


Figure 2.2 Hypothetical parallel segregated circuits connecting the basal ganglia, thalamus and cortex. The circuits are named according to the primary cortical target of the output from the basal ganglia. Striatal subregions are contained in the green boxes. In reality, the loops are not fully segregated but connected via axon collaterals to allow information flow between functional units. ACA, anterior cingulate area; APA, arcuate premotor area; CAUD, caudate; b, body; h, head; DLC, dorsolateral prefrontal cortex; EC, entorhinal cortex; FEF, frontal eye fields; GPi, internal segment of globus pallidus; HC, hippocampal cortex; ITG, inferior temporal gyrus; LOF, lateral orbitofrontal cortex; MC, motor cortex; MDpl, mediulis dorsalis pars paralarnellaris; MDme, medialis dorsalis pars magnocellularis; MDpc, medialis dorsalis pars parvocellularis; PPC, posterior parietal cortex; PUT, putamen; SC, somatosensory cortex; SMA, supplementary motor area; SNr, substantia nigra pars reticulate; STG, superior temporal gyrus; VAmc, ventralis anterior pars magnocellularis; Vapc, ventralis anterior pars parvocellularis; VLm, ventralis lateralis pars medialis; VLo, ventralis lateralis pars oralis; VP, ventral pallidum; VS, ventral striatum, cl, caudolateral; cdm, caudal dorsomedial; dl, dorsolateral; 1, lateral; ldm, lateral dorsomedial; m, medial; mdm, medial dorsomedial; pm, posteromedial; rd, rostrodorsal; rl, rostrolateral; rm, rostromedial; vm, ventromedial; vl, ventrolateral. Adapted from Mink, 2003.

2.2. Dopamine

2.2.1 Dopamine systems

The mammalian brain receives its entire dopamine (DA) supply from a population of neurons whose cell bodies reside in the ventral tegmentum, substantia nigra and retrorubral cell groups of the midbrain and send widespread forebrain projections to diverse efferent structures (Fuxe et al., 1970). These neurons are of tremendous clinical importance due to their diverse projections and ability to modulate excitatory synaptic activity in several different brain areas. Indeed, neurological and psychiatric disorders such as Parkinson's disease, Huntington's disease, schizophrenia, Tourette's syndrome, and drug addiction are all intimately associated with dysfunction of either midbrain DA neurons themselves, or of brain regions that receive heavy dopaminergic input. Midbrain DA neurons are typically divided into three major systems based on morphology, anatomical origin and connectivity. These underlying neuroanatomical differences confer each system with specific functional roles: the mesolimbic system is involved in reward and motivation, the mesocortical system is involved in cognition and higher cortical processing, and the nigrostriatal system is involved in voluntary movement and sensorimotor integration (Haber and Gdowski, 2004). In addition to these systems, a DA pathway that terminates in the thalamus has also been identified that originates from the hypothalamus, periaqueductal grey matter, ventral mesencephalon and the lateral parabrachial nucleus. This was reflected in immunolabeling of the human and macaque monkey thalamus by dopaminergic markers tyrosine hydroxylase, DA and DA transporter (Sanchez-Gonzalez et al., 2005).

The DA pathways originate in subdivisions of the substantia nigra (SN) and the adjacent ventral tegmental area (VTA). The SN, named after the dark melanin pigment that is formed by the intracellular auto-oxidation of DA, is subdivided into the pars compacta (SNpc) and pars reticulata (SNpr) (Halliday and Tork, 1986). It is difficult to visualize a clear demarcation between the SNpc and SNpr in primates, due to the invasion of DA neurons and their dendritic

arbours into the SNpr (Haber and Fudge, 1997). The SNpc merges with the medially-adjacent DA cell groups of the VTA and collectively, these midbrain DA neurons are divided into dorsal and ventral tiers. The cells of the dorsal tier include both the dorsal SNc and the contiguous VTA, and the cells of the ventral tier include the densocellular and ventral groups of the SNc. In primates, the dorsal tier DA neurons project to the ventromedial striatum, the bed nucleus of the stria terminalis, septal nuclei, the amygdala, the prefrontal cortex (PFC), and the hippocampus. Thus, dorsal tier neurons project primarily to limbic and cognitive areas of the cortex, producing the mesolimbic and mesocortical DA systems. The ventral tier neurons project throughout the motor striatum (with the exception of the ventral striatum), but have sparse cortical projections, and thus make up the nigrostriatal system.

2.2.2 Dopamine neuron physiology

Results from individual experiments using different methods suggest that DA plays a role in behavioural functions as diverse as movement, reward, punishment, salience, learning, cognition, and many other processes, which may be related to the different time courses of DA signaling (Schultz, 2007). DA neurons show two predominant patterns of firing activity termed tonic and phasic (Grace, 1991). The phasic and tonic firing of DA neurons influence extracellular DA concentrations differently (in different locations) and are controlled by different mechanisms. Tonic activity consists of a regular spike firing pattern of 1–6 Hz that DA neurons usually exhibit in the absence of salient stimuli (Grace and Bunney, 1984;Schultz et al., 1997). Tonic firing patterns maintain basal extracellular levels of DA in afferent regions, and can be affected by visceral stimuli that can moderately increase or decrease efferent DA levels to provide a “tone” on DA receptors (Grace, 1991). These levels recorded using in vivo microdialysis are on the order of 0.3 to 15 nM in the striatum and PFC (Lapish et al., 2007). Tonic firing contributes more to extrasynaptic DA levels, and is modulated by presynaptic limbic and cortical glutamatergic inputs (Grace, 1991;Howland et al., 2002). In addition, the population

activity of VTA DA neurons may also affect tonic DA levels (Grace, 1991). Phasic activation of DA neurons increases their firing rates to 20 Hz (Grace and Bunney, 1984), which results in significant and long-lasting increases in extracellular DA concentrations (Phillips et al., 2003). Phasic firing is thought to contribute mainly to synaptic (as opposed to extrasynaptic) DA levels, is mediated primarily by bursting events at the level of the cell body (Grace, 1991) and is believed to lead to a much larger DA release than when these neurons fire in a slow, irregular single spike mode (Floresco et al., 2003). The time course (seconds) and localization of DA released by burst firing is restricted by a high-affinity and rapid reuptake system. This bursting activity is thought to represent a key component of reward signaling (see Section 2.3).

2.2.3 Dopamine receptors

Post-synaptically, DA exerts its modulatory effects through binding to metabotropic G-protein coupled receptors, of which there are five subtypes, termed D_1 through D_5. The classification scheme is based upon the DA receptor's ability to either stimulate (D_1-and D_5) or inhibit (D_2, D_3, D_4) adenylyl cyclase activity. In the striatum, stimulation of DA receptors on MSNs initiates intracellular signaling cascades that 'excite' or 'inhibit' MSNs by modulating the gating and trafficking of voltage-dependent and ligand-gated (ionotropic) ion channels embedded in the dendritic membrane, thereby dictating the way in which MSNs respond to glutamatergic cortical input. In this way, DA receptors exert a regulatory influence over the glutamate-mediated 'tone' in the striatum (Onn et al., 2000). Through stimulation of adenylyl cyclase, D_1 and D_5 DA receptors activate cyclic AMP (cAMP) second messenger pathways, leading to intracellular kinase signaling cascades resulting in the transcription of genes that encode the membrane proteins and enzymes necessary to mediate long-term changes in intrinsic plasticity (e.g. changes in properties of ionic channels on dendrites that regulate neuronal excitability), and ionotropic and metabotropic glutamate receptor-mediated changes in synaptic plasticity (Seamans and Yang, 2004). Activation of D_2, D_3, and D_4 DA receptors suppresses

cAMP, one effect of which is to inactivate glutamate NMDA receptors on the post-synaptic membrane. *In situ* hybridization of D_1 and D_2 receptor mRNA indicates highest levels of both receptors in the striatum, NAC and olfactory tubercle. D_2 receptor mRNA is also found in high levels in midbrain DA neurons where it presumably encodes autoreceptors. D_3 receptors are found in lower levels in the striatum, but higher in the NAC than D_2 receptors (Sealfon and Olanow, 2000).

2.2.4 Dopamine and the basal ganglia

DA plays a critical modulatory role in the cortical-basal ganglia circuits. It can be broadly generalized that DA acts as a neuromodulator – rather than a mediator – of excitatory synaptic neurotransmission. In the striatum, which is the major input structure of the basal ganglia, axon terminals from nigrostriatal DA neurons form asymmetric synapses with excitatory corticostriatal afferents on the necks of dendritic spines on intrinsic striatal medium spiny neurons (MSNs). At least two types of MSNs have been identified which express different profiles of DA receptors: striatonigral, 'direct' pathway medium spiny neurons express high levels of D_1 receptors and project directly to neurons that interface between the basal ganglia and the rest of the brain, namely, neurons of the SNpr and internal segment of the globus pallidus (GPi). In these neurons, GABA is co-expressed with substance P and dynorphin. By contrast, striatopallidal, 'indirect' pathway medium spiny neurons express high levels of D_2 receptors (together with GABA and enkephalin) and their axons project to the external segment of the globus pallidus (GPe), whose neurons in turn project to the interface nuclei (SNr and GPi), either directly or via the subthalamic nucleus (Surmeier et al., 2007)(Figure 2.3). However, the pathways may not be totally segregated, as collateral axonal branches have been demonstrated (Smith et al., 1998).

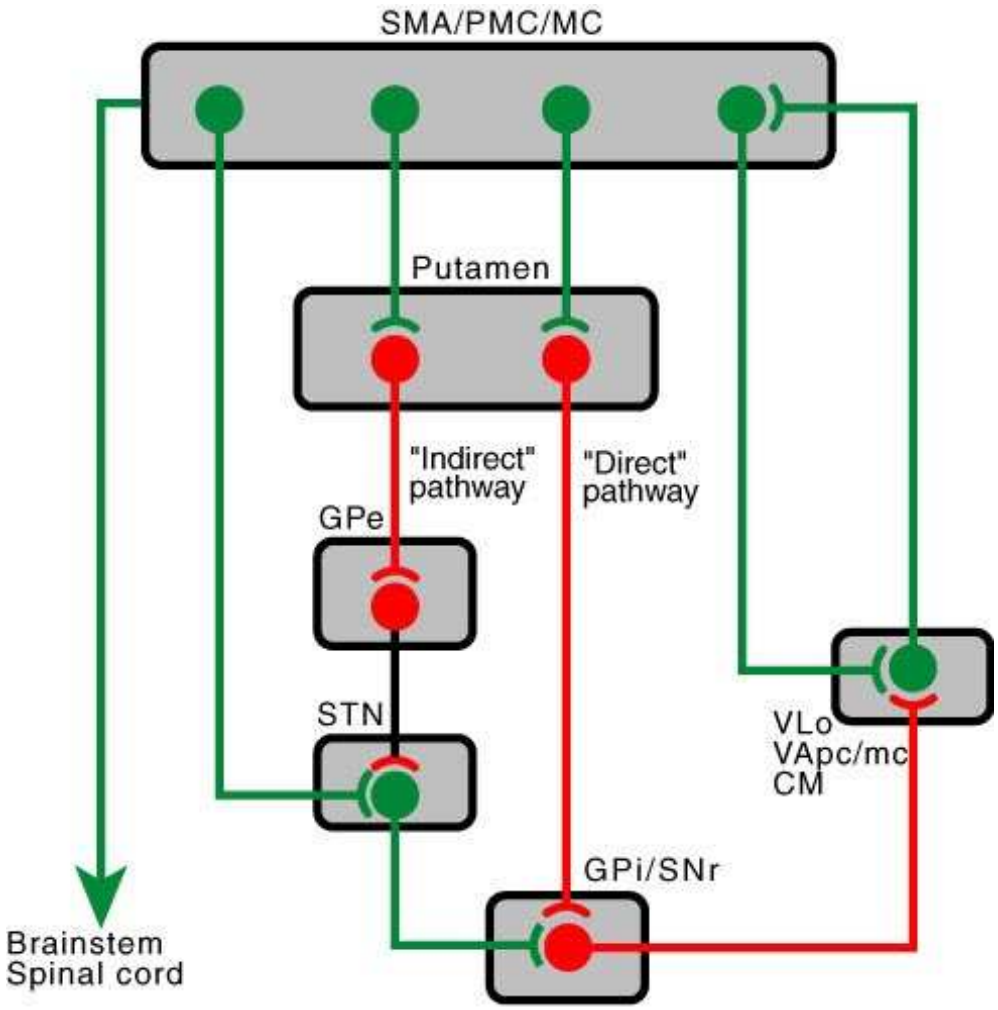


Figure 2.3 Simplified schematic of the intrinsic anatomy of the motor cortico-striatal-thalamo-cortical circuit containing the direct and indirect pathways. Red arrows indicate inhibitory (GABA–ergic) connections; green arrows, excitatory (glutamatergic) connections. CM indicates centromedian nucleus of thalamus; GPe, external segment of the globus pallidus; GPi, internal segment of the globus pallidus; MC, primary motor cortex; PMC, premotor cortex; SMA, supplementary motor area; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; VApc/mc, ventral anterior nucleus of thalamus pars parvocellularis; VLo, ventrolateral nucleus of thalamus pars oralis. Adapted from Mink, 2003.

Overall, the two output pathways are hypothesized to have opposing effects on motor function, where activation of neurons from the direct and indirect pathways respectively facilitate and suppress thalamic drive to the cortex. The two pathways are thought to be in balance such that increased activity in the direct pathway causes decreased GPi/SNpr output and increased activity in the indirect pathway causes increased GPi/SNpr output. By adjusting the balance, the activity of cortical targets of the basal ganglia can be modulated up or down. With respect to the well-studied motor-loop, it has also been proposed that the direct pathway is involved in fine motor control and complex motor functions, and the indirect pathway exerts a more generalized control over gross motor function (Onn et al., 2000). Yet another hypothesis is that DA exerts a focusing effect on MSNs in the striatum, the net effect of which is favoring the processing of one of many competing motor programs issued from the primary motor cortex and associated cortical regions through the basal ganglia circuits. In this way, the output of the basal ganglia acts focally to select desired motor mechanisms and broadly inhibit competing motor mechanisms to allow movement to proceed without interference (Mink, 1996). It is clear that DA modulates glutamatergic effects on corticostriatal inputs to the striatum, although it is not precisely understood how DA modulates the direct and indirect pathways. The classic model proposes that DA has a dual effect on striatal neurons, causing excitation of D_1 receptor-bearing neurons in the direct pathway and inhibiting D_2 receptor-bearing neurons of the indirect pathway (Obeso et al., 2000). However, the growing evidence that D_1 and D_2 receptors co-localize on the same MSNs (Aizman et al., 2000), as well as the fact that DA neurons also innervate extrastriatal targets within the basal ganglia indicates that this model is overly simplistic. In any case, disorders in which brain DA levels are severely compromised, such as Parkinson's disease, compellingly show the critical role that DA plays not only in modulating the motor functions of the basal ganglia, but also the executive and emotional functions, which can also be abnormal in Parkinson's.

2.3 Reward Processing in the Mammalian Brain

2.3.1 Introduction

In addition to modulating the circuits controlling voluntary movement, midbrain DA neurons have several other functions in the brain, including motivation and reward processing. 'Rewards' are defined as stimuli which, when administered to an organism following a correct or desired response, produce repeated approach behaviours or the repetition of responses (Bishop M.P. et al., 1963;Olds and Milner, 1954). Thus, a reward is an operational concept used to describe the positive value that an organism attributes to an object, behaviour or internal physical state (Breiter and Rosen, 1999). The ability of an organism to detect, approach, and interact with (i.e. consume, in the case of food rewards) the rewarding stimuli in its environment is a fundamental component of goal-directed behaviour, and requires the integration of cognitive, motivational and motor circuits, in which DA plays a crucial modulatory role. The mesocorticolimbic DA system and its terminal areas including the orbitofrontal cortex, ventral striatum, amygdala, and others, are particularly associated with reward processing in the brain.

2.3.2 Reward signaling by dopamine neurons

Electrophysiological studies in non-human primates demonstrate that 65 to 80% of DA neurons (primarily in the VTA) show phasic activation in response to primary liquid and food rewards, visual, auditory and somatosensory reward-predicting stimuli, and intense, novel stimuli (Horvitz, 2000;Schultz, 2000;Ljungberg et al., 1992). Rather than signaling the absolute presence of a reward, DA neuron activity has been proposed to code the discrepancy between the predicted reward and the actual reward, which is termed the 'prediction error' (Mirenowicz and Schultz, 1994;Schultz, 1998). Thus, DA neurons are activated when rewards occur without being predicted or are better than expected, and are depressed when predicted rewards are omitted or are worse than predicted. DA neurons are therefore able to produce a global teaching signal that can be broadcast to widespread striatal and prefrontal cortical structures indicating the

availability of environmental stimuli with positive motivational value to the organism (Schultz 2007). The phasic responses of DA neurons are consistently stronger to either rewards or reward-predicting conditioned stimuli that are associated with higher reward magnitude, probability, and expected value (Fiorillo et al., 2003;Tobler et al., 2005). Following training, and if reward magnitude is kept constant, phasic DA cell firing varies monotonically with the probability of reward delivery, i.e. DA neuron activity increases linearly with increasing probability (p=0 to p=1) (Figure 2.4, centre panel), or according to utility theory, the expected value of the reward, defined as the product of the probability or reward delivery and the magnitude of the reward (Figure 2.4, left panel).

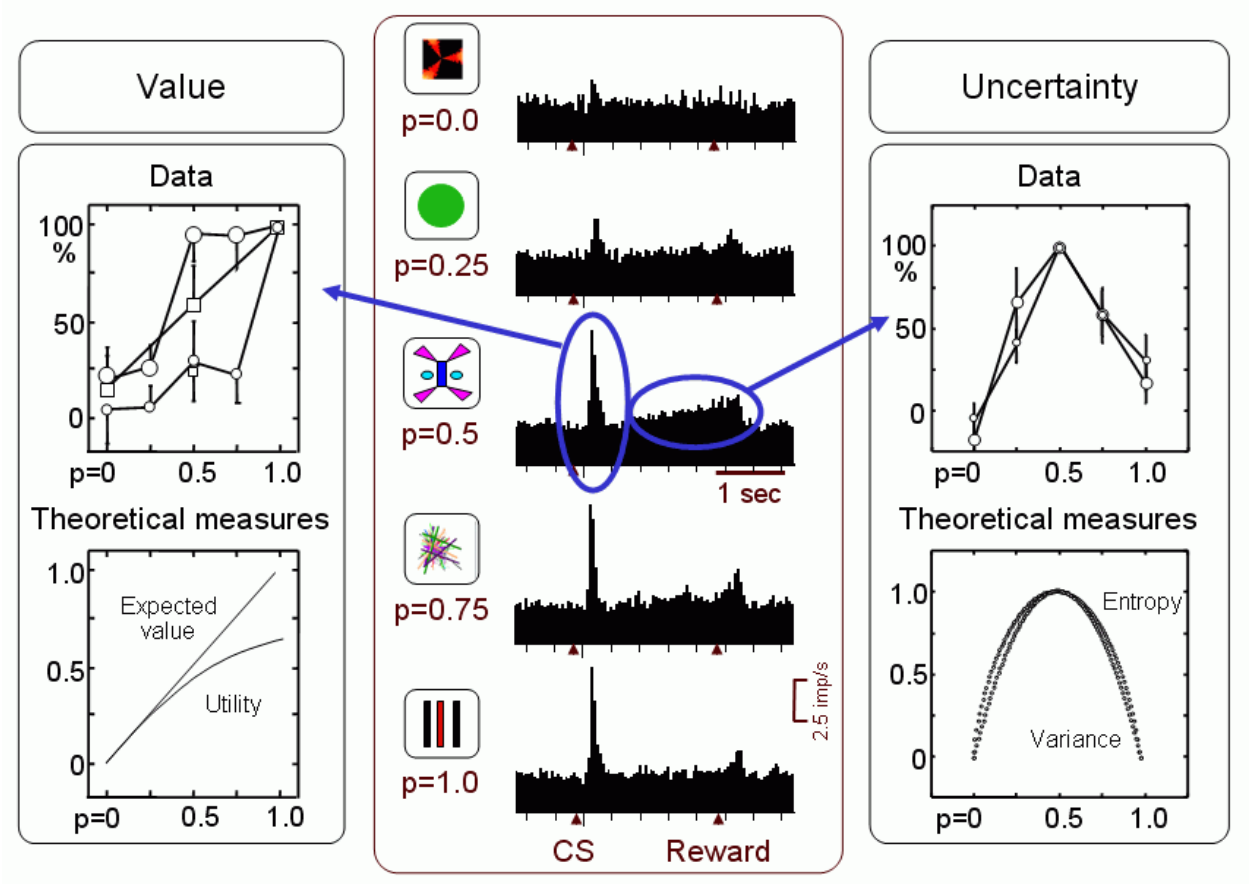


Figure 2.4 Separate coding of reward value and uncertainty by DA neurons in non-human primates. Five different conditioned stimuli predict all-or-none reward at different probabilities. Centre panel: Averaged neuronal population responses in two monkeys. The initial, phasic response to the conditioned stimulus (CS) increases monotonically with the probability of the reward predicted by the CS (increasing from top to bottom). Left panel: The nearly monotonic increase in the population responses for several stimulus sets ('Data') may encode expected value

or utility (below). The more sustained response between CS and reward (centre) encodes uncertainty by showing a peak at p=0.5. Right panel: Sustained population response (top) co-varying with entropy and variance (and standard deviation) (bottom; entropy scale in bits, variance scale normalized to maximum). Adapted from http://www.scholarpedia.org/article/Reward_signals.

Computational neuroscientists have summarized the error signal carried by DA neurons into a mathematical temporal difference model (Montague et al., 1996). This algorithm is essentially a value function that relates the expected value of a reward at a particular point in time to the time-discounted sum of rewards that can be earned in the infinite future (McClure et al., 2003). If a reward occurs after that point in time that is better than the initial prediction of the expected value, then the algorithm produces a positive result (positive error prediction), and the converse occurs if the second reward is worse than the prediction (negative error prediction). According to this model, DA serves two functions in reward signaling: it is required for learning to predict future rewards as well as being involved in biasing action selection toward situations predictive of future reward (McClure et al., 2003). Another (and not mutually exclusive) influential hypothesis of DA function in reward is the incentive salience hypothesis, which states that DA's role in reward is the attribution of incentive value to stimuli in the environment or behavioural acts. Berridge and Robinson describe this process as the transformation of a "liked" stimulus into a "wanted" stimulus that drives the organism to engage in goal-directed, motivated behaviour (Berridge and Robinson, 1998). This hypothesis has recently been incorporated with the temporal difference model and reward prediction by McClure *et al.* (2003). Finally, the phasic responses of DA neurons in response to salient stimuli have also been proposed to serve a 'bottom-up' function of alerting the organism to attend to stimuli of interest. Based on the finding that efferent projections from the superior colliculus likely convey early visual input to nigrostriatal DA neurons, Redgrave and Gurney (2006) proposed that the resulting phasic activity in DA neurons serves an alerting function to the organism, switching attentional and

behavioural selections to unexpected, behaviourally important stimuli (Redgrave and Gurney, 2006).

In the natural environment, rewards usually occur with some degree of uncertainty. If the reward value is held constant, and if an animal is trained to associate certain conditioned stimuli with discrete probabilities (p) of reward delivery, more than one third of DA neurons show a relatively slow, sustained and moderate activation between the onset of the reward-predicting stimulus and the delivery of the reward (Figure 2.4, centre panel) (Fiorillo et al., 2003). These tonic DA responses are maximally active at $p = 0.5$, decline both at $p = 0.25$ and $p = 0.75$, and are virtually zero at both extremes of the probability distribution ($p = 0$ and $p = 1$) (Figure 2.4, left panel). This response was hypothesized to reflect the uncertainty associated with reward expectation, as uncertainty can be expressed as the variance, or entropy of the probability distribution, which is an inverted-U-shaped function with a peak at $p = 0.5$ (intuitively, it can be understood that an outcome is most uncertain when the likelihood of its occurrence is 50%, and most certain to occur or not occur, at 100 and 0%, respectively). These findings have recently been extended to humans using functional magnetic resonance imaging (fMRI); although DA neuron activity could not be assessed directly, midbrain BOLD signals tracked the error prediction signal transiently and demonstrated more sustained activity that correlated with uncertainty (Dreher et al., 2006). Direct measuring of DA levels in animals using microdialysis has provided another hypothesis for sustained increases in DA during reward paradigms. Mogenson and Phillips (1976) were the first to propose that DA played a role in motivated behaviour (Mogenson and Phillips, 1976), and this hypothesis has expanded into the current incentive motivation hypothesis of DA function, that relates tonic increases in synaptic DA to the current motivational state of an organism (Fibiger and Phillips, 1986;Ikemoto and Panksepp, 1999;Phillips et al., 2007). Regardless of the mechanism, through different temporal profiles of neural activity, DA neurons have the capacity to code the occurrence, expectation, and

uncertainty or saliency of reward-related stimuli as the organism learns to optimize motivated, goal-directed behaviours.

2.3.3 Dopamine and reward expectation

DA release in the ventral striatum, which is the principle target of mesolimbic DA neurons, is associated with reward prediction and incentive motivation, as has been extensively demonstrated in animals (Phillips et al., 1989;Schultz et al., 1992;Schultz et al., 1997;Schultz, 1998;Garris et al., 1999;Phillips et al., 2003). The phasic burst firing of DA neurons occurs on the millisecond time scale, while the tonic firing occurs over seconds, or perhaps minutes, and so these different properties of DA neurons are difficult to demonstrate in humans using the slower time resolution of most neuroimaging techniques. Despite these limitations, the expectation of rewarding stimuli has been associated with an increased BOLD signal in the ventral striatum as measured by fMRI in several studies (Breiter and Rosen, 1999;Delgado et al., 2000;Pagnoni et al., 2002;Dreher et al., 2006;Elliott et al., 2000;Knutson et al., 2001a). An fMRI study in cocaine addicts showed increased activation of the NAC during the pre-infusion period for both saline and cocaine, in which there was a 50% expectancy condition for receiving cocaine (Breiter and Rosen, 1999;Breiter et al., 1997). The fact that the same degree of activation occurred during the pre-infusion period in both conditions indicates that the NAC activity may reflect a computation of expectancy. Volkow and colleagues (2006) used fluorodeoxyglucose (FDG) PET to measure brain glucose metabolism during the expectation of receiving methylphenidate in drug naive subjects. The subjects were told that methylphenidate could be experienced as pleasant, unpleasant or devoid of subjective effects to eliminate specific expectancy effects. When subjects expected to receive methylphenidate but received placebo, significant metabolic increases were seen in the ventral cingulate gyrus and NAC, and the effect was largest in subjects who had not yet experienced the active medication, suggesting that these structures are involved in expectation for what the authors termed "uncertain drug effects"

(Volkow et al., 2006). This possibility is supported by other imaging studies indicating increased activity of the ventral striatum during the expectation of other types of rewards, including monetary rewards (Elliott et al., 2000;Delgado et al., 2000;Knutson et al., 2000;Breiter et al., 2001;Dreher et al., 2006;Knutson et al., 2001a), primary rewards (Berns et al., 2001;O'Doherty et al., 2002), and drug rewards (Leyton et al., 2002). Changes in BOLD signal or glucose metabolism in the ventral striatum are not specific to altered DA release and could reflect changes in glutamatergic transmission from cortical or other limbic structures that may be modulated by DA (Knutson and Gibbs, 2007).

Some PET studies have shed light on this area, where it is possible to quantify a direct increase in DA release in response to reward. Small and colleagues (2003) demonstrated increases in striatal DA release have been in response to primary food reward (Small et al., 2003). Other groups have examined DA release using PET in response to monetary reward: Koepp and colleagues (1998) measured DA release using [^{11}C] raclopride (RAC) PET while subjects played a video game for monetary reward while in the PET scanner (Koepp et al., 1998), and Zald and collegues (2004) demonstrated increased DA release in the dorsal striatum during a variable-schedule card selection task as compared to a sensorimotor control task (Zald et al., 2004). With respect to pharmacological rewards, Leyton and colleagues (2002) demonstrated that a low oral dose of amphetamine in drug-naïve subjects caused a decrease in RAC binding in the ventral striatum which correlated with the subjects' "drug wanting" and with the personality trait of novelty seeking (Leyton et al., 2002). Other studies have also shown ventral (Oswald et al., 2005;Martinez et al., 2003;Drevets et al., 2001) and dorsal (Schlaepfer et al., 1997;Volkow et al., 1999;Volkow et al., 2001) striatal DA release in response to psychostimulants. The expectation of caffeine in habitual coffee drinkers was shown to increase DA release in the thalamus as estimated by a change in RAC binding (Kaasinen et al., 2004), although the responses in the dorsal or ventral striatum did not reach significance.

2.3.4 Measuring synaptic dopamine in humans with [^{11}C] raclopride PET

Currently, the only method by which DA can be quantified and imaged in the human brain is through the use of PET. There has been tremendous interest in developing PET tracers specific to the DA system due to its involvement in neurological and psychiatric disorders. Because DA cannot cross the blood-brain-barrier, neuroimaging studies of the DA system in the living brain have largely relied on indirect measures, such as labeling DA receptors, DA transporters, precursors of DA synthesis, or compounds which bind to the enzymes that degrade synaptic DA. Furthermore, cerebral blood flow or glucose metabolic changes as a result of manipulation of the DA system can also be investigated with PET.

Raclopride is a DA $D_{2/3}$ receptor antagonist with a moderate affinity for D_2 receptors (K_d = 1000-2000 p*M*) (Seeman et al., 1989). Although it exhibits high specificity for $D_{2/3}$ receptors, its relatively lower affinity subjects it to competition from endogenous DA. This feature has been exploited to provide an index of changes in synaptic DA levels. As mentioned in the previous section, RAC PET has been used to measure endogenous DA release in response to pharmacological (Volkow et al., 2001;Leyton et al., 2002;Volkow et al., 2003) and behavioural (Koepp et al., 1998;Zald et al., 2004) interventions, both in healthy controls and in patient populations. The ability of endogenous DA to displace RAC binding is outlined in the occupancy model shown in Figure 2.5. An increase in synaptic DA as a result of some intervention displaces bound RAC, thereby reducing the signal, whereas if synaptic DA levels are depleted, a greater amount of RAC is able to bind $D_{2/3}$ receptors, increasing the signal. On the basis of microdialysis studies in non-human primates it has been estimated that a 10% reduction in availability of D_2 receptors for RAC binding reflects a five-fold increase in synaptic DA levels (Breier et al., 1997).

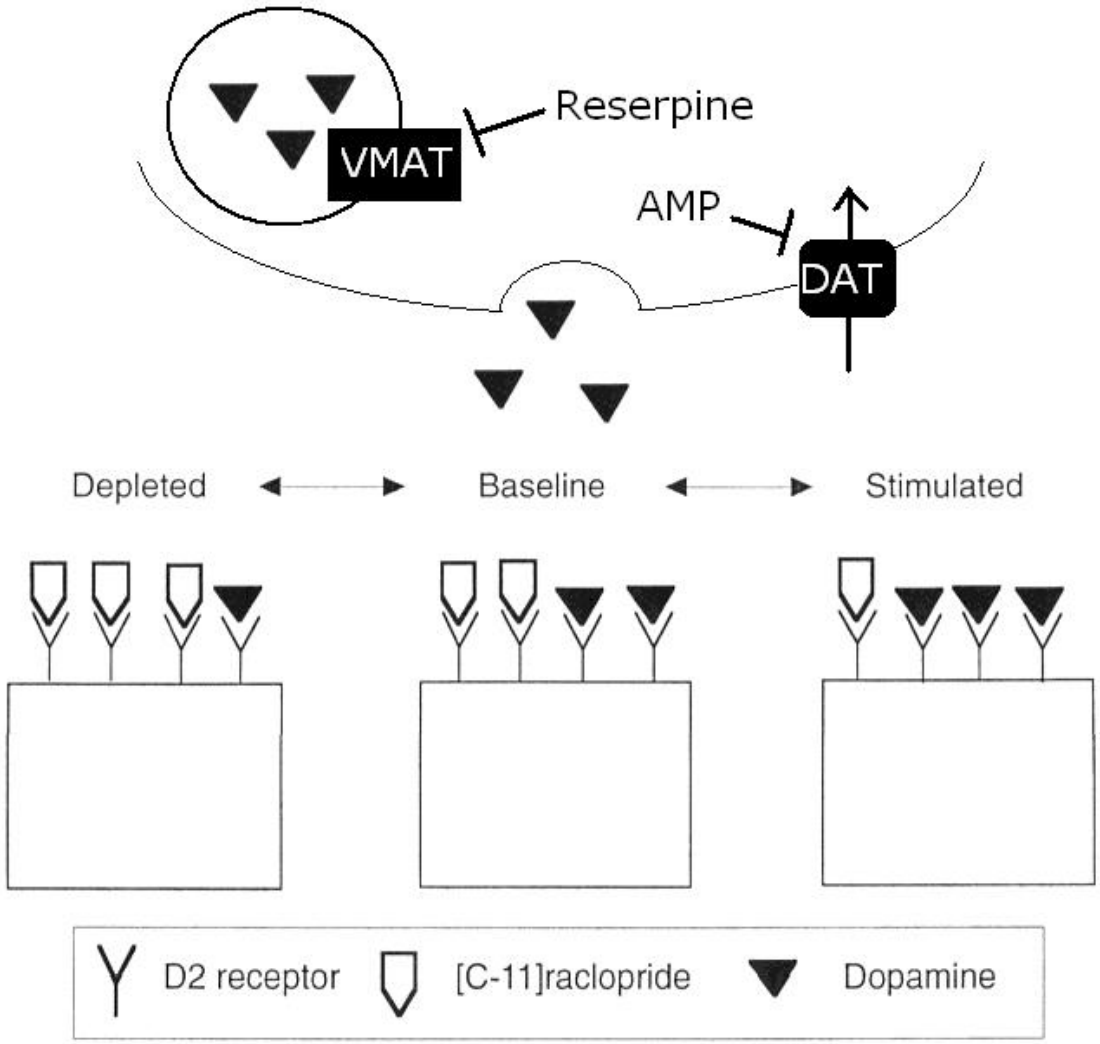


Figure 2.5 Cartoon of the classic occupancy model for RAC. Drugs that deplete DA levels by blocking vesicular monoamine transporters (VMAT), such as reserpine, permit more RAC binding to D_2 receptors. Conversely, drugs like amphetamine (AMP) that reverse the DA transporter (DAT) increase levels of synaptic DA and effectively displace RAC, decreasing its observed binding potential. Adapted from Laruelle, 2000.

Reliable RAC displacement measurements have generally been restricted to the striatum, which contains the highest concentration of both D_1 and D_2 receptors (Sealfon and Olanow, 2000). The ability to measure DA release in extra-striatal regions, such as the prefrontal cortex, has been limited by the relatively low density of receptors (0.3 – 4 pmole/g range) (Volkow et al., 1996), small volumes of interest, and variation in receptor-ligand kinetics from those which govern DA signaling in the striatum. Within the striatum, $D_{2/3}$ binding as measured by RAC is heterogeneous, with higher binding in the dorsal striatum than in the ventral striatum (Drevets et al., 1999;Mawlawi et al., 2001;Drevets et al., 2001;Martinez et al., 2003). This regional heterogeneity agrees with the D_2 total receptor densities shown in human postmortem studies (Camps et al., 1989;Gurevich and Joyce, 1999).

Since PET measures the total radioactivity in brain regions, quantitative analysis requires the differentiation of specific radioligand binding from the background of nonspecifically bound and free radioligand. The most common approach is to use a kinetic analysis where the possible sites of tracer-tissue interactions are estimated by a model defined by three compartments: the specifically bound tracer compartment (i.e. brain tissue of interest), the non-specifically bound tracer compartment (i.e. all other brain tissue, termed the reference region), and the circulating tracer compartment (i.e. plasma). The modeling depends on the existence of a reference tissue region with a negligible concentration of specific tracer binding sites; for example the cerebellum is used in RAC studies as it contains extremely low concentrations of DA $D_{2/3}$ receptors. Using this type of kinetic model reduces the temporal information obtained with dynamic PET scanning into a few parameters related to the biological processes under investigation. The two parameters of interest in neuroreceptor studies are the distribution volume ratio (DVR) and the binding potential (BP). The DVR is the ratio of the radioactivity in the reference region to the activity in the specifically bound tracer region, which is generally related to the BP as DVR = BP+1. Thus, the goal of compartmental kinetic modeling is to

determine a single DVR or BP value within a specified region-of-interest (ROI, e.g. the striatum), that represents tracer uptake in that region over the duration of the PET scan. All statistical analyses are then carried out on the BP values, for example measuring DA release in response to levodopa in PD patients.

For tracers which bind reversibly to their targets (e.g. RAC), two commonly used approaches are the tissue input graphical Logan method (Logan et al., 1996) and the simplified reference tissue (RTM) method (Gunn et al., 1997;Lammertsma and Hume, 1996). The methods make different assumptions, make estimates of different parameters, and have different sensitivities to statistical noise in the data (Sossi et al., 2007). The inputs to the models, however, are the same: the time-activity curves (TAC) from a region of interest (ROI, e.g. the striatum), and the reference region. Both methods provide BP values that are highly correlated, but the Logan method has been shown to introduce a downward bias in the BP values using high-resolution PET with RAC, particularly in cases where there is a significant amount of subject head motion (Sossi et al., 2007). Thus, whereas the Logan method is suitable for ECAT data where the images are less noisy (Chapter 3), it is not suitable for higher-resolution scanners such as the HRRT (Chapters 4 and 5).

2.4 Parkinson's disease

2.4.1 Introduction

Parkinson's disease (PD) is a chronic, progressive neurological disorder that affects approximately 100 000 Canadians. The prevalence increases with age, affecting 0.1% of people over the age of 55, 1% of people over 65, and 10% of people over 80 (Rajput, 1992;Tanner and Aston, 2000). Both sexes are affected roughly equally with a slight predominance in males (Lang and Lozano, 1998). PD is characterized by the selective degeneration of nigrostriatal DA-producing cells that modulate voluntary movement. Although classically seen as a movement disorder, it is now apparent that the symptoms of PD extend far beyond motor control, and often, these autonomic, cognitive and mood symptoms can affect quality of life to an even greater extent than the motoric symptoms. For example, it is well-recognized that up to 40% of PD patients have co-morbid depression, which may even precede the onset of motor symptoms as a presenting symptom (Leentjens et al., 2003). Cognitive changes can occur in PD, and can be as subtle as mild impairment in memory or can develop into frank dementia. There is some evidence that in some patients, the cognitive decline occurs in parallel with the decline in motor function (Owen et al., 1992). In addition, problems sleeping frequently occur, as do autonomic troubles such as severe constipation or problems regulating body temperature. Thus, in addition to the hallmark motor features of PD, including tremor at rest, rigidity, difficulties initiating movement (akinesia) and/or slowness of movement (bradykinesia), and postural instability, PD patients suffer a constellation of other symptoms that are not as clearly associated with central DA depletion. Interestingly, the manifestations of PD can be highly variable from one individual to the next; some may have severe bradykinesia with minimal rigidity, others may have the opposite, some may have marked tremor and little bradykinesia or rigidity, and still others maybe afflicted by all equally.

There is no biological marker that designates an unequivocal diagnosis of PD, and the gold standard remains clinical diagnosis, which, as determined by neuropathological examination, has a diagnostic accuracy of around 90% (Hughes et al., 2001). Approximately 10% of cases are genetic, resulting from mutations in encoded proteins involved in protein degradation and maintaining mitochondrial function (Abou-Sleiman et al., 2006), and the remainder of cases are termed 'sporadic.' Idiopathic PD, the most common form of Parkinsonism, can generally be distinguished from other types of Parkinsonism if the patient has an asymmetric presentation of symptoms, a resting tremor, and demonstrates good symptomatic response from levodopa, although these features could also be seen individually in other Parkinson-like neurodegenerative disorders such as multiple system atrophy. It should also be noted that the classic 4-6 Hz resting tremor is absent in up to one quarter of cases of PD (Lang and Lozano, 1998). Therefore, in the less straightforward cases the diagnosis evolves over time.

2.4.2 Neuropathophysiology

The pathology of PD is associated with the selective degeneration of DA-producing neurons in the SNpc of the ventral midbrain, although other monoaminergic brainstem nuclei (both catecholinergic and serotonergic) are also affected. Furthermore, not all DA projections are equally vulnerable; nigrostriatal DA neurons are most susceptible, exhibiting a cell loss of 50-80% at the onset of symptoms. The resulting DA depletion in the putamen is thought to account for the akinesia seen in PD. Mesocorticolimbic DA projections exhibit degeneration as the disease progresses, and this, together with degeneration of other non-DA neurons might contribute to other, non-motor symptoms also seen in PD including the aforementioned cognitive and mood alterations. Interestingly, this ventrolateral-to-medial spatial pattern of cell loss is opposite to that which occurs in normal ageing (Lang and Lozano, 1998). The other hallmark pathological feature of PD is the presence of neuronal intracellular protein inclusions – termed Lewy bodies – in both brainstem and cortical nuclei. Lewy bodies are not specific to PD as they

are also seen in other neurodegenerative disorders including dementia with Lewy Bodies (DLB), multiple system atrophy, Alzheimer's disease and occasionally also in progressive supranuclear palsy, corticobasal degeneration, motor neuron disease, and Down's syndrome (Rajput and Robinson, 2005). The mechanism of formation and the pathological role of Lewy Bodies in PD are not well understood, with theories ranging from a nonspecific role that is unrelated to disease pathogenesis, to a compensatory, protective mechanism to sequester toxic proteins, to being the agent responsible for nigrostriatal DA cell loss.

In PD, nigrostriatal DA depletion results in reduced inhibition of GPi in the direct pathway, as well as reduced inhibition of the STN in the indirect pathway; both result in a reduced thalamic drive to the motor cortex, altering the control and execution of voluntary salient movement. Reduced DA tone in the striatum is though to 'destabilize' the smooth functioning of the other corticobasal ganglia circuits as well, one consequence of which is the production of large increases in neuronal synchronization and oscillatory activity in the basal ganglia loops (Bergman et al., 1998).

2.4.3 Treatment

Current treatment for PD focuses on symptomatic control, as there is as yet no cure or therapy known to prevent or delay the progression of DA cell loss. The gold standard of treatment is exogenous levodopa, the direct biochemical precursor to DA, given in conjunction with a peripheral decarboxylase inhibitor to prevent catabolic breakdown in peripheral tissues before the drug crosses the blood-brain barrier. Since levodopa bypasses the rate-limiting enzyme in DA biosynthesis (tyrosine hydroxylase), its administration accelerates DA synthesis in the surviving DA neurons and replenishes the depleted synaptic DA stores. DA receptor agonists are also effective, however most patients will eventually require the addition of levodopa for optimal symptom control. Long-term treatment with levodopa, together with the progression of the disease, is associated with a number of complications. Following an initial

"honeymoon phase" when patients generally experience consistent, long-lasting benefit from each dose, and can even miss doses with little deleterious effect, they may eventually develop shorter medication responses that are tightly time-locked to each dose, resulting in fluctuations in motor responses (Goudreau and Ahlskog, 2005). Motor fluctuations occur in 40% of patients treated with levodopa for four to six years duration (Goudreau and Ahlskog, 2005). End-of-dose deterioration, or "wearing off" of the beneficial effect before the next dose is due, occurs at the mildest end of the spectrum, with more abrupt transitions from a mobile to an immobile state (the "on-off" effect) occupying the severe end. In addition, other factors can contribute to the variability in clinical response, including bioavailability, rate of gastric emptying, absorption, and blood-brain barrier transport (Goudreau and Ahlskog, 2005). These factors become more important with more advanced disease, likely because the altered central kinetics render patients highly dependent upon continuous bioavailability, as it is presumed in earlier disease there is greater buffering capacity (de la Fuente-Fernandez et al., 2004). Interestingly, the degree of patient insight into symptoms during these fluctuations is variable; some patients have specific sensory cues which signal that their dose is wearing off, such as dystonic leg cramping or the return of their tremor, while others cannot feel the medication wearing off or taking effect, despite the clear presence of motor fluctuations that would be noticed by an objective observer. As the therapeutic window between symptom control and motor complications narrows, pharmacotherapy becomes less effective and surgery (such as subthalamic nucleus deep-brain stimulation) becomes an important avenue for treatment.

CHAPTER III: Investigation of dopamine release in response to overt and covert subthalamic nucleus deep-brain stimulation in Parkinson's disease: a pilot study

3.1 Introduction

3.1.1 Subthalamic nucleus deep-brain stimulation

Subthalamic nucleus deep-brain stimulation (STN-DBS) is currently the most common surgical procedure performed for the treatment of advanced Parkinson's Disease (PD). In the later stages of Parkinson's, patients often encounter motor complications as a result of prolonged dopamine (DA) replacement pharmacotherapy which include involuntary movements known as dyskinesias, and motor fluctuations in which patients cycle between periods of good mobility ("on" periods) and impaired mobility ("off" periods). As these complications cannot be adequately managed with medication, surgery becomes an important treatment option. DBS surgery involves introducing an electrode into the sensorimotor portions of the STN, and high-frequency stimulation is applied via an implanted, externally programmable pulse generator. In appropriate patients, DBS, which can be performed bilaterally, can markedly reduce the intensity and duration of "off" periods, increase the duration of "on" periods, and effectively reduce dyskinesias (DeLong and Wichmann, 2007).

The initial selection of the STN as a target for surgical intervention in humans was based on evidence in animal models of PD of hyperactivity in the STN and internal segment of the globus pallidus (GPi), and that lesions in these structures ameliorated parkinsonian symptoms in the animals (Wichmann et al., 1994b;Wichmann et al., 1994a;Bergman et al., 1998). This observation was consistent with the "rate model" of the neuropathology of PD that predicts that the motor deficits seen in Parkinson's are due to preferential activation of the indirect pathway as a result of DA depletion, causing excessive inhibition of thalamocortical drive. The use of high-frequency stimulation of the STN replaced lesioning, as it was found that both procedures produced similar phenotypic effects and STN-DBS had the additional benefit of being reversible.

Physiological and anatomical evidence points to the STN as an important target in the basal ganglia circuitry gone awry in PD, yet the precise therapeutic mechanism of STN-DBS remains unclear.

3.1.2 STN-DBS and the placebo effect in Parkinson's disease

Patients who have undergone STN-DBS surgery for their PD provide a valuable model in which to study the placebo effect due to the reversible nature of the stimulation, and the ability to turn the stimulators on and off covertly. This enables the investigators to attribute any symptom changes to either the effect of the stimulation itself, or to the patient's beliefs. Several studies have investigated the motor symptoms of PD patients when their stimulators are turned on and off while manipulating the verbal instructions given to them as a method to influence patient expectations (Mercado et al., 2006;Colloca et al., 2004;Pollo et al., 2002). This "overt versus covert" model has been used as a means to study the placebo effect itself (Colloca et al., 2004). Depending on the paradigm, the patient is kept unaware as to the status of their STN stimulator while a blinded investigator measures their motor performance on a given task, such as movement velocity of the hand (to measure bradykinesia) or joint rigidity. At the same time, another investigator surreptitiously adjusts the stimulator settings to provide effective or ineffective stimulation. By doing this, the effect of verbal suggestion on motor symptoms can be assessed independently of the effect of stimulation. For example, if the patient's stimulator is turned off in a covert fashion when they believe it to be on, any improvement in motor performance would be entirely due to the placebo effect. In addition, to avoid the use of frank deception due to ethical concerns, the amplitude of the stimulator's current can be adjusted either up or down while a corresponding verbal suggestion is given, e.g. the stimulator is reduced to 20% of its original amplitude while the patient is told that it is fully on (Pollo et al., 2002). Such paradigms also afford an opportunity to study the nocebo phenomenon, in which the patient is told that their motor performance will worsen because they are turning down the stimulation,

while the stimulator actually remains on. The term 'nocebo effect' has also been used to describe the situation in which the patient exhibits a worsening in his or her condition in response to a placebo (Kennedy, 1961). Benedetti and colleagues demonstrated that motor performance in PD patients worsened with the induction of a negative verbal expectation, yet the induction of a positive verbal expectation blocked this nocebo effect (Benedetti et al., 2003b). Thus, the patient's expectation that they would have improved motor performance reversed the motor worsening in response to the opposite (negative) suggestion.

Studies of this type have demonstrated that verbal instructions are able to modify motor performance in PD patients, and it has been shown that patients with STN-DBS demonstrate improved motor functioning when they are told they will do well and the stimulator is turned off, compared to when they are told they will do poorly (Pollo et al., 2002;Benedetti et al., 2003b;Mercado et al., 2006). In a previous clinical study conducted in our centre, it was found that awareness of the status of the STN-DBS stimulator affected the motor symptoms in opposite directions: when patients were told their stimulators were on, they demonstrated greater clinical improvement compared with when the stimulators were on but the patients were blinded to the status of their stimulators, and when the stimulators were turned off and they were informed, the patients worsened clinically to a greater degree than when they were blinded (Mercado et al., 2006). The authors reported that approximately 35% of the magnitude of the active STN DBS effect was due to the awareness of the stimulation. Placebo-induced expectations have been shown to modulate not only the motor response to STN-DBS but also both the mean rate and firing pattern of STN neurons (Benedetti et al., 2004). It is thought that that the benefits of STN stimulation in PD are not mediated by DA release (Hilker et al., 2003;Strafella et al., 2003), however, the placebo-derived benefits of STN stimulation may be DA related, as has been shown in the placebo effect in response to pharmacological (de la Fuente-Fernandez et al., 2001b) and repeated transcranial magnetic stimulation therapy (Strafella et al., 2006). This

suggests that expectation of both worsening and improvement can modify the response to stimulation and might have a DA correlate. To investigate this possibility, we used ^{11}[C] raclopride (RAC) positron emission tomography (PET) to measure DA release in PD patients with STN-DBS when they are blind to whether their stimulators are on or off, and compared the results to when they were aware of the stimulator condition. Based on the results from the previous clinical study, and the finding of placebo-induced DA release in the ventral striatum, we hypothesized that the level of RAC binding, indicating synaptic DA release, would be different in aware and blind conditions. Specifically, we tested if the degree of DA release in the ventral striatum would be greater in patients aware that their stimulators are on compared to when they are blind, and could potentially be lower when they were aware that the stimulators were off compared to when they are blind.

3.2 Methods

3.2.1 Subjects

5 patients diagnosed with idiopathic PD who had undergone STN-DBS surgery were recruited from the Vancouver General Hospital Surgical Centre for Movement Disorders by a neurosurgical fellow and/or the neurosurgeon. All subjects gave written informed consent. The study was approved by the UBC Clinical Research Ethics Board (Appendix A). Patients were not depressed (Beck Inventory of Depression score < 12) (Beck et al., 1988) and were free of cognitive impairment (Mini Mental State Exam score > 26) (Folstein et al., 1975).

3.2.2 Study design

The study was designed to replicate as much as possible in the PET scanner the clinical study, thus the design was very similar to that described in Mercado *et al.* (2006). The patients underwent four RAC PET scans over the course of two consecutive days. The patients were randomized to one of four groups which determined the order of their scans according to the

design shown in Table 3.1, and contained different combinations of the 4 conditions: Blind-OFF, Blind-ON, Aware-OFF, Aware-ON. Within the groups, the order of the days was counter-balanced.

	DAY 1		DAY 2	
	Blind Condition		Aware Condition	
Group 1	OFF	ON	OFF	ON
Group 2			ON	OFF
	Aware Condition		Blind Condition	
Group 3	OFF	ON	OFF	ON
Group 4	ON	OFF		

Table 3.1 Study Design. The four conditions (Blind-OFF, Blind-ON, Aware-OFF, Aware-ON) are shown as a function of group. OFF/ON indicates the status of the STN-DBS stimulator.

The order of the Aware and Blind days was randomized. For the scans conducted in the blind condition, the order was not randomized, i.e. the scans were always conducted in the OFF condition first. The reason for this was to reduce the risk of unblinding the subjects, as some patients with STN-DBS can "feel" when their stimulators are turned on. Patients were withdrawn from anti-parkinson medication 12-18 hours overnight before both scanning days, and their STN-DBS stimulators were turned off overnight.

3.2.3 Manipulation of Expectation

Manipulation of expectation during the study was carried out using verbal instructions. For the Aware conditions, subjects were told, "We are now turning your stimulator on/off." For the Blind conditions, subjects were told, "We are now *adjusting* your stimulator. It could be either on or off." All stimulator adjustments were performed at the time of RAC injection, i.e. at the beginning of the PET scan. For the two scans performed with the stimulator on, the stimulation parameters were those previously determined to be effective for that patient (i.e. amount of current and amplitude). To further reduce unblinding, the stimulator was adjusted to turn on with current ramped up over 8 seconds (the maximum time interval permitted on the

Medtronic stimulator device). This was done to reduce the sensory cues that some patients experience if the current is increased abruptly.

3.2.4 Outcome measures

The aim of the present study was to replicate a previous clinical study in patients while undergoing RAC PET and examine the effects of altered expectation on striatal DA release when patients were blind as to whether their stimulators are on or off, compared to when they were aware of the stimulator condition. Thus, objective (i.e. clinical) measures were required to quantify both the effect of STN-DBS stimulation and the placebo effect on motor symptoms. In order to achieve this, a blinded examiner conducted a brief modified version of the UPDRS III (Unified Parkinson's disease Rating Scale, Motor section, Appendix B) (Fahn et al., 1987) 30 minutes into the PET scan (i.e. 30 minutes post-tracer injection). The UPDRS exam was modified for use while the patient lay in the PET scanner, thus only measures of rigidity, tremor, and bradykinesia in the upper limbs and measures of rigidity and tremor only in the lower limbs were considered (Modified UPDRS, mUPDRS, Appendix C). The absolute RAC BPs were compared against each other for each condition: $RAC_{on/blind}$, $RAC_{on/aware}$, $RAC_{off/blind}$, and $RAC_{off/aware.}$

3.2.5 PET scanning protocol and image analysis

All scans were performed using an ECAT 953B/31 tomograph (Siemens Canada, CTI, Knoxville, TN, USA) operating in 3D mode (septa retracted). 16 sequential frames over 60 minutes were obtained, starting at the time of injection of 5 mCi of RAC (specific activity >1000 Ci/mmol at ligand injection). From the emission data (30–60 min) an integrated image with 31 planes (each 3.37-mm thick) for each subject was obtained. The five axial planes in which the striatum was best visualized were averaged. On this time- and spatially-summed image, one circular region of interest (ROI) of 61.2 mm^2 was positioned on the head of each caudate

nucleus, and three circular ROIs of the same size were placed without overlap along the axis of each putamen (from rostral to caudal: P1, P2 and P3); ROI position was adjusted to maximize the average radioactivity within each ROI. To identify the ventral striatum, the images were displayed in the coronal orientation and the Talairach and Tournoux brain atlas was used to select three planes in which the ventral striatum was best visualized. On the image summed over these planes, two circular ROI of 61.6 mm^2 were placed bilaterally on the ventral striatum of each hemisphere. The background activity was obtained from a single elliptical ROI (2107 mm^2) on the cerebellum. The binding potential ($BP = B_{max}/K_d$) was determined using a graphical approach and a reference (cerebellar) tissue input function (Logan et al., 1996).

3.2.6 Statistical analysis

RAC BPs in the dorsal and ventral striatum were compared between conditions to investigate the differences in synaptic DA levels. For each region (head of caudate, rostral, intermediate and caudal putamen, and ventral striatum) and for the mUPDRS, repeated measures analysis of variance (ANOVA) were carried out, accounting for stimulation (ON vs. OFF), awareness (blind vs. aware), and their interaction. As the studies were conducted over 2 days, the effect of day was also adjusted for as an additional covariate (ANCOVA).

3.3 Results

3.3.1 Subjects

The clinical characteristics of the patients are presented in Table 3.2. Subject 1 had a history of depression but was not on antidepressant medication at the time of the study. Subject 3 reported using recreational marijuana twice a week and the occasional use of cocaine, but denied cocaine use for 8 months prior to the study.

Subject	M/F	Age (y)	Duration of PD (y)	Duration of STN-DBS (y)	Medications for PD, daily dose
1	F	45	7.6	2.3	Levodopa 600 mg, Entacapone 600 mg
2	M	53	10	2.5	Levodopa 700 mg, Pergolide 10 mg
3	M	50	19.4	0.6	none
4	M	64	10	0.8	Levodopa 200 mg, Domperidone 20 mg, Pergolide 4 mg
5	M	73	18	0.5	Bromocriptine 22.5 mg

Table 3.2 Clinical characteristics of the PD patients. The daily dose of levodopa is given in equivalents of immediate release levodopa/carbidopa.

3.3.2 Clinical response to STN DBS

The mean + SEM mUPDRS scores in the four conditions are presented in Figure 3.1. A main effect of stimulation was observed ($p = 0.01$, repeated measures ANOVA), indicating the effectiveness of STN-DBS stimulation (mUPDRS scores, Aware: 18.8 ± 3.7 OFF to 6.0 ± 2.3 ON; Blind: 17.8 ± 4.8 OFF to 5.6 ± 1.6 ON). No significant effect of awareness was observed.

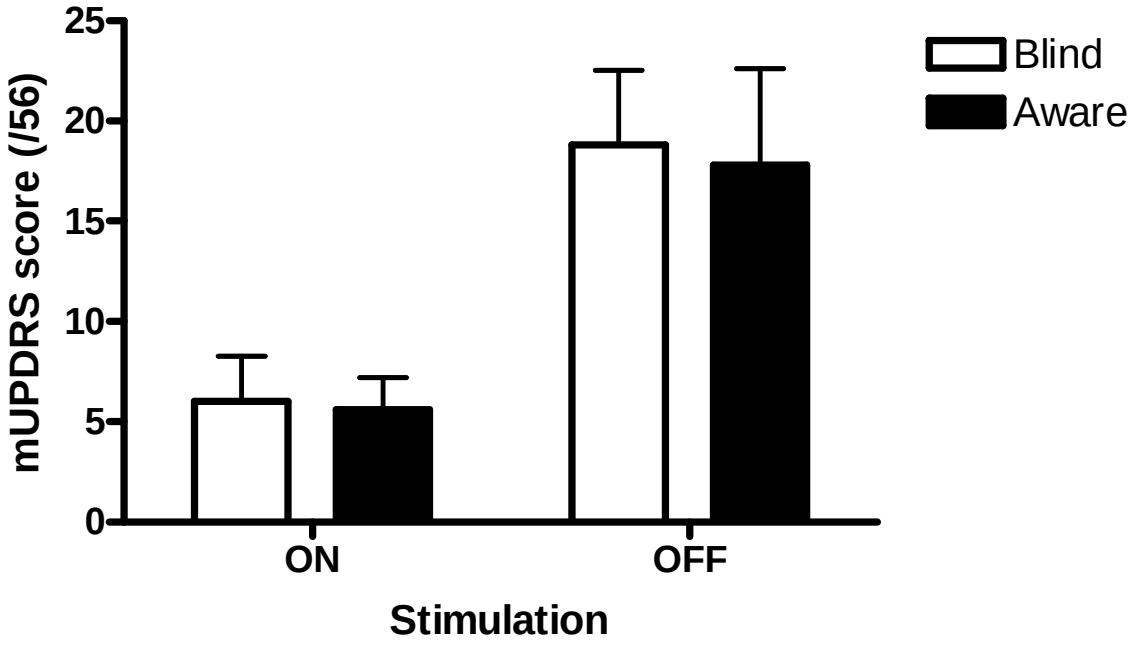


Figure 3.1 Effect of STN-DBS stimulation (mean + SD) on mUPDRS motor scores for 5 PD patients in the four experimental conditions, as measured by a blinded examiner at 30 min. into the PET scans. A significant effect of stimulation was found ($p = 0.01$), but not of awareness nor of the interaction.

3.3.3 [^{11}C]Raclopride results

The RAC BPs + S.E.M. in each condition in each striatal subregion are presented in Figure 3.2 below. No main effect of stimulation was detected in any subregion, indicating that the STN-DBS stimulation had no impact on the degree of DA release.

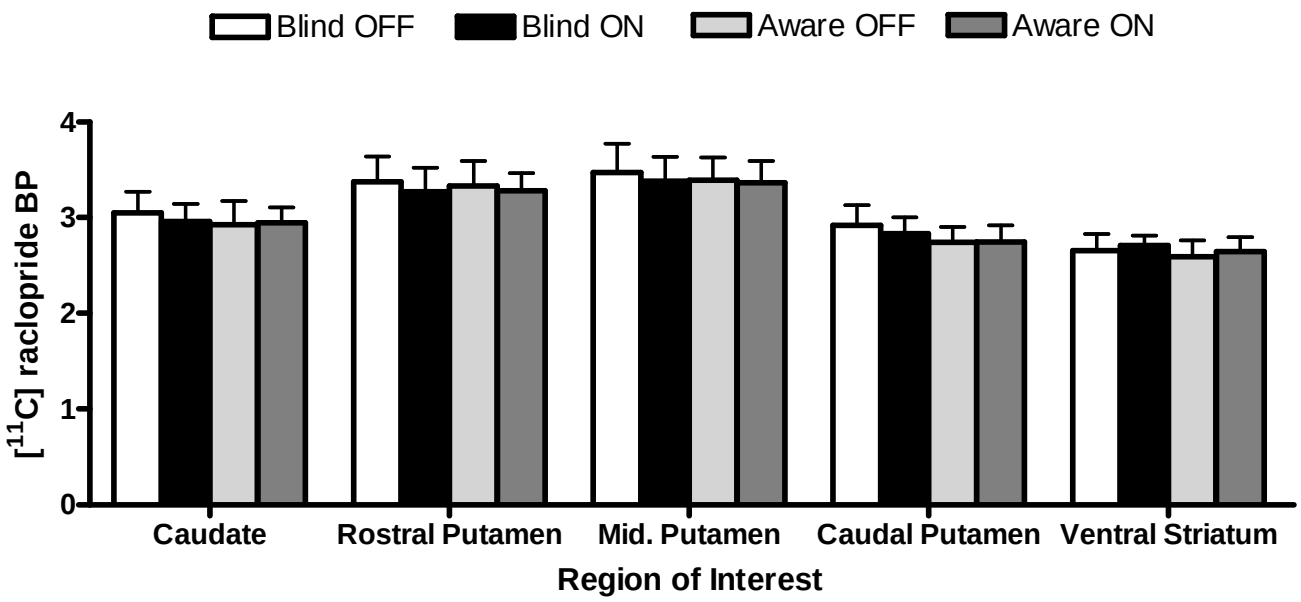


Figure 3.2 Striatal RAC binding potential (mean + SD) of 5 PD patients scanned in each condition. A significant main effect of awareness was observed in the caudal putamen ($p < 0.01$), although no significant effects of stimulation, awareness or their interaction were observed in other striatal subregions.

Separate two-way repeated measures ANCOVAs conducted in each striatal subregion indicated a significant main effect of awareness in the caudal putamen only ($p = 0.0029$) (Figure 3.3). In this region, the RAC BP in the Aware condition was decreased compared to the Blind condition, in both the ON and OFF conditions, indicating a greater amount of synaptic DA (means ± S.E.M.: ON, 1.83 ± 0.17 Blind versus 1.74 ± 0.17 Aware; OFF, 1.92 ± 0.2 Blind versus 1.74 ±0.16 Aware). For the ON condition, Blind – Aware corresponded to a 4.6% decrease in RAC BP, and for the OFF condition, Blind – Aware corresponded to a 9.2% decrease in RAC BP. Post-hoc pairwise comparisons indicated a significant effect of awareness in the OFF condition ($p = 0.0044$) (Figure 3.3).

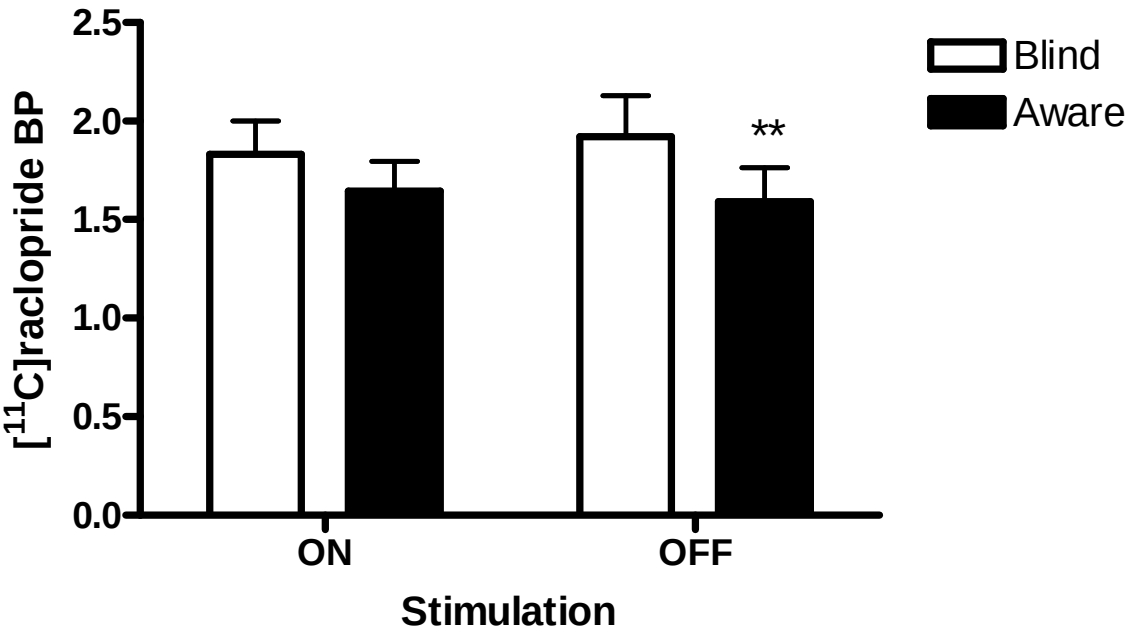


Figure 3.3 Effects of STN-DBS and awareness on RAC binding potentials (mean + SEM) in the caudal putamen. The main effect of neither stimulation, nor the interaction between awareness and stimulation reached significance. Post-hoc pairwise comparisons indicated a significant effect between Blind OFF and Aware OFF ($p = 0.0044$).

When the RAC BP values were averaged over the entire putamen, no significant effect of awareness or stimulation was seen. The results for the ventral striatum did also not reach statistical significance. Both of these are shown in Figure 3.4.

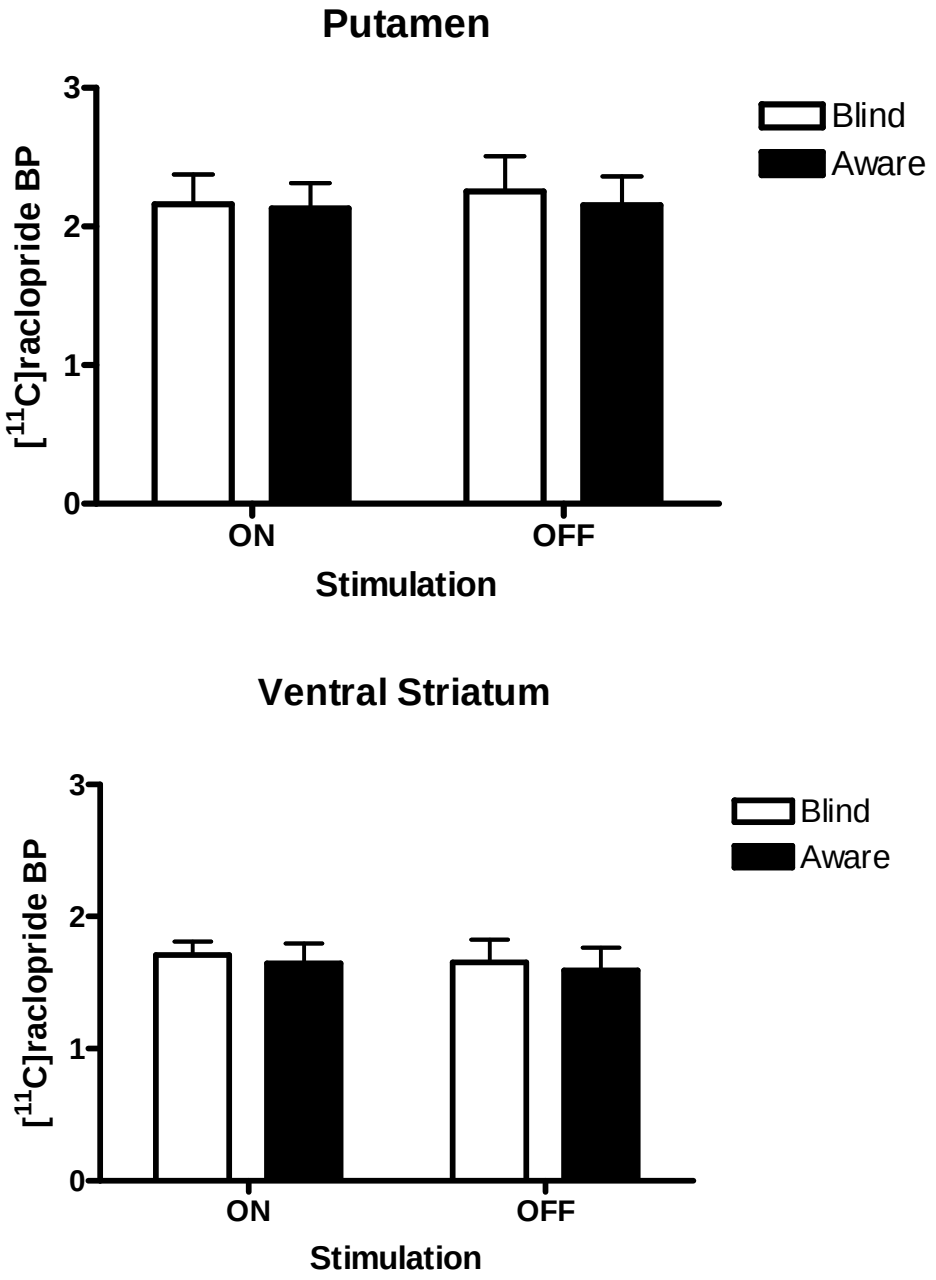


Figure 3.4 Effects of STN-DBS and awareness on RAC binding potentials (mean + SEM) in the putamen (top panel) and ventral striatum (bottom panel). Neither awareness nor stimulation had an effect on RAC BPs in either region.

3.4 Discussion

This was a pilot study conducted in five PD patients who had undergone STN-DBS surgery for advanced PD. The objective was to use overt and covert treatment to ascertain if we could detect a DA correlate for the results of our previous clinical study, in which it was shown that awareness of the status of the STN-DBS stimulator can modulate the clinical response to STN-DBS stimulation in opposite directions, depending on whether the stimulator is on or off. As the placebo effect in PD has been shown to be related to striatal DA release, we hypothesized that awareness of being "on" would induce greater DA release than being blind to the stimulator's condition. The present findings indicate that this was not the case, as no difference was detected when the patients were blind or aware and the stimulators were on. We also hypothesized that a decrease in synaptic DA might occur when the patients were aware that the stimulators were off, which was also not the case in this study. Thus, neither awareness nor stimulation significantly altered the amount of synaptic DA as measured by RAC PET.

The main possible reason for this negative result lies in the design of the study itself and the success of the overt and covert STN-DBS manipulations. The profound clinical effects of DBS made it difficult to maintain blinding, and thus manipulate awareness during the PET scans. Although STN-DBS stimulation produced a significant clinical improvement (Figure 3.1), the lack of an awareness effect on the mUPDRS motor scores may indicate a potential ceiling effect, again due to the strong impact of STN-DBS. DBS is a highly effective treatment for the motor symptoms of PD (Kleiner-Fisman et al., 2003;Lang et al., 2003;Ashkan et al., 2004), and we found that the patients could often tell when their stimulators were being turned on. We attempted to reduce the potential for unblinding by always having the Blind-OFF condition first, so that the patient would not feel themselves being "turned off" in between conditions. We also ramped up the amplitude of the current gradually over 8 seconds (the longest delay provided by the Medtronic device) to mask the abrupt sensation of being turned off and on. However, some

patients still reported that they were able to tell the status of their stimulator. One could interpret the results in another way: the lack of success in manipulating awareness in the patients and the corresponding lack of change in DA release could itself provide further proof for the importance of expectation to placebo-induced DA release in PD.

It is possible that we did not actually induce a placebo effect because we were not truly using sham stimulation. The overt/covert paradigm for studying the placebo effect attributes the difference in response between overt and covert administration of therapy to the placebo effect (Benedetti et al., 2003a). In this experimental approach, deception is not used to manipulate the subjects' expectations, i.e. they are not told that they are receiving active treatment when they are in fact receiving placebo. In this study, the Blind condition involved telling the patients that their stimulators could be either on or off, therefore they were uncertain as to the status of the stimulators and would not necessarily have any expectation of improvement. As expectation of clinical benefit has been proposed as a key mechanism underlying placebo-induced DA release in the ventral striatum in PD, it is therefore not surprising that we did not detect any differences between the blind and aware conditions. In addition, the stimulators were "adjusted" at the time of radiotracer injection, and the time-course of the DA correlate of expectation may need a greater length of time to develop in order to be detected using RAC PET. Indeed, it is possible that our overt/covert manipulations were successful, but did not induce a sufficient magnitude of DA release beyond the test-retest variability of RAC.

We did demonstrate that the clinical effects of STN-DBS are not medicated by DA release, which is consistent with other studies (Hilker et al., 2003;Strafella et al., 2003). Current pathophysiological models of basal ganglia organization suggest that PD is a state characterized by hyperactivity of the glutamatergic excitatory action of the STN over the output nuclei of the basal ganglia (globus pallidus pars interna (GPi) and substantia nigra pars reticulata (SNr)), thereby propagating an excessive inhibitory influence in the thalamus, cortex, and brainstem

(Wichmann and DeLong, 1996). Although the mechanisms are unclear, it is hypothesized that STN-DBS reduces or inactivates either the neurons of the STN or their excitatory glutamatergic projections. Placebo injections of saline administered when patients were conditioned to expect apomorphine have been shown to change the mean rate and firing pattern of STN neurons in PD patients (Benedetti et al., 2004), and it is assumed that this reflects placebo-induced striatal DA release. However, as the benefits of STN-DBS are presumably mediated downstream to stimulation of striatal DA receptors, it may well be that the effect of placebo effect STN-DBS may involve non-dopaminergic mechanisms.

3.5 Conclusion

This study provides further support for the observation that the therapeutic effects produced by STN-DBS are not mediated by increased DA release. The beneficial clinical effects seen as a result of manipulation of expectation might be DA-related, but this could not fully be assessed as a result of the difficulty in maintaining blinding due to the profound effects of STN-DBS on the motor symptoms in PD. Alternate experimental designs may be required to fully assess the placebo effect in this setting.

CHAPTER IV: Development and implementation of the methodology for the analysis of high-resolution (HRRT) PET data

4.1 Introduction

This Chapter describes my role in developing the image analysis methods of high resolution positron emission tomography (PET) data which are now in routine use in the UBC PET Program. A background of the basic principles of PET is presented, including the limitations of the data and how they are corrected for, followed by a description of the systematic experiments that were conducted in order to refine the steps of the data analysis.

4.1.1 Overview

PET is one of the most effective methods for the non-invasive measurement of physiological functioning in awake, human subjects. Whereas magnetic resonance imaging (MRI) or x-ray computed tomography (CT) provide detailed anatomical information, PET imaging enables the quantification of physiological parameters such as blood flow, glucose metabolism, and receptor binding characteristics. Clinical PET imaging is routinely used in oncology to diagnose and differentiate malignant from benign tumors, and also has applications in cardiology, neurology, psychiatry and pre-clinical pharmaceutical studies.

PET images are acquired by detecting the decay of positron-emitting radioisotopes with short half-lives such as carbon-11 (20 min), nitrogen-13 (10 min), oxygen-15 (2 min), and fluorine-18 (110 min), although isotopes with longer half-lives are also used, such as copper-14 (12.7 hours) and iodine-124 (4.2 days). Several fundamental components of biological molecules (i.e. C, N, O and F) are also positron-emitting isotopes, and can therefore be readily incorporated either into compounds normally used by the body such as glucose or water, or into molecules that bind to receptors or other sites of drug action. Such labeled compounds are known as radiotracers (or, simply, tracers). PET scans begin with either the injection (intra-venous) or inhalation of the tracer into the subject, where it is rapidly taken up into the

circulation and distributed to its binding site. Some radiotracers distribute in tissues by partially following the metabolic pathways of their natural analogues; others bind with specificity in the tissues containing the particular receptor proteins for which they have affinity. The chemical synthesis of radiotracers consists of two steps: first, production of the radioisotope itself from its native element, and second, the substitution of the radioisotope into the drug molecule. This is a highly complex chemical synthesis requiring the use of a cyclotron that must be in close proximity to the PET scanning facility due to the short half-life of the tracers. Several radioligands have been developed for brain imaging, such as precursors to neurotransmitters, pre- and post-synaptic receptors, and vesicular transporters in nerve terminals. The data generated using these different radioligands can thus provide insights into the biological integrity and functioning of neurotransmitter systems of interest.

4.1.2 Organization of dynamic PET data

Dynamic PET imaging refers to the ability to measure the regional concentration *in vivo* of a radiotracer in a particular tissue in a subject over time. Prior to reconstruction, the PET data are sampled into smaller data sets, or time-frames, typically of progressively increasing length from the start of the emission scan (i.e. at the time of tracer injection) to the end of the scan. The length of the scan is defined by the tracer kinetics and the length of the time frame is determined by a requirement that a sufficient number of counts be acquired to construct a meaningful image (defining the shortest frame length). Depending on the biological question of interest, if the counts in an identical region in the brain (a region of interest, or ROI) are sampled across each frame and plotted over time, the resulting graph is a time-activity curve (TAC) (Figure 4.1). In the case of dopamine (DA) receptor imaging with [^{11}C] raclopride (RAC), at the time of tracer injection (t=0) the mean activity rises sharply within the putamen as the tracer is distributed to the binding sites by the circulation, and then gradually levels off as the tracer binds to DA D_2 receptors and equilibrium is reached.

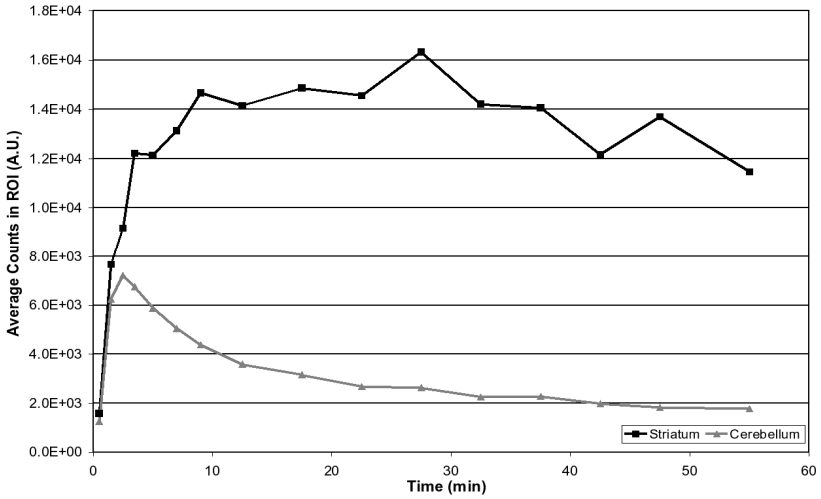


Figure 4.1. Time-activity curves for an ROI placed on the putamen (top trace) and cerebellum (reference region, bottom trace) of a PD patient injected with a 10 mCi dose of RAC. The emission data were split into 16 frames as follows: 4x60s, 3x120s, 8x300s, and 1x600s.

4.1.3 Basics of Signal detection

Positron emission is a type of radioactive beta decay, in which a proton decays into a neutron, a neutrino and a positron (β^+, the antimatter counterpart of an electron). The emitted positron travels a short distance from its parent nucleus (the positron range), interacting with atomic electrons in its immediate vicinity before colliding with an electron and causing an annihilation reaction (Figure 4.2). This reaction occurs when the positron has lost sufficient kinetic energy through scattering by surrounding electrons. The positron range varies between isotopes, but is generally so small that its contribution to the degradation of the spatial resolution is ignored.

Figure 4.2 has been removed due to copyright restrictions.

This figure contained a cartoon image of positron-electron annihilation coincidence detection.

The source of the image was:

http://www.heartandmetabolism.org/images/HM34/ HM3407gr1.gif

Figure 4.2 Schematic of annihilation coincidence detection. Following positron decay, an emitted positron (e^+) travels a finite distance before colliding with an electron (e^-). This annihilation produces two high-energy (511 keV) photons (γ-rays) which propagate at 180° to each other and are detected in coincidence by the scanner's scintillation detectors.

The annihilation of the positron and the electron produces two 511 keV gamma rays (high energy photons) that propagate in nearly opposite directions (Figure 4.2). These photons are detected in coincidence by multiple detector heads that are organized in a geometrical cylinder surrounding the gantry. Each head is composed of many scintillation detectors, the number and size of which largely determine the spatial resolution of the scanner. The scintillation detector consists of a solid crystal with a high atomic number that is coupled to a photomultiplier tube, which serves to amplify the light generated as the photons strike the crystals and convert it to an electronic signal. If two photons are detected within a short time window, an event is recorded along the line connecting the two detectors, termed the line of response (LOR) (Figure 4.3).

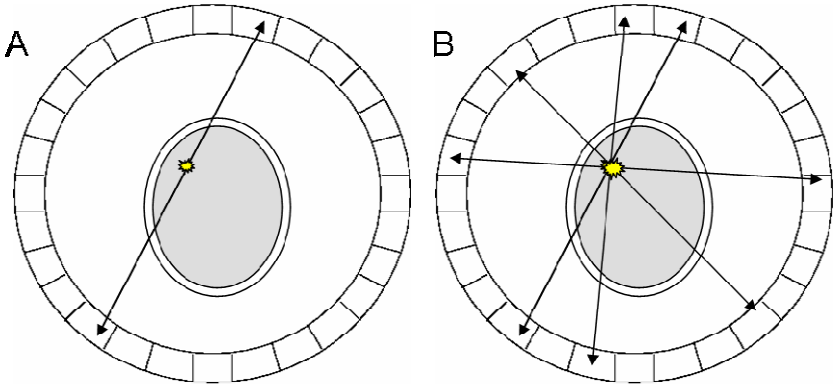


Figure 4.3 Simplified schematic of lines-of-response (LORs) within a PET scanner gantry. Gamma (γ) rays, produced by the annihilation of a positron and an electron, propagate in opposite directions and strike the detector ring. The resulting line that connects the two events is termed the LOR (A). Over the course of many such events, several LORs are created which intersect through the radioisotope distribution (B).

Summing many such events over the duration of the scan results in the accumulation and intersection of the LORs through the radioisotope distribution (Figure 4.3 B). If the proper calibration is then applied (which converts the count rate per voxel into the activity concentration per voxel), an image is generated that reflects the concentration of the radiopharmaceutical within the tissue of interest. The goal of the reconstruction of PET data is to therefore transform the raw counts from the detectors into a meaningful biological image that illustrates the *in vivo* regional or local tissue concentration of the radiotracer. This quantity can then be related to a physiological parameter of interest – such as dopamine receptor occupancy - by applying a mathematical, linear compartmental model.

4.1.4 Issues with data collection and interpretation

There are several physical phenomena that complicate PET imaging. Producing accurate PET images relies upon accurate coincidence detection, and not all of the events that are detected in coincidence originate from the same annihilation. This can result in the incorrect positioning of events, thereby creating false LORs which can cause image degradation and compromise quantitative accuracy. Physical causes of image degradation include photon attenuation,

detection of scattered and random events, and non-uniformity in detector pair sensitivity, which are briefly explained in the following sections.

Attenuation of annihilation photons

The presence of material (i.e. brain tissue and skull) in the field-of-view of the scanner causes photon travel to be attenuated. The photons can lose so much energy that they do not exit the skull and fail to be detected, are below the threshold of energy discrimination, or they are sufficiently slowed down such that they fail to be detected within the coincidence timing window. Importantly, for a given attenuating material, γ-ray attenuation depends only on the total thickness of the material (Zanzonico, 2004). Attenuation can thus be relatively easily corrected for, because the thickness of the attenuating material can be measured, and is in fact the largest correction made to PET image data. This is achieved by performing a transmission scan prior to the emission scan, where an external positron-emitting rod source located in the gantry of the scanner is rotated around the field of view with and without the subject in the scanner. The attenuation correction factor can then be derived from the ratio of the counts acquired in these respective scans.

Detection of scattered events

One or both of the annihilation photons may undergo scattering prior to being detected. This occurs when the photon interacts with an outer shell electron in its path, causing it to lose energy and change direction, i.e. become deflected. Those annihilations for which one or both photons are deflected, but both are still detected, are termed scattered events. Scattering results in the incorrect positioning of an LOR, as depicted in Figure 4.4b (Zanzonico, 2004). In 3D PET imaging, the percentage of scattered events in all measured counts ranges from 30-55% (Thompson, 1988). Since photons lose energy when they are scattered, a portion of such events can be rejected by narrowing the energy window that is accepted by the coincidence detection

system (termed energy discrimination). However, if the window is too narrow, true coincidences can be falsely rejected, thus additional corrections must be applied.

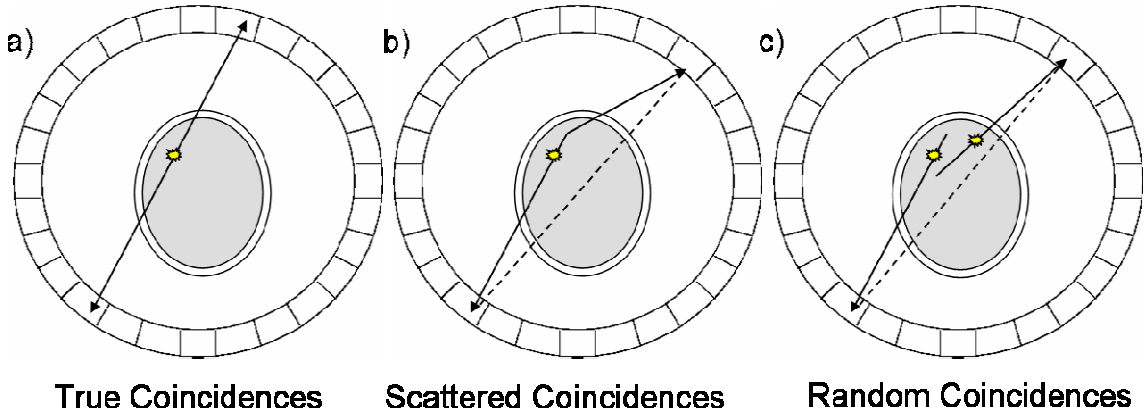


Figure 4.4 Plots indicating annihilated photons from an event detected with a) true coincidence, b) scattering, and c) random coincidence. The last two result in mispositioning of the LOR along which the event is detected.

Detection of random events

Frequently, only one of a pair of annihilation photons will be detected. This can occur if the orientation of the annihilation results in one photon propagating out of the field of view, if it is scattered out of the field of view, or if it passes through the detectors but fails to be detected. The other photon that is detected is therefore termed a single. A possible consequence of this is that two photons arising from separate annihilations are detected within the same timing coincidence window, in what is termed a random or accidental event. This can also result in the incorrect positioning of an LOR (Figure 4.4c). The count rate of random events can be reduced by narrowing the coincidence timing window. The singles rate during a scan is proportional to the amount of injected radioactivity, and the random rates vary with the square of the activity, and thus is an important consideration when calculating the maximum injected activity dose (Ollinger and Fessler, 1997). The arrival of photons due to random coincidences is uniformly distributed in time, whereas the true coincidences will fall within the coincidence timing window. Thus, random events can be corrected for by collecting data in a second coincidence

timing window that is offset in time such that it collects no true coincidences (Ollinger and Fessler, 1997). Subtracting this value from the coincidences arriving in the original timing window effectively removes the contribution of random coincidences to the data.

Non-uniformity in detector pair sensitivity

The sensitivity of a PET camera is defined as the measured event rate per unit of activity, and is determined by two factors: 1) the geometric efficiency of the scanner, which is the fraction of emitted radiations that strike the detectors, and 2) the intrinsic efficiency of the detectors, which is the fraction of radiations striking the detectors that are stopped and counted by the detectors (Zanzonico, 2004). The sensitivity is not uniform throughout the field-of-view of the scanner. Furthermore, crystal imperfections, differences in photomultiplier tube gains, and variations in the electronics used to detect the photomultiplier tube signals all contribute to variability within elements of a detector, as well as between detector blocks. To correct for this, a source with a known number of emissions is scanned and compared to the detected number of emissions and a correction factor is then calculated to account for the discrepancies. This "normalization scan" is a time-intensive process that can take up to one week for high-sensitivity scanners such as the HRRT (long acquisition times are required to obtain sufficient counts from the lower activity sources that must be used to avoid detector saturation) and must be performed every 3-4 months. Therefore, subjects cannot be scanned during these regular scanner maintenance times.

Other corrections applied to PET data

In addition to those described above, other correction factors must also be applied to PET image data. These include deadtime correction, decay correction, and branching factor correction. The deadtime is the length of time required for the counting system to fully process an event, during which no other events can be recorded (Zanzonico, 2004). At clinically administered doses of activity, deadtime count losses are generally minimal, thus only a small

correction factor is usually applied. The decay correction accounts for the exponential decrease in radioactivity as a function of time. Thus scanning of the same object at a later time will result in fewer annihilation photons, and therefore, fewer reconstructed counts in the image. Correction for decay involves scaling the reconstructed image counts, depending on the radioisotope used. Finally, branching correction accounts for that small proportion of the radiotracer that undergoes electron capture and not positron decay (e.g. the branching ratio for carbon-11 is 99%, which means that the vast majority undergoes beta-decay resulting in positron emission and a negligible amount decays by other means).

4.2 The High Resolution Research Tomograph

4.2.1 Introduction

The Vancouver high resolution research tomograph (HRRT), shown in Figure 4.5, is a state-of-the-art high sensitivity, high resolution scanner. It is one of 17 HRRT scanners in the world (3 of which are in Canada) and because of the gantry size is dedicated to brain research only. Instead of the usual circular detector ring design, the HRRT consists of 8 flat panels of crystals set in an octagonal geometry to facilitate coupling of the crystal to the photomultiplier tubes (Figure 4.5).

Figure 4.5 The high resolution research tomograph (HRRT) from the front end with the cover removed, revealing the octagonal ring design.

With an overall spatial resolution of 2.4 mm and an extended axial field-of-view, the image data generated by the HRRT encompasses the entire head and neck of the subject in exquisite anatomical detail (Figure 4.6).

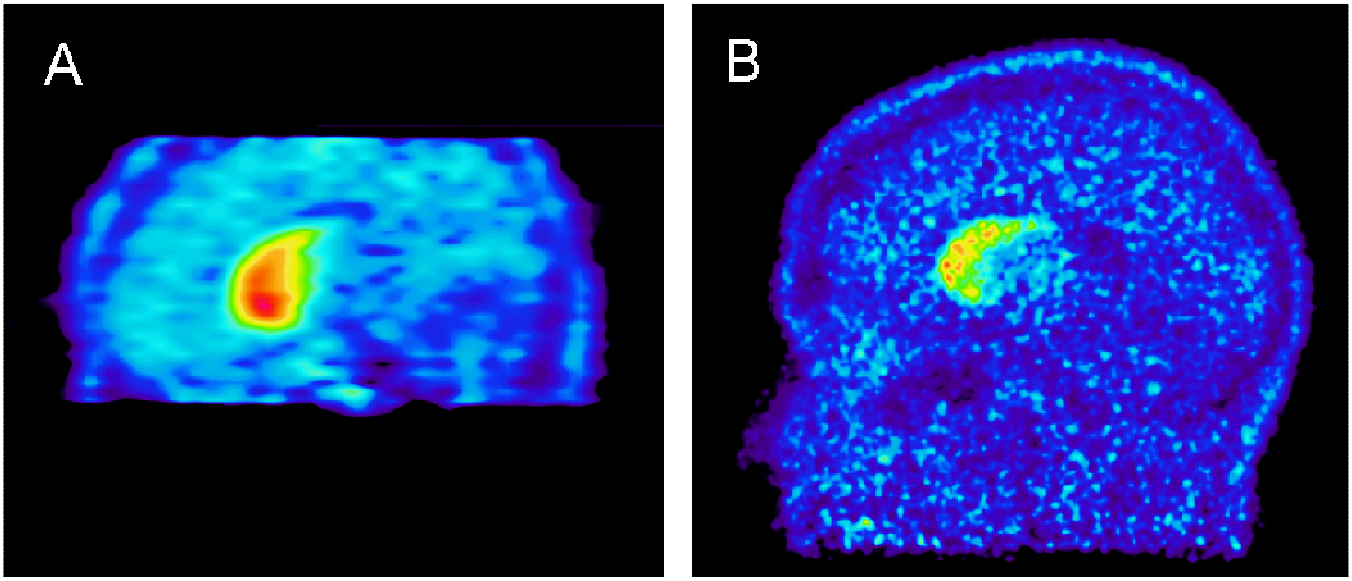


Figure 4.6 Sagittal [^{11}C]raclopride PET images of a PD patient taken using the A) ECAT 953B and B) HRRT scanners. The extended axial view of the HRRT can clearly be seen, as the patient's entire head and neck are visible.

Some performance characteristics of the HRRT are outlined in Table 4.1, as compared to the ECAT 953B tomograph, the other scanner currently in use at the UBC PET Program, and which was used for the pilot study described in Chapter 3. The vast increase in crystals and possible LORs in the HRRT over that of the ECAT provide an idea of the massive increase in the size of the data set, and corresponding computing time required to reconstruct the images. Of particular relevance to the analysis of the image data is: 1) the increase in the axial field-of-view in the HRRT, which enables the full extent of the subject's head to be seen, and 2) the increase in spatial resolution, allowing brain regions to be identified with greater precision.

Specifications	ECAT 953B/31	HRRT
Radial Field-of-View	11 cm	31.2 cm
Axial Field-of-View	7.6 cm	25.1 cm
Number of Planes	31	206
Plane Thickness	3.37 mm	1.22 mm
Sensitivity	2%	6%
Voxel Dimensions	2.61 x 2.61 x 3.37 mm^3	1.22 x 1.22 x 1.22 mm^3
Resolution (centre)*	5.0 mm	2.4 mm
Total No. of Crystal Elements	6144 BGO	119 808 LSO/LYSO
No. of Lines of Response	8×10^6	4.49×10^9
Size of data set	16 MB	1 GB

Table 4.1. Specifications for two PET scanners currently in use at the UBC PET Program. BGO, Bismuth Germanate; LSO/LYSO, Cerium-doped lutetium/-yttrium oxyorthosilicate. * The resolution as measured by the full-width-at-half-maximum of the point-spread function at the centre of the field-of-view.

4.2.2 Rationale for new image analysis methodology

Given the improved data quality provided by the HRRT, we wanted to refine our processing and analysis methods to take full advantage of the increased spatial resolution as well as account for the challenges in dealing with the different characteristics of the scanner. In particular, we discovered that patient motion posed a much more significant problem to the HRRT data and degraded the image quality to a more noticeable extent than on ECAT data, as even small motions (1-3 mm) are on the order of the spatial resolution of the scanner. In addition, the increased spatial resolution offered an opportunity to delineate subcortical structures with a high degree of certainty (for example, separation of the caudate nucleus and putamen by the internal capsule), and we wanted to incorporate this new anatomical specificity into the quantification of our regions-of-interest (ROIs). Finally, these aforementioned processes required the exploration of different analysis tools and software, as the HRRT data format and file architecture were entirely different from those of the ECAT. The following sections describe the development of the methodology that is now currently in use at the UBC PET program to analyze HRRT data.

4.3 Overview of PET image data analysis

In order to describe how the new analysis methods were designed and implemented, an understanding of the steps involved in the image data processing is required. The process is outlined in Figure 4.7 below.

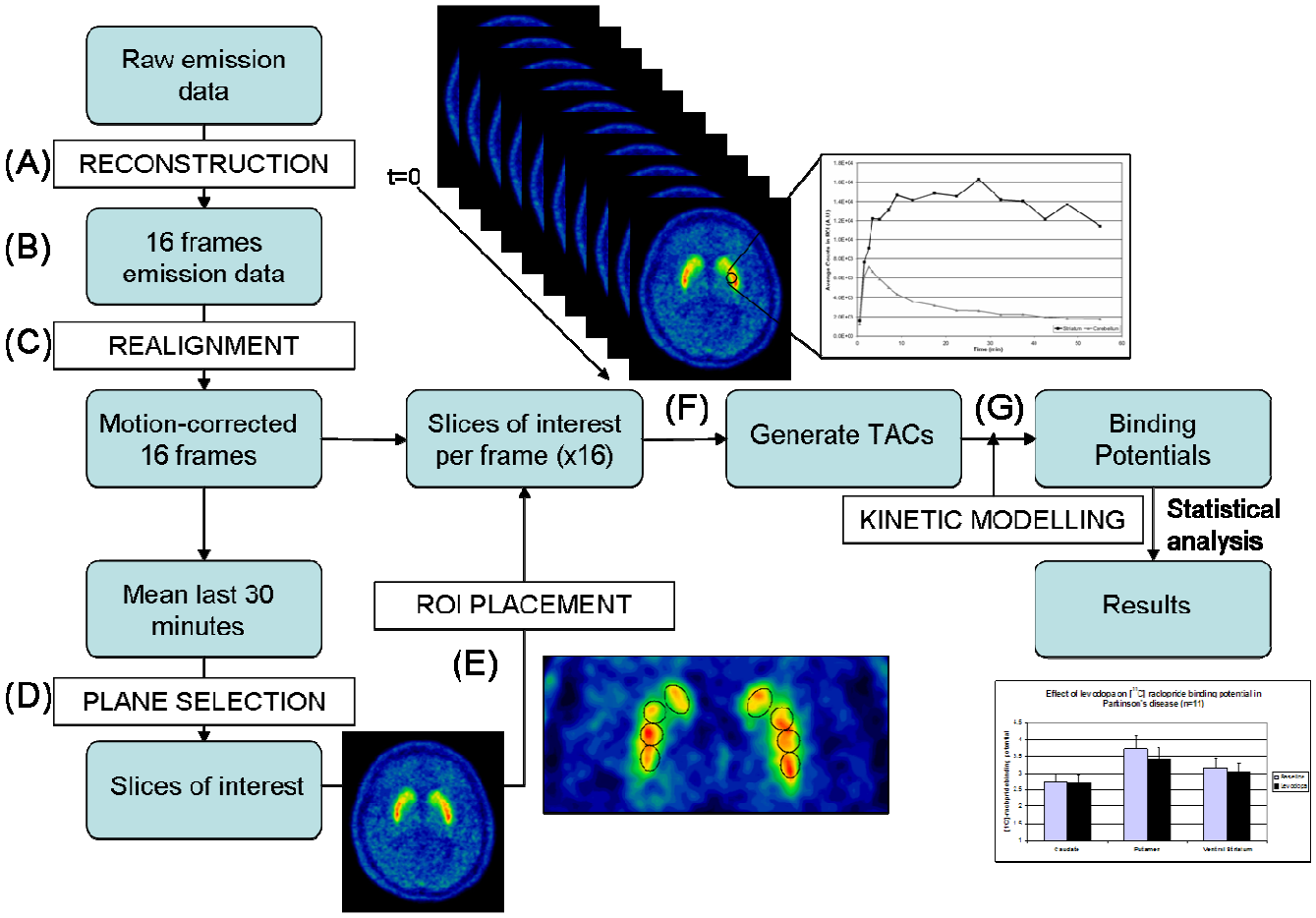


Figure 4.7 Flowchart of the steps involved in ROI-based HRRT PET data analysis, from image reconstruction (A) to calculation of the binding potentials (G), using a RAC PET scan as an example. Blue boxes represent the different conformations of the data, and the white rectangles indicate the manipulations that were made to the data. See text for description of the steps.

Briefly, raw emission data are reconstructed using a statistical algorithm containing the correction factors outlined in Section 4.1.3 (Figure 4.7, A). The result of the reconstruction is 16 separate time frames of image data in 16 separate files (Figure 4.7, B). Patient motion that occurs between frames is corrected for by realigning each frame to the mean image of the last 30 minutes of the scan (Figure 4.7, C). The investigator then selects the planes in which the brain structures of interest are best visualized, also using the mean image (including all planes) of the last 30 minutes of the scan when the tracer uptake is best visualized (Figure 4.7, D, bottom image). The investigator then manually places regions-of-interest (ROIs) on the striatum, in the case of RAC (Figure 4.7, E). If the inter-frame motion correction was successful, the ROIs should fall in the same place on each frame. A TAC is generated for each ROI (one is shown in Figure 4.7, F) by calculating the mean activity within the ROI in each frame. A TAC is also generated for the reference region for the tracer, which is the cerebellum in the case of RAC. These TACs are used as the inputs into a kinetic model which calculates the binding potentials of the tracer within the ROIs, defined as B_{max}/K_d . Although this framework had already been established for the analysis of ECAT data, the HRRT data presented unique challenges that required adaptations at every step. These adaptations are described in the following sections.

4.4 Validation of HRRT human data

4.4.1 Introduction

The data presented in Chapter 5 were the first human data to be collected using the HRRT. Prior to that, the physicists responsible for the scanner conducted several phantom studies in order to ascertain if the imaging data accurately reproduced the known activity used in the phantoms. In doing so, they were able to identify the basic corrections that needed to be applied in order to obtain reliable quantification. Phantom studies are useful because the "truth," i.e. the concentration and location of the tracer, is known. However, they do not encompass the

complexity of human data and therefore their use is restricted to identifying and solving only a certain number of problems. Human data present different challenges, over and above those that can be solved with phantom studies. For example, the time course of tracer concentration is measured in human data (the TAC), versus a static measurement in phantoms. Human data are subject to non-uniform photon attenuation, which affects the scatter correction, and so the reconstructions include a wide dynamic range of count rates. These issues are not encountered in phantom studies. In addition, there is significant inter-subject variability in baseline RAC binding potentials in the population (Farde et al., 1995). Furthermore, there were yet no published results demonstrating if and how the increased resolution of the HRRT affected RAC binding potential values. Therefore, at that point, we did not know if the results we obtained were due to the resolution improvements in the HRRT, or were due to the fact that some unknown corrections had not been applied to the data.

4.4.2 Methods

Given that we simply did not know what to expect in terms of absolute binding potential values using the new tomograph, it was necessary to assess the measures obtained based on expected biological responses and comparison to literature values obtained using tomographs with lower resolution. In order to achieve this, RAC data sets from five PD patients were used. These patients were scanned as part of the study presented in Chapter 5, and the data sets consisted of a baseline RAC scan where the patients had been withdrawn from their medication, and a RAC scan one hour following the administration of oral levodopa. Literature values for the percent decrease in RAC binding in PD patients in response to levodopa are approximately 10% (Pavese et al., 2006;de la Fuente-Fernandez et al., 2004). We needed to use a change in binding potential as the benchmark measure - rather than the absolute baseline binding potential – due to the variability in the population, and in chronic PD patients in particular. The data sets were

analyzed according to the schematic in Figure 4.6, and binding potentials were calculated and compared to literature values.

4.4.3 Results

The improvement in data quantification over time is illustrated in Figure 4.8. Levodopa-induced DA release in the striatum from the same five data sets was measured at various time points over the course of one year, three of which are shown below. The changes observed between the values obtained in June 2006 and January 2007 were mainly due to refinements made to the data reconstruction, and incorporation of the proper correction factors, whereas the changes observed between January 2007 and May 2007 are mostly due to refinements made to the frame-to-frame realignment methods described in Section 4.6. Having access to image data from a study with an expected outcome based on neuroscience was critical in determining the accuracy in data quantification. Thus, we expected that levodopa would induce DA release in the striatum of the PD patients, thus the RAC binding potentials (BP) following the drug administration would be lower than baseline. Based on this tenant, we were able to attribute wildly different BP values to potential problems in the pre-processing of the data, which helped in the identification of problems in the data analysis.

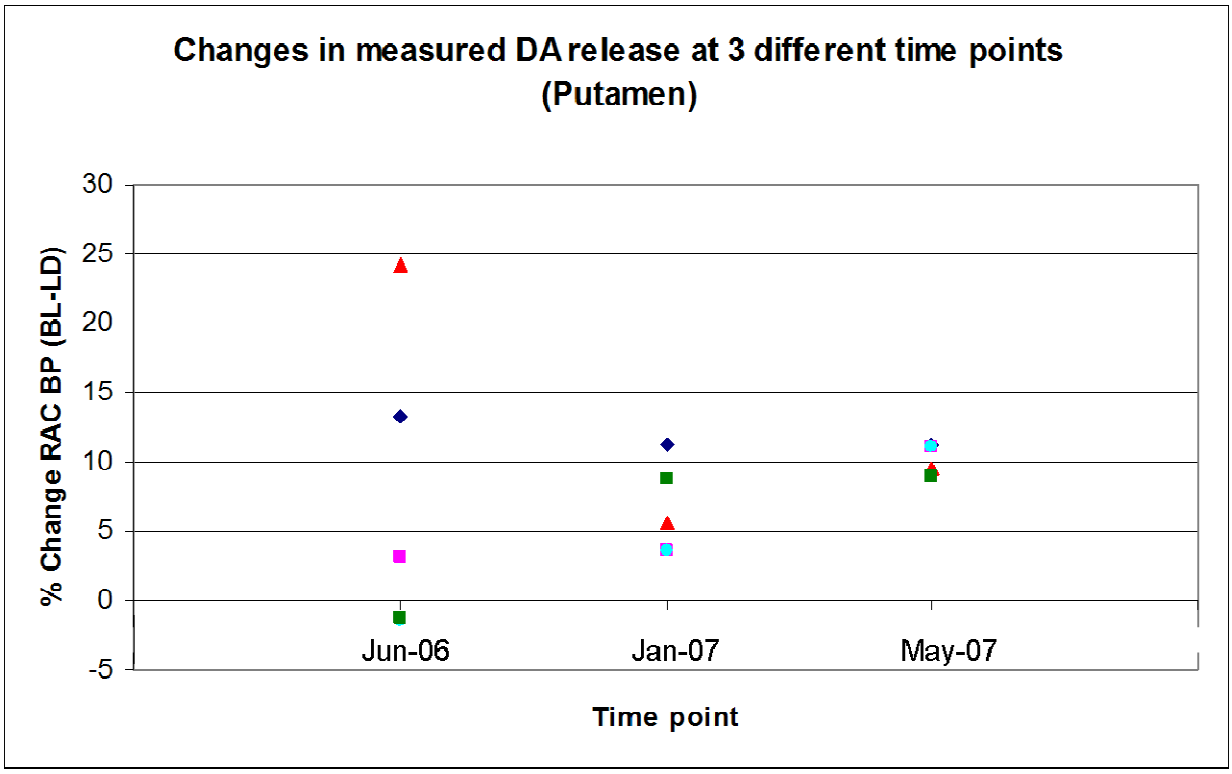


Figure 4.8 DA release as estimated by 100*($RAC_{baseline}$ – $RAC_{levodopa}$)/$RAC_{baseline}$ in the putamen of 5 PD patients with analysis performed at three time points over the course of one year (June 2006-07). In June 2006, with a suboptimal data quantification algorithm a wide, biologically unrealistic variation is observed between subjects in response to levodopa. Results are much more consistent after improvements in data reconstruction and realignment methods, and comparable to published literature values of approximately 10% reduction in RAC binding in response to levodopa (Pavese et al., 2006;de la Fuente-Fernandez et al., 2004).

4.5 Implementing the methodology for motion correction of HRRT data

4.5.1 Introduction

Patient motion during the PET scan can be a major source of image degradation, and is especially problematic when studying those suffering from movement disorders such as PD. PET scans can be long, for example, 60 minutes for a typical RAC emission scan, in addition to a 10-minute transmission scan and the time required for patient positioning means that the patient is in the scanner for 75-90 minutes. Furthermore, many protocols require that the patient be off medication, which can also contribute to excessive motion. Thus, patient motion is an inevitability, as complete subject immobilization would require sedation which is clearly

undesirable, both in terms of the ethical implications of using anesthetics in PD patients, as well as the potential effects of anesthetics on RAC binding (Dewey et al., 1993). As severe head movement restriction would cause significant patient discomfort (including anxiety and claustrophobia), current measures to reduce motion involve 'gentler' head restraint techniques, such as the use of thermoplastic masks or Velcro straps. As can be seen in Figure 4.9, these devices still allow some motion (anywhere from 1-20 mm), but serve both as a reminder to the subject to try not to move, as well as an aid in repositioning a subject undergoing repeated scans.

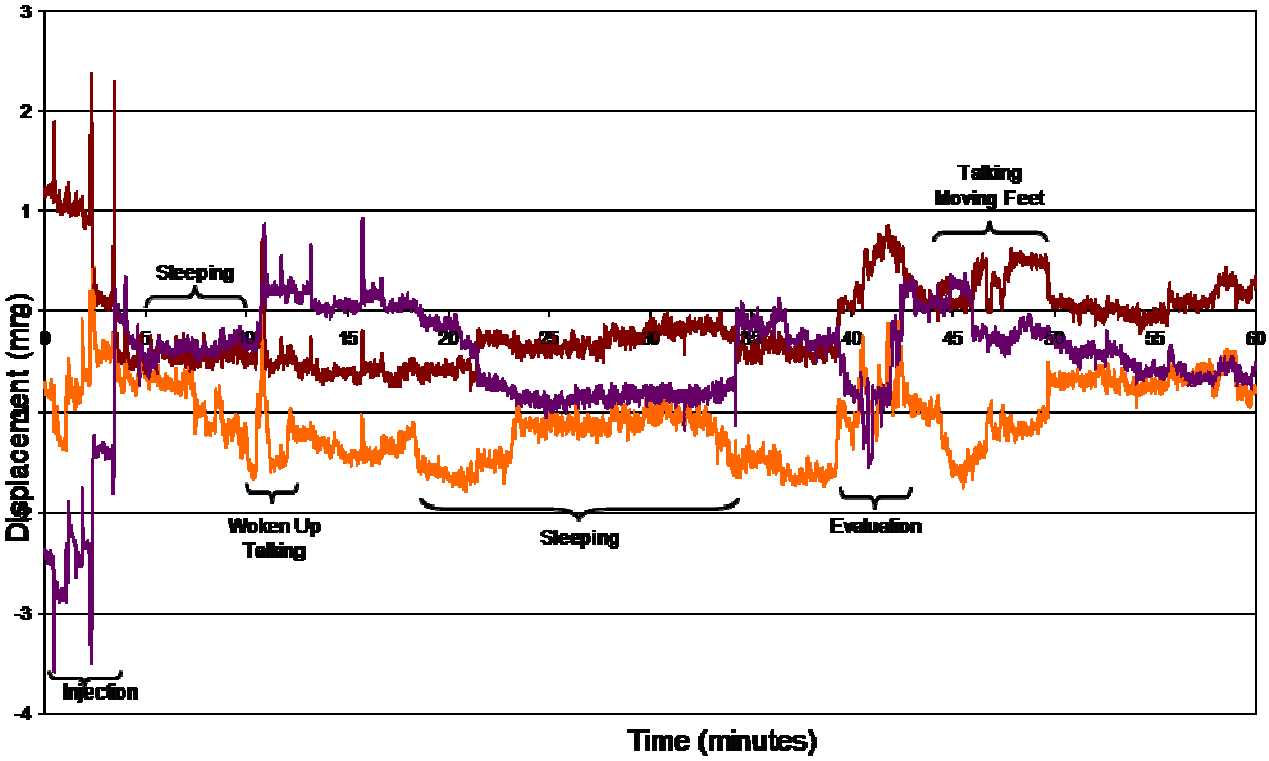


Figure 4.9 Displacements in (mm) made by a PD subject wearing a thermoplastic mask restraint during the duration of a 60-minute RAC PET scan, along the horizontal (purple), vertical (orange), and axial (red) axes. Observations of what the patient was doing at the time of motion are included.

Numerous studies have demonstrated the impact of motion on data quantification. Based on Figure 4.9, it can be seen that even the smallest head movements (5mm) are close to or even greater than the spatial resolution of the HRRT, which can cause image degradation and problems in radiotracer quantification. The impact of motion on the results ultimately depends

on the ability to apply a suitable correction. The most common method, and that which is least likely to degrade the data, is frame-to-frame realignment post-reconstruction. However, this method had not been validated on HRRT data, and it was unknown if it would 1) be effective, and 2) be worth the significant computation times required. Thus, we wanted to first qualify and quantify the impact of patient motion on the HRRT data, and implement a robust method to correct for it, and determine if this correction method was necessary and effective.

4.5.2 Methods

Currently, the standard motion correction technique applied to PET data is performed post-reconstruction and involves realigning the frames to a common target, using an algorithm that minimizes the differences between voxels. The target frequently used is the mean image of the final 30 minutes of the scan. It should be noted that this method only corrects for motion in-between frames, and not within frames. In order to correct for within-frame motion, the subject's head position must be tracked throughout the duration of the entire scan. This was accomplished using the Polaris Motion Tracking system, which records the position of 4 retro-reflective spheres attached to the subject via a thin, neoprene swim cap (Bloomfield et al., 2003). Using this system, we measured the amount of patient motion and inferred how different brain regions would be affected by that motion. When the motion plots were examined, it became evident that the degree of motion would almost certainly have a negative impact on the quality of the data. In addition, having access to the motion plots meant that we knew *a priori* which imaging data would be subject to the most motion in order to test the motion correction protocols we developed. An example of the motion tracking can be seen in Figure 4.10.

We selected a commercially available, standard package for registration of PET data (Automated Image Registration, AIR) (Woods et al., 1993) and undertook an iterative approach in determining the optimal parameters for HRRT frame-to-frame realignment. At each iteration, we tested various cost functions, different minimization strategies, and a variety of sampling,

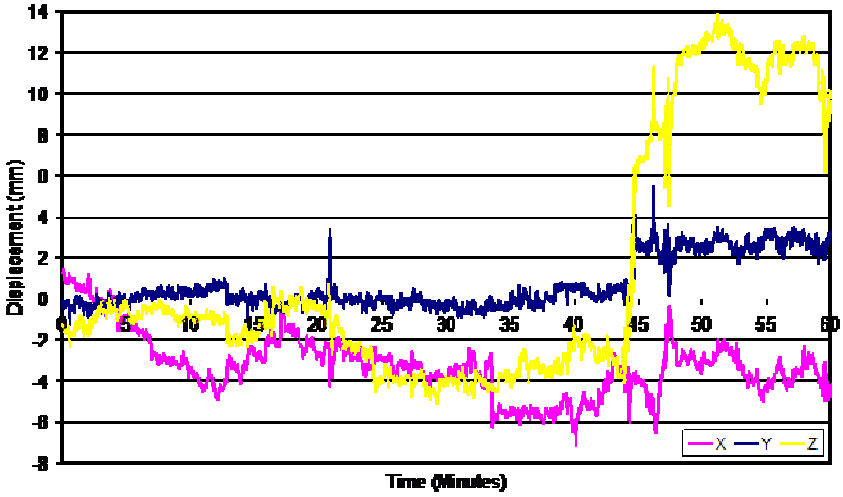
editing and smoothing of the image data, representing the different variables used by the algorithm. As a starting point, we used the values which were validated in the literature using a PET scanner of lower resolution (Woods et al., 1993). To determine the efficacy of the algorithm, the images from both low- and high-motion test cases were carefully visually inspected following each iteration. These tests were carried out on a subset of data from PD patients that were collected as part of another protocol (see Chapter 5). If the realignment was successful, ROIs placed on one frame should carry over into the same position on subsequent frames. The position of the ROIs across frames was then visually inspected, as well as the TACs, to qualitatively determine the success of the realignments. Finally, binding potentials were calculated (for example, baseline RAC binding versus levodopa) to ensure that the results made biological sense.

4.5.3 Results

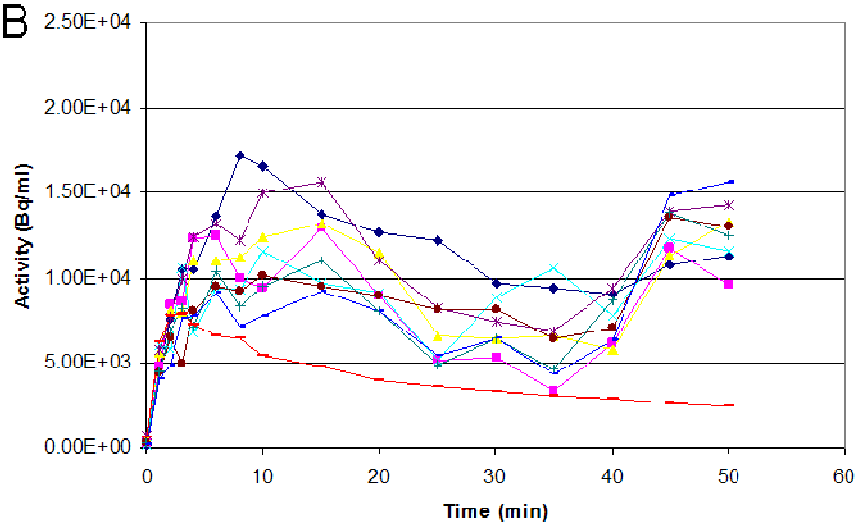
Qualitative impacts of realignment

Several versions of the realignment algorithm were tested over the course of two months, until a version was selected which was effective at registering frames within both low- and high-motion cases. The clear impact of realignment was seen on the TACs generated for ROIs placed on the striatum, shown in Figure 4.10. This subject exhibited substantial amounts of motion during the scan, as can been seen in the motion tracking plot, and frame-to-frame realignment restored the TACs to a normal pattern.

A


Displacement (mm)
Time (Minutes)
X
Y
Z

B


Activity (Bq/ml)
Time (min)

C

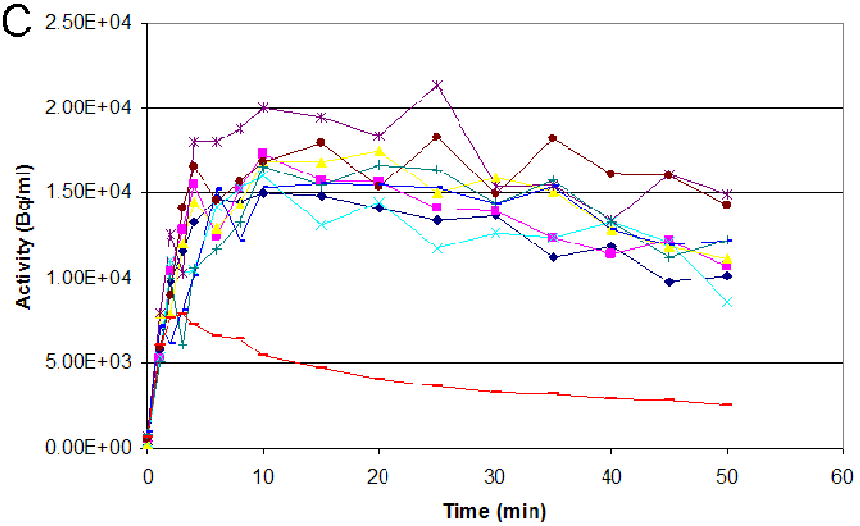

Activity (Bq/ml)
Time (min)

Figure 4.10 The effects of motion during a 60-minute RAC PET scan on image data quantification. The motion tracking plot is shown for a PD subject (A), as well as the resulting TACs for 8 striatal ROIs and one cerebellar ROI before (B) and after (C) frame-to-frame realignment. (A) This subject exhibited substantial motion in all directions (x,y,z) during the scan, and during the last 15 minutes in particular, as evidenced by the blue, pink and yellow traces. TACs were generated for 8 striatal ROIs (upper traces in B,C), and one cerebellar ROI (bottom trace in B,C). Without realignment, the TACs display an uncharacteristic upward pattern toward the end of the scan (B). Following realignment, the expected pattern is restored for all ROIs.

Quantitative impacts of realignment

The impact of frame-to-frame realignment on the RAC BPs was much greater in the high motion data. Two representative cases, one with low motion and one with high motion, are shown in Figure 4.11. In both cases, the realignment between frames restored the characteristic 10% decrease in RAC BPs in response to levodopa in the putamen.

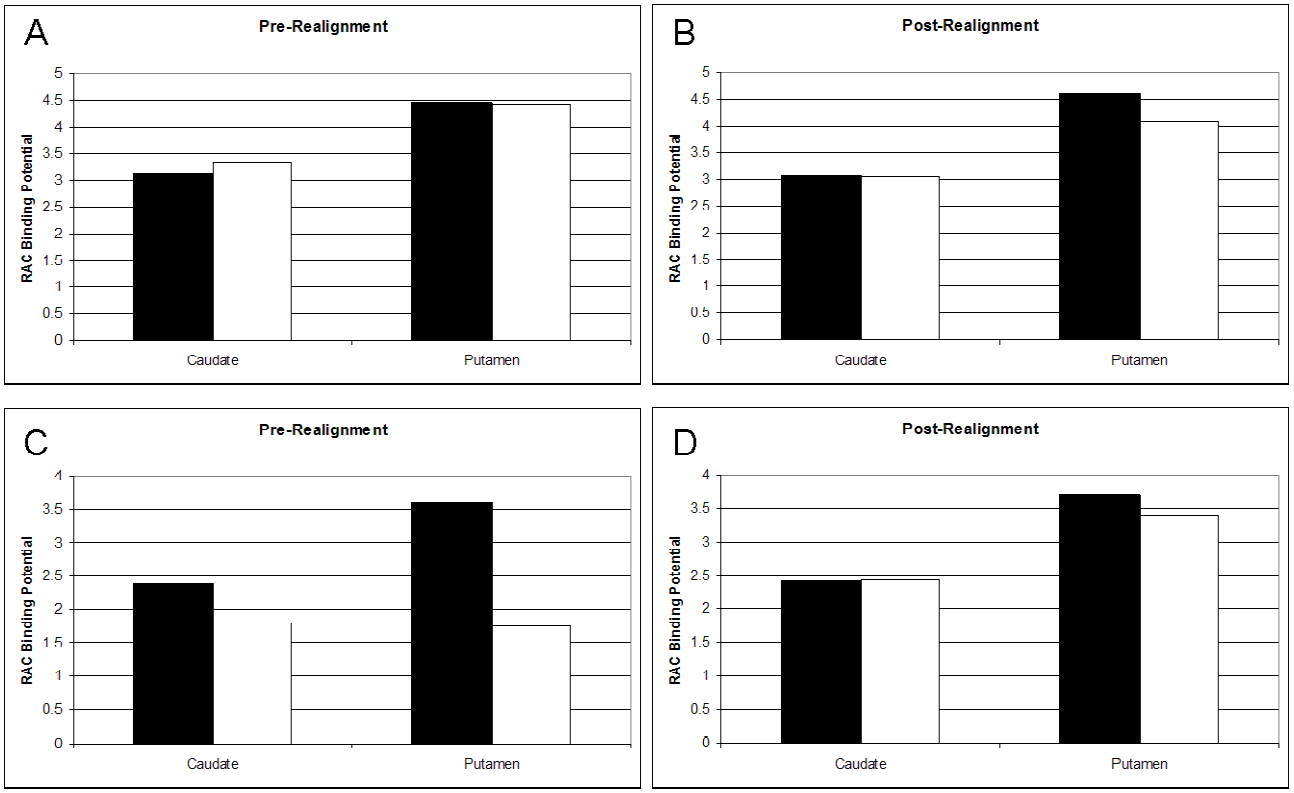


Figure 4.11 Striatal RAC BPs in a PD patient with low motion (A,B) and high motion (C,D) scans, at baseline (black bars) and following oral levodopa (white bars), before (left panels) and after (right panels) frame-to-frame realignment. The impact of motion on the BPs can been seen in the pre-realignment panels, where there no effect of levodopa is seen in the low-motion case

(A) and a biologically unlikely effect (52% decrease in RAC BP in the putamen) is seen in the high-motion case (B). Realignment was effective in restoring biologically meaningful results; an 11% (C) and 8% (D) decrease in putaminal RAC BPs in response to levodopa.

4.5.4 Discussion

Frame-to-frame realignment of the PET data proved to be an effective method of motion correction. The impact was more pronounced in scans in which the patient exhibited a large amount of motion, which is almost certain to occur when scanning patients with movement disorders such as PD. As a result of this work, this method of motion correction has now been implemented in our centre and is used for all HRRT data with a high rate of success (see Appendix D for the final protocol). One benefit of this method of registration is that multiple scans from the same subject can be registered to the same target, for example, a baseline scan. This feature is particularly useful for RAC scans which require at least one baseline and one intervention scan in order to measure DA release. If both images are in the same position, the same ROI placement can be used, reducing the analysis time significantly (this method was used in Chapter 5).

Although effective, this method of frame-to-frame realignment is not without drawbacks. First, it corrects only for motions that occur between frames, and not those within frames. This can be addressed by incorporated the motion tracked information directly into the data reconstruction, which is currently being implemented in our program. Second, the data are interpolated, which degrades the data to a small extent. This must be taken into consideration if further registrations are made, for example, if PET-MRI co-registration is used to draw ROIs. Finally, it can be difficult to visually detect very small errors in the registration, and at the moment, there is no mechanism that has been developed to test the success of realignment other than visual inspection. However, this may not be a large problem for ROI-based analysis, since any realignment discrepancies will be easily spotted during creation of the TACs.

4.6 Implementing a methodology for region-of-interest-based data analysis

4.6.1 Introduction

When attempting to quantify tracer binding, an ROI-based approach necessitates defining the areas of interest within the brain *a priori*. RAC PET scans provide very clear signals of tracer uptake in the striatum, but less so for extrastriatal sites. Most problematic to the analysis of PET scans is the fact that the images are a measure of functional information, and may not directly represent the underlying anatomy. This can be addressed by co-registering a subject's PET scan with their anatomical, T1-weighted MRI to confirm the ROI placements. However, if the activity concentration of the tracer is clear, as is the case for RAC images, ROIs can be determined without anatomic reference. In fact, it has been shown that for RAC PET scans, drawing ROIs directly onto the PET images provides results which are virtually identical to those derived when MRI co-registration is used (Wang et al. 1996).

Each new generation of PET scanners is accompanied by an improvement in image resolution, from the PETT III (1976) which had a resolution of 10.35 mm FWHM (Hoffman, 1976) to the HRRT (2002) boasting a resolution of less than 2.4 mm FWHM (Wienhard, 2002). The impact of improved resolution on image quality is easily visible in Figure 4.12 which shows images from tomographs with progressively higher resolution capabilities (A–C).

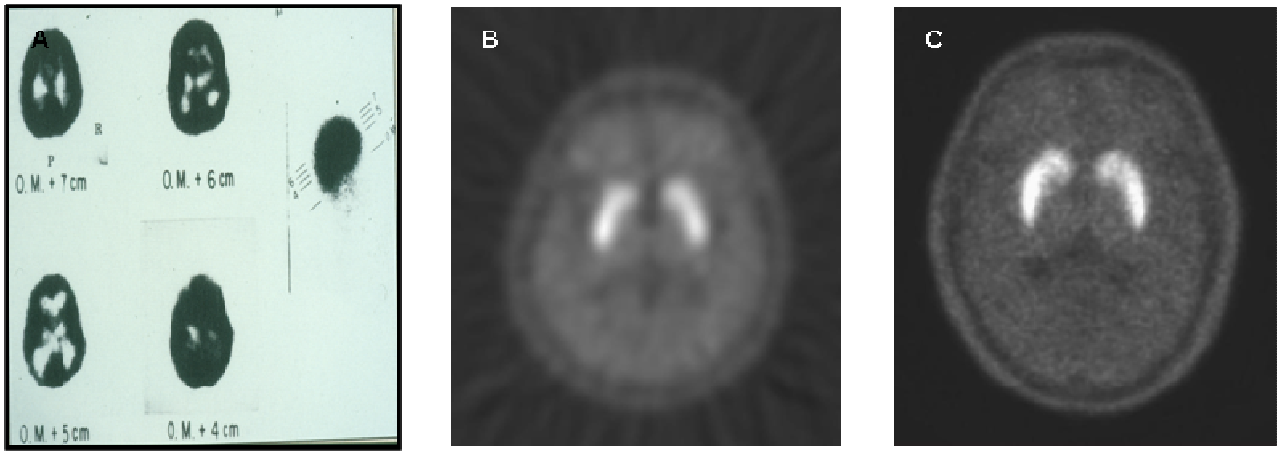


Figure 4.12. PET images of the human brain with scanner resolution increasing from left to right. Images are from the PETT III (A), ECAT 953 (B), and HRRT (C).

Paradoxically, increasing the resolution of the images introduces more challenges in defining ROIs, as the limitations to the technique become more obvious. Figure 4.13 illustrates an ECAT image with its corresponding ROI template on the dorsal striatum. When this same template is placed on the HRRT image, the increased resolution reveals that the ROIs are capturing the internal capsule as well. Clear boundaries of the caudate and putamen are visible in the HRRT image and so the ROIs can be adjusted, whereas in the ECAT image the ROIs are simply laid tip-to-tail as no differences between the structures is visible. Furthermore, the almost seven-fold increase in the number of planes over that of the ECAT meant that criteria had to be developed for selecting the planes to include in the analysis.

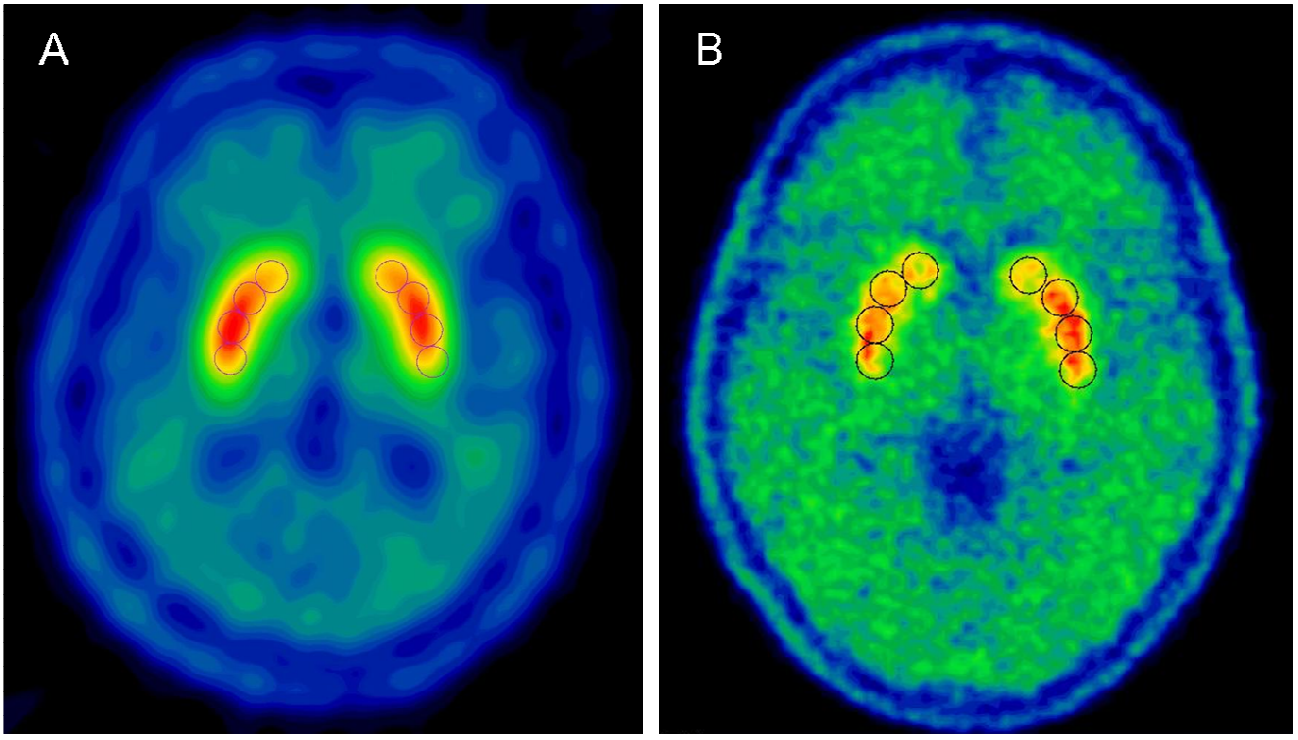


Figure 4.13 RAC images from the (A) ECAT 953B and (B) HRRT with the standardized ROI template from the ECAT overlaid on the striatum.

In order to take full advantage of this increased spatial resolution and ability to delineate subcortical structures, it was necessary to refine the method of ROI-based analysis. Specifically, two aspects needed to be addressed: what shape of ROIs should be used, and which planes

should be included. Since the operator can greatly affect the outcome, we needed to define metrics that could be used to incorporate some objectivity and reproducibility into the decisions. We used knowledge of the neuroanatomy of the striatum to define the volume and areas of the ROIs, as well as the number of planes to include, and knowledge of the biology of the nigrostriatal DA system to validate the methods.

4.6.2 Methods

These investigations were carried out using four representative RAC PET scans (2 baseline, 2 post-levodopa administration) from 2 PD patients, collected as part of another protocol (see Chapter 5). At the time, we did not have access to anatomical MRIs for the subjects, thus all investigations were performed directly on the HRRT data. The data were realigned according to the protocol outlined in Figure 4.6. Mean images of the last 30 minutes of the scans were generated in order to best visualize the striatum, as the majority of the tracer activity represents selective binding to DA $D_{2/3}$ receptors during this time. These mean images were used for plane selection, ROI template construction, and ROI placements for the comparisons between raters. All investigations were carried out on transaxial (horizontal) sections.

ROI Shape Selection

The total volume of the striatum is approximately 20 cm^3 in an adult (Firnau 1986), although not all of this is sampled in the analysis. According to Mai *et al*. (1997), a very rough estimate of the area of the caudate and putamen in a horizontal section at the level of the midpoint of the striatum is approximately 180 mm^2 per side, or 360 mm^2 bilaterally. We chose the same template used for the analysis of ECAT data as a starting point (Figure 4.13). This template consists of 4 consecutive large circles placed end-to-end over the anterior-posterior extent of the striatum (one on the head of the caudate and three on the putamen). Then, three additional templates were constructed using a combination of circles and ellipses. Ellipses were

only used for the head of the caudate nucleus and the posterior putamen, in accordance with the anatomical shape that can be seen in Figure 4.14.

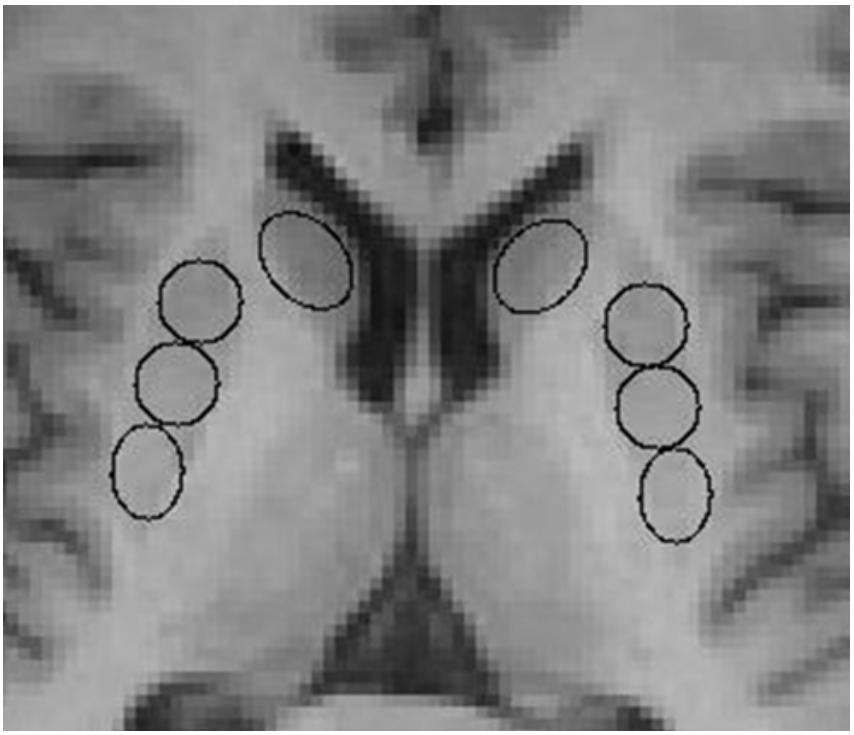


Figure 4.14 T1-weighted MRI of a PD subject depicting the striatum with elliptical ROIs overlaid on the head of the caudate nucleus and the posterior putamen.

The areas were varied systematically to include a large and a small version of each shape: we hypothesized that larger ROIs would result in less inter-rater differences as there are fewer options for ROI placement, while smaller ROIs are easier to place on areas of high tracer uptake. The shapes and areas of the four templates, labeled T1-T4 are shown in Table 4.2, and depicted in Figure 4.15.

Template	Caudate (C)	Anterior Putamen (P1)	Intermediate Putamen (P2)	Posterior Putamen (P3)	Total Area (mm^2)
T1	89.12 ellipse	69.81 circle	69.81 circle	50.5 ellipse	558.49
T2	69.81 circle	47.53 circle	47.53 circle	47.53 circle	424.81
T3	74.27 ellipse	47.53 circle	47.53 circle	50.5 ellipse	439.67
T4	69.81 circle	69.81 circle	69.81 circle	69.81 circle	558.49

Table 4.2. Areas and shapes of the four candidate ROI templates, T1-T4, for the dorsal striatum. All areas are in mm^2. The total area shown is for both sides (i.e. 8 ROIs). The total volume of the ROIs used would depend on the number of planes selected.

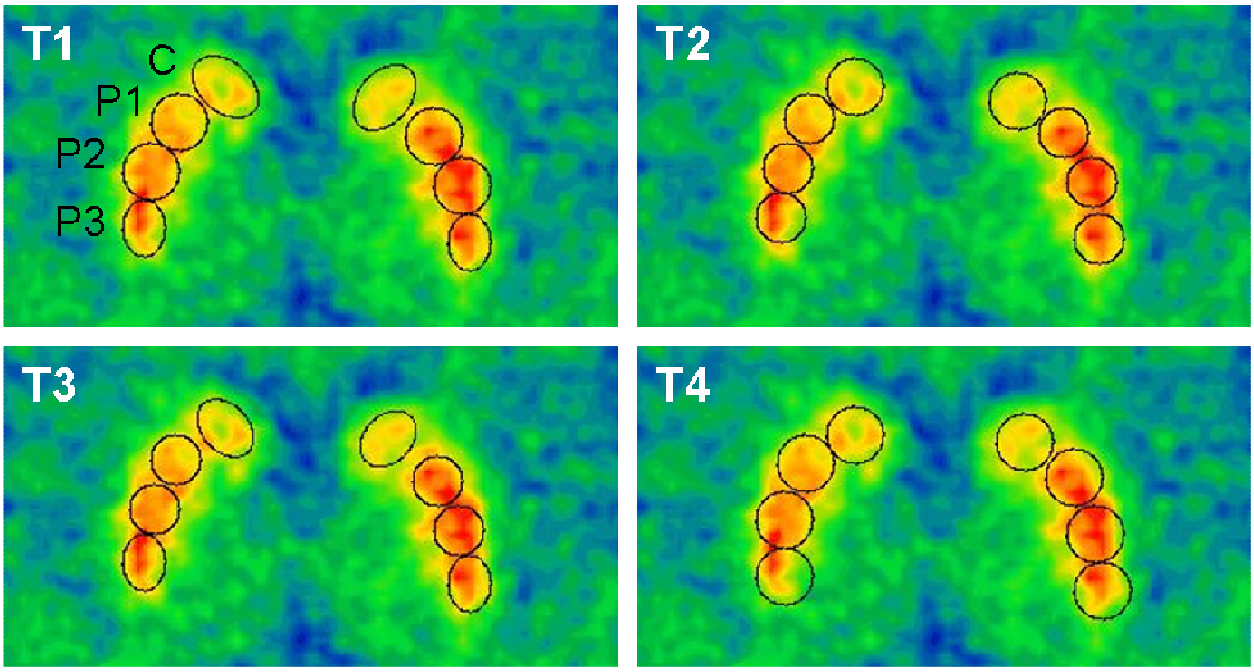


Figure 4.15 Candidate ROI templates used for analysis the dorsal striatum of RAC scans. Templates (T1-T4) are shown on the same mean image of 9 planes in horizontal section through the striatum. The head of the caudate nucleus has one ROI (C, top of each panel), and the putamen has three running from anterior to posterior (P1, P2 and P3). T1 and T3 use a combination of ellipses and circles (T1, larger and T3, smaller), and T2 and T4 use only circles (T4, larger and T3, smaller). T4 is the same template used for ECAT image analysis. Blue areas indicate lower tracer uptake and red areas indicate high levels of tracer uptake.

It was necessary to devise metrics in order to assess if one template provided superior results. The ROI templates were therefore compared on the grounds of validity and reproducibility. Validity was examined in two ways: the face validity of the templates was assessed by determining the ease of template use, as well as how well they represented the underlying anatomical structures, and the biological validity was assessed by determining how well the different templates reflected the biological measure of DA release. In addition, the absolute values of the binding potentials from the different templates were compared to determine which shapes and sizes provided higher results, which is indicative of a better ability to capture the structure of interest with less contamination from partial volume effects. Finally, reproducibility was examined by comparing the results performed on the same data sets separately by two

independent raters. The RAC BPs for each ROI for each template were then calculated using the same cerebellar TAC as the input function. The outcome measures used were the percent differences between raters in the BPs. These analyses were carried out using both 9 and 12 planes.

4.6.3 Results

Face Validity

We found that the templates that included ellipses on the head of the caudate and the posterior putamen were easier to place, and better reflected the underlying anatomy. The difference in shapes and sizes of the ROIs restricts where they can be placed along the anterior-posterior axis of the striatum. For example, it can be seen in Figure 4.15 that due to the large size of the circles in T4, the P3 ROI is capturing only the most posterior aspect of the putamen (the "tail"), and a larger proportion of colder area. This is in contrast to T1 or T3, in which P3 is a smaller ellipse, and due to the conformation of the rest of the template, is able to capture a hotter region that more closely follows the shape of the activity. When the same templates are placed on the MRI, it can be seen that the larger circles fail to conform to the anatomy of the posterior putamen or the head of the caudate (Figure 4.16).

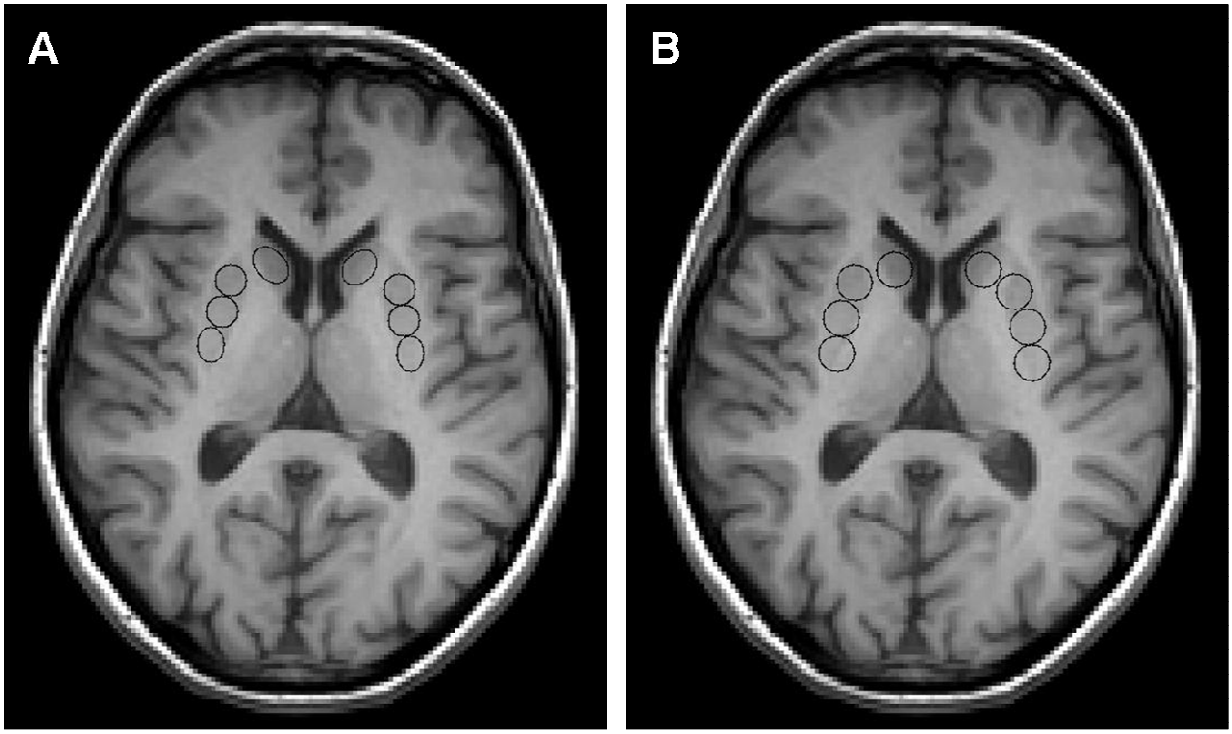


Figure 4.16 T1-weighted MRIs from the same PD subject showing how T3 (A) and T4 (B) reflect the anatomy of the striatum.

Biological Validity

We assumed that if a particular ROI template is better than another, the ROIs within it would better capture the activity in the striatum, i.e. the hotter areas. We found higher BP values using the templates with the smaller circles and ellipses, indicating less contamination due to partial volume effects. Interestingly, both ellipses and circles on the head of the caudate provided essentially the same results.

In terms of the number of planes, we found that using 12 planes provided lower average BP values for each ROI. Since we were capturing a larger extent of the striatum (superior to inferior), the edge planes (i.e. at the top and bottom) contained lower amounts of tracer uptake, and thus resulted in a lower average activity. This was confirmed post-hoc using coregistered T1-weighted MRIs, where it could be seen that ROIs placed the edge planes contained little grey matter of the putamen.

Reproducibility

The range of the percent difference of the BPs generated per ROI between raters using 9 planes was 0.86 – 5.17 %. The results using 12 planes were similar (range in percent differences was 0.70 - 7.41%). The results are shown in Figure 4.17. Overall, T4 provided the lowest difference in BP values between raters. We assumed that the reason for this is that it is the template with the largest ROIs (large circles) and there are fewer options for ROI placement. The highest degree of inter-rater variability was seen in the placement of P3. Interestingly, for this ROI, T3 provided more consistent results between raters. This could reflect the fact that the ellipse more closely matches the shape of the posterior putamen in a horizontal section and it was therefore more evident where it should be placed. Otherwise, the results were fairly consistent between raters for the other templates for all of the ROIs (Table 4.3).

	T1		**T2**		**T3**		**T4**	
Planes	**9**	**12**	**9**	**12**	**9**	**12**	**9**	**12**
Mean ± S.E.M.	3.54 ± 0.41	3.13 ± 0.51	2.59 ± 0.45	2.38 ± 0.36	2.46 ± 0.32	2.91 ±0.26	2.56 ± 0.61	2.76 ±0.75
L Caud	4.33	2.82	3.91	2.24	2.92	3.30	1.04	2.01
L P1	3.94	1.19	2.51	1.35	3.68	1.82	1.79	2.67
L P2	2.33	2.08	1.26	1.48	1.59	2.81	1.68	1.52
L P3	3.63	5.09	2.21	3.51	2.32	2.09	5.17	7.43
R Caud	2.26	3.98	2.19	2.84	3.05	4.02	1.39	0.60
R P1	4.69	3.37	3.06	2.07	2.94	3.64	3.88	1.60
R P2	2.13	1.67	0.88	1.45	0.88	2.69	0.86	2.29
R P3	5.04	4.87	4.67	4.12	2.32	2.92	4.69	3.98

Table 4.3 Percent differences in BPs between raters for each ROI (far left hand column) for all four candidate templates. Percent differences were calculated according to the following formula: $(BP_{rater\ 1} - BP_{rater\ 2})/BP_{rater\ 1} * 100$. Results for both 9 and 12 planes are shown. The values for all ROIs in a column were averaged to determine the mean difference for each template (row labeled Mean ± S.E.M.).

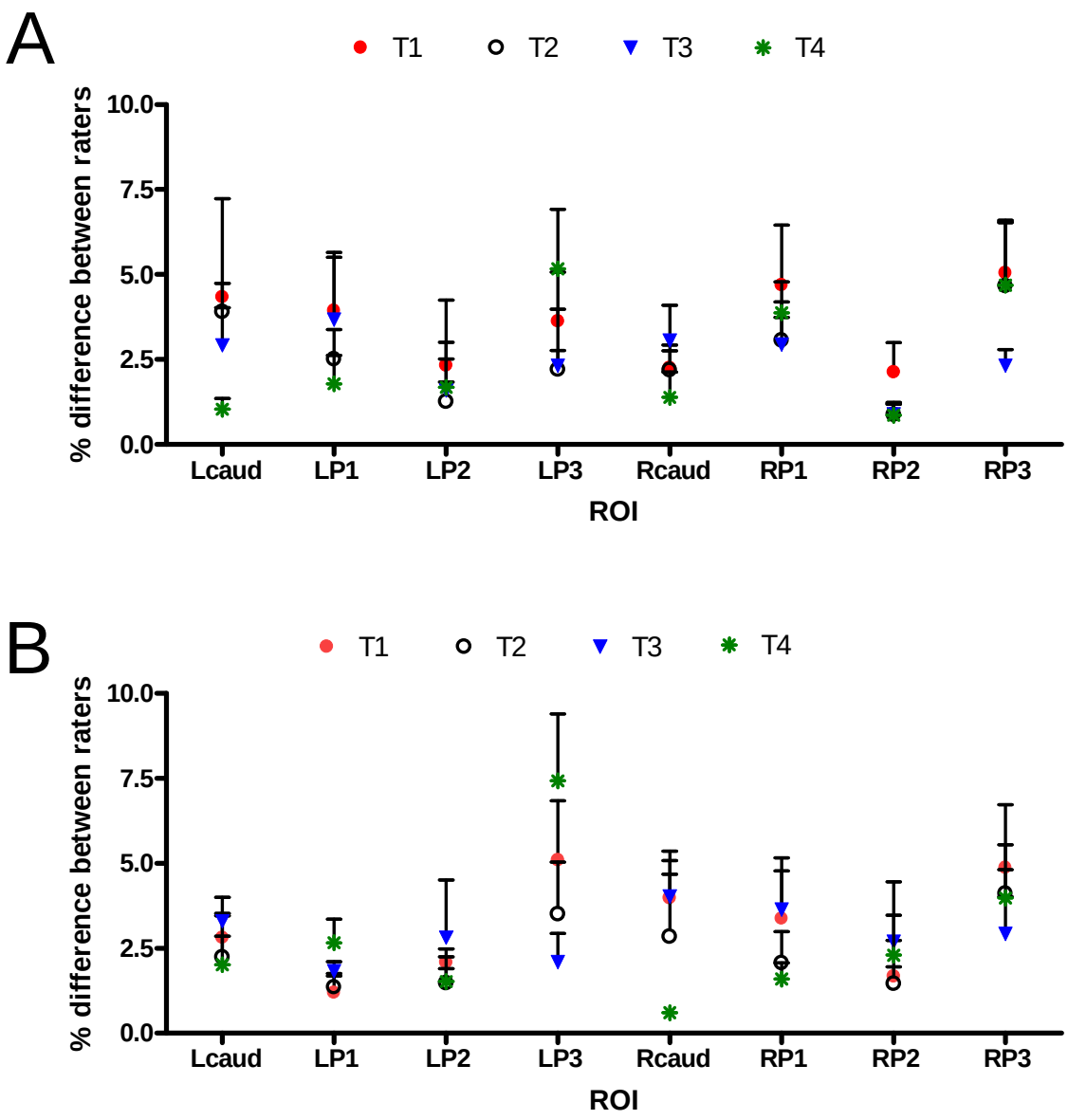


Figure 4.17 Mean + SEM inter-rater variability expressed as a percent difference between BP values generated for each ROI by two separate raters for each ROI template. Percent differences were calculated according to the following formula: $(BP_{rater\ 1} - BP_{rater\ 2})/BP_{rater\ 1} *100$. Results for A) 9 and B) 12 planes are shown. The greatest difference between raters can be seen in P3.

4.6.4 Discussion

Assessing the validity of the different ROIs proved to be a difficult task, as on the whole, the templates were quite similar and thus any differences in quantification of the BPs would be subtle. An ROI-based analysis using the approach where ROIs are placed directly on the PET scans requires consistency between subjects. The use of geometrical shapes ensures that the BP values from subjects are comparable as they are extracted from ROIs of the same area. In addition, any ROI placement protocol must satisfy the following criteria:

1) Valid – the ROIs must capture the structures of interest, both in terms of structure and function, and reflect the underlying biology; and
2) Reproducible – the method must be able to provide the same or very similar results between two independent raters.

Since the results we obtained using the different templates were on the whole quite similar, we decided to choose the template that offered the highest degree of face and biological validity, and that which we found easiest to use. Thus, we opted to use 9 planes for ROI placement, and T3 was selected as the ROI template which provided the combination of being the easiest to use, as the shape best reflected the activity seen in the RAC images, and gave consistently higher BP values.

4.7 Conclusion

This Chapter describes the evolution of the data analysis methodology of HRRT PET data. When the process began, there were yet no published results demonstrating if and how the increased resolution of the HRRT affected RAC binding potential values. In addition, the methods in place for the ECAT data were not suitable for the increase in quality and size of the HRRT image data. We essentially had to start from the beginning, not knowing if the results we obtained were due to the resolution improvements in the HRRT, or were due to the fact that some unknown corrections had not been applied to the data. The processes described in this

chapter resulted in the development and implementation of the standard method of ROI-based analysis of image data from the HRRT that is now in routine use in this Centre. Other methods of analysis are currently being considered and investigated, including voxel-based statistical analyses using parametric images of BP values, as well as other approaches to ROI-based analyses using the individual subjects' co-registered MRIs.

CHAPTER V: Separate contributions of uncertainty and expected reward value in the mechanism of the placebo effect in Parkinson's disease

5.1 Introduction

The placebo effect is commonly detected in trials of therapies for PD, where patients demonstrate clinical improvement in response to pharmacological placebos (de la Fuente-Fernandez et al., 2001b;de la Fuente-Fernandez et al., 2002), sham deep-brain stimulation (Benedetti et al., 2003b;Colloca et al., 2004;Pollo et al., 2002), and sham surgery (McRae et al., 1996;McRae et al., 2004;Watts et al., 2001). Importantly, PD is an excellent model in which to study the placebo effect due to the ability to objectively assess clinical improvement; a blinded examiner can measure changes in motor function in response to placebo using standard clinical scales. Indeed, PD patients can present marked and sustained improvements on objective measures with placebo treatment even in rigorously controlled and blinded trials (Goetz et al., 2000).

Several studies have demonstrated the critical role of expectation in the mechanism of the placebo effect, and expectation has been shown to be associated with release of DA (de la Fuente-Fernandez et al., 2002;Kaasinen et al., 2004;Scott et al., 2008), changes in brain glucose metabolism (Mayberg et al., 2002;Volkow et al., 2003) or changes in subthalamic nucleus (STN) neuronal firing (Benedetti et al., 2004). Most relevant to this work, manipulation of expectation affects the clinical motor performance of PD patients (Mercado et al., 2006;Colloca et al., 2004;Benedetti et al., 2003b;Benedetti et al., 2004;Pollo et al., 2002). The beneficial effects of placebo-induced expectation in PD extend beyond improvement in motor symptoms, as it has been shown that quality of life following fetal transplantation for PD is determined not by the actual surgical procedure (transplant or sham) that was performed, but rather by the patient's belief as to which group s/he was assigned to (McRae et al., 2004). We previously hypothesized that the expectation of therapeutic benefit which is elicited by a placebo can be likened to the

expectation of a reward, particularly in patients suffering from chronic illness who are required to take frequent doses of medication (de la Fuente-Fernandez and Stoessl, 2002;de la Fuente-Fernandez et al., 2002). Thus, in their ability to stimulate positive expectations, placebos can be rewarding in their own right.

It is now established that the placebo effect in PD is mediated by dopamine (DA) release in the dorsal and ventral striatum (de la Fuente-Fernandez et al., 2001b;Strafella et al., 2006). Placebo-induced DA release as measured by [^{11}C] raclopride (RAC) PET has been shown in response to saline when patients were expecting to receive the injectable DA receptor agonist apomorphine (de la Fuente-Fernandez et al., 2001b) and sham repetitive transcranial magnetic stimulation (rTMS) (Strafella et al., 2006). Furthermore, the degree of DA release in the dorsal striatum has been associated with the perceived improvement in motor symptoms of the patient. Our previous study indicated that placebo responders, as determined by subjective measures (i.e. self-report following the PET scan), demonstrated greater DA release in both the caudate nucleus and putamen compared to non-responders (de la Fuente-Fernandez et al., 2001b). However, a related study conducted in PD patients exposed to sham rTMS failed to find a statistically significant difference in the degree of DA release between responders and non-responders, although did demonstrate greater DA release in the putamen contralateral to the more symptomatically affected side (Strafella et al., 2006). Thus, the degree of placebo-induced DA release in the motor areas of the striatum – the primary region of nigrostriatal DA depletion in PD – appears to be graded, reflecting in part the symptomatic requirements of the patient, whether they are consciously perceived or not.

In contrast to the dorsal striatum, the degree of placebo-induced DA release in the ventral striatum may be independent of the perceived benefit felt by the patients. In both our previous study (de la Fuente-Fernandez et al., 2002) and in Strafella et al. (2006), all patients displayed increased DA release regardless of whether they detected a subjective placebo effect.

Mesoaccumbens DA neurons play a crucial role in reward signaling, and human neuroimaging studies have replicated animal studies in demonstrating NAC involvement in the expectation of primary rewards (O'Doherty et al., 2002), secondary reinforcers (Breiter et al., 2001;Knutson et al., 2000;Knutson et al., 2001b), and drug rewards (Leyton et al., 2002). This literature prompted the reward hypothesis of the placebo effect, which states that placebo effects are produced in part by the expectation of benefit which is akin to the expectation of reward, resulting in striatal DA release (de la Fuente-Fernandez and Stoessl, 2002;de la Fuente-Fernandez et al., 2002). This idea has been supported by a recent study in placebo analgesia, which demonstrated both DA and endogenous opioid release in the NAC following placebo administration with the expectation of analgesia (Scott et al., 2008).

De la Fuente-Fernandez et al. (2001) used a paradigm in which PD patients expected active medication for 3 out of 4 scans, thus for each scan the perceived likelihood of symptom improvement was potentially 75% (allowing for the use of varying doses of active medication). In the Strafella (2006) study, patients had a 50% expectation of receiving "real" rTMS. Thus in both studies, there was a significant component of expectation in the paradigms which was able to stimulate striatal DA release. However, it remains unknown if the amount of placebo-induced DA release can be modulated by the patients' strength of expectation. The phasic reward-related activity of midbrain DA neurons depends on reward availability, that is, these neurons encode the probability and magnitude (i.e. value) of the reward that is predicted by a conditioned stimulus in a monotonic fashion; the greater the value of the reward, the greater the amplitude and frequency of burst firing (Tobler et al., 2005). Intuitively, one could speculate that the placebo effect could be maximized when the expectation of benefit is high, whereas if the predicted likelihood of receiving active treatment is low, there will be little (if any) placebo-derived benefit. Thus, as the degree of expectation is modulated, there might be a linear relationship between expectation and striatal DA release. Another possibility is that placebo-

induced DA release represents the uncertainty associated with the subject not knowing if s/he is receiving active medication or placebo. A population of midbrain DA neurons has been shown to increase tonic DA activity in a manner that corresponds to the degree of uncertainty associated with reward prediction (Fiorillo et al., 2003). This slower DA response has an inverted U-shaped dose response curve that is maximal at a probability of 50%, which is the point of maximum uncertainty. If this applies to the placebo effect, it is possible that the greater the uncertainty of benefit associated with placebo administration, the greater the placebo-induced DA release. Based on these findings, we hypothesized that the degree of placebo-induced DA release in the striatum of PD patients could be modulated in either a monotonic or an inverted-U dose-response fashion, and might additionally depend on the degree of clinical improvement expected by the subject. Based on the results of our previous study, we predicted a bilateral release of DA involving both the dorsal and ventral striatum which would suggest involvement of both nigrostriatal and mesolimbic DA pathways (de la Fuente-Fernandez et al., 2002).

The objective of the current experiment was to determine if the degree of placebo-induced DA release in PD could be modulated by the strength of expectation of benefit. We used verbal instructions to manipulate the patients' expectations of improvement, telling them their explicit probability of receiving levodopa versus placebo. Four probabilities (25, 50, 75, and 100%) were selected as the independent variables which would enable us to detect either a linear or an inverted U-shaped dose-response relationship between reward expectation (i.e. clinical improvement) and DA release in the dorsal and ventral striatum. In addition to the biochemical placebo effect (i.e. DA release), we also measured the clinical placebo effect in the subjects, as this can be objectively tested by a blinded examiner. Finally, the subjectively perceived placebo effect was measured by patient self-report.

5.2 Methods

5.2.1 Subjects

35 patients diagnosed with idiopathic PD were recruited from the Movement Disorders Clinic at UBC Hospital. Five subjects withdrew from the study due to claustrophobia or the discomfort associated with PET. The 30 remaining subjects completed the study (men, n=25, women, n=5). The mean ± SD age was 62 ± 7.6 years and disease duration was 9.9 ± 3.6 years based on the time of symptom onset. We selected patients with mild to moderate disease severity (mean Hoehn & Yahr score of 2.2 ± 0.5) since non-disabling symptoms may not derive as much benefit from placebo as those with more severe disease from which they require symptomatic relief. Patients were free of depression (Beck Inventory of Depression mean score 6.3 ± 2.3) (Beck et al., 1988) and cognitive impairment (Mini-Mental Status Examination mean score of 29.2 ± 1.1) (Folstein et al., 1975), and were taking levodopa as part of their medication regimen for PD. All patients gave written informed consent. The study was approved by the UBC Clinical Research Ethics Board (Appendix E).

5.2.2 Study design

The study design is depicted in Figure 5.1. The experiment took place on two consecutive days whenever possible, and patients were withdrawn from all anti-parkinson medication 12-18 hours prior to being scanned. All subjects underwent three RAC PET scans. On the first day, a baseline scan was performed, followed by a scan that began one hour following the oral administration of standard-release 250/25 mg levodopa/carbidopa (Sinemet), which was delivered in an open fashion. On the second day, the subjects were randomly assigned to one of four groups, A through D, which determined the verbal instructions they were given regarding the likelihood of receiving active drug (levodopa) for the scan: (A) 25% probability of receiving active drug, (B) 50% probability of receiving active drug, (C) 75% probability of receiving active drug, and (D) 100% probability of receiving active drug. In actual fact, all

subjects were given placebo. The group allocation was not revealed to the patient until the time of placebo administration. The active medication given for the second scan was crushed and put into capsules so as to look identical to the placebo capsules (as it was not possible to obtain placebo tablets which looked identical to levodopa as marketed). All subjects were additionally given 20 mg domperidone 30 minutes prior to both levodopa and placebo in order to prevent peripherally mediated side effects of levodopa such as hypotension or nausea.

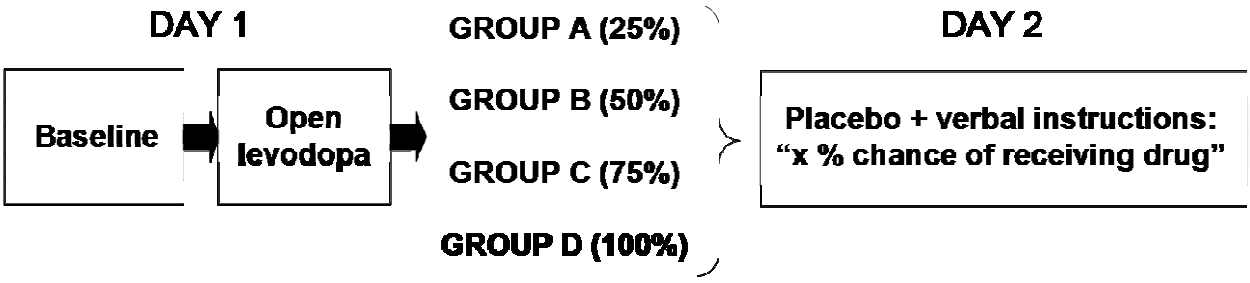


Figure 5.1 Study Design. White boxes represent the three RAC PET scans. It can be seen here that all subjects were treated in an identical fashion until they were randomized to separate groups on Day 2.

5.2.3 Objective ratings

Additional measures were taken in order to detect both objective and subjective placebo effects. At the beginning of each day, the patients' baseline motor function was assessed by a blinded examiner using the Unified Parkinson's Disease Rating Scale (UPDRS Part III, Appendix B) (Fahn S, Elton RL. 1987). The same, blinded examiner conducted an abridged version of the UPDRS at the midpoint (30 minutes post-RAC injection) of each PET scan (Modified UPDRS, mUPDRS, Appendix C). The UPDRS was modified to include only measures of tremor, bradykinesia and rigidity in the upper limbs, and tremor and rigidity only in the lower limbs in order to minimize the impact of head motion on the PET data.

5.2.4 Subjective ratings

The subjects were asked following both the levodopa and placebo scans if they felt any improvement in their PD symptoms following the medication, and to rate that improvement, if any, using an arbitrary scale from 0 to 3 (0 = no improvement, 1 = mild, 2 = moderate, 3 = strong) (Subject Self Reporting Form, Appendix F). The subjects who reported perceiving symptom improvement (defined as mild and greater) were defined as placebo responders. The subjects were also asked whether they thought they received active medication or placebo following the placebo scan.

5.2.5 Expected reward value

Expected reward value (ERV) is defined as the product of the probability of reward delivery (p) and the reward magnitude (MAG): ERV = p x MAG. In this study, the probability was dictated by the group allocation (25 – 100%). The reward was the clinical benefit experienced by the patient elicited by the placebo, which the patients thought could be the same dose of levodopa that they received on Day 1. Thus, in this case, the magnitude of reward would be the patients' degree of symptom improvement in response to levodopa on Day 1. Since we had both objective and subjective measures for the clinical benefit in response to levodopa, the magnitude of the reward could be defined in two different ways: MAG_{obj} was defined as $mUPDRS_{baseline} - mUPDRS_{levodopa}$, and MAG_{subj} was defined as the patient's perception of symptom improvement as measured by self report (0 to 3, none to strong). Therefore, the ERV calculations were defined as follows:

$$ERV_{obj} = \text{Group} \times MAG_{obj}$$

$$ERV_{subj} = \text{Group} \times MAG_{subj}$$

5.2.6 Manipulation of expectation

In this experiment, expectations were manipulated verbally, and it was essential that the patient clearly understood their probability of receiving levodopa. The groups were given the

following instructions to specifically illustrate the probability of receiving active drug in order to most convincingly manipulate expectation:

"You have been randomly assigned, like pulling numbers out of a hat, to Group A. As you read in the consent form, this means that you have a 25% chance, or 1 in 4 chance, of receiving active Sinemet, exactly the same dose that you were given yesterday for the second scan. We took one real Sinemet pill, and three placebos and shook them up and withdrew one. This is what we are giving you. You will be told what you have been given after the scan is complete."

Following this, the patients were then asked to confirm that they understood their chances of receiving medication.

Since this study required the use of deception, the consent form given to the patients upon recruitment (potentially as early as three months in advance of the time of scanning) represented the true beginning of expectation manipulation (Consent Form, Appendix G). The consent form stated:

"The purpose of this study is to examine the different factors that contribute to a person's response to the treatment of their Parkinson's disease. The study requires the use of some deception, and as a result the full purpose of the study cannot be revealed to you at this time. However, nothing that has been described above about the purpose is false. We have simply omitted some details. These will be described to you once the study has been completed. At that time, we will fully debrief you about the background, purpose and methods that were used during the experiment and answer any questions that you may have."

Thus, the patients were told that deception would be used, but that we could not inform them as to the nature of the deception. This tactic is virtually identical to the one described in Miller et al. (2005), and is considered ethically acceptable for studies such as this (Miller et al., 2005;Miller and Wendler, 2005).

5.2.6 Debriefing

Immediately following the completion of the experiment, the subjects were debriefed as to the true purpose of the study and the nature of the deception used. The subjects were informed that they were given placebo for the final scan, and in fact could never have received levodopa for that final scan (Debriefing Form, Appendix H). They were told that deception was necessary in order to be able to measure how their expectations affected their brain DA levels,

and that if they were aware that they were receiving placebo, their expectations would be zero, which would fail to produce a placebo effect and defeat the purpose of the study. As a precaution, and in light of the small Parkinson's community in British Columbia, subjects were additionally asked not to reveal the study design to any fellow patients – either friends, or support group acquaintances – as there would be a chance that they could potentially be recruited into the study and their data would be invalidated. They were also advised that if they objected to the approach that was taken, their data would be removed from further analysis.

5.2.7 Positron emission tomography and image analysis

All scans were performed using a high-resolution research tomograph (HRRT, CTI/Siemens) operating in three-dimensional (3D) mode. A 10-minute transmission scan using a rotating radioactive source (^{137}Cs) was performed at the beginning of each scan for attenuation correction. Head motion was minimized by the use of an individually molded thermoplastic mask. In addition, head motion was tracked in a subset of patients using the Polaris Motion Tracking system (Bloomfield et al., 2003), which required the subject to wear a thin neoprene swim cap under their thermoplastic mask. Following the attenuation scan, the RAC scan began with the bolus injection of 10 mCi of RAC (mean ± SD specific activity = 4312 ± 1869 Ci/mmol at ligand injection) into the left antecubital vein over 60 seconds, and emission data were then acquired over a period of 60 minutes in 16 frames of progressively increasing duration. Emission data were reconstructed using a statistical algorithm (Ordinary Poisson 3D– OSEM) that contained corrections for scatter, attenuation, random events and normalization (Politte and Snyder, 1991). Emission data were then corrected for motion by inter-frame realignment using Automated Image Registration (Woods et al., 1993). The levodopa and placebo images were registered to the baseline image to facilitate region-of-interest (ROI) placement within subjects.

Region-of-interest placement

From the emission data (30–60 min), an integrated image with 206 planes (each 1.211 mm thick) was obtained for each subject. For the dorsal striatum, elliptical and circular ROIs were placed on baseline integrated images on 9 consecutive transaxial slices (total thickness 10.89 mm) in which the caudate nucleus and putamen were best visualized (as seen in Section 4.6.4). As ROI placement on so many planes is practically difficult and data from single slices tend to be noisy, these 9 planes were regrouped into three mean images of 3 planes each for ROI placement. Individual ROIs were adjusted to maximize the average activity on each image. For ventral striatum analysis, an integrated image in the coronal plane was created from the emission data (30-60 minutes), and 6 consecutive coronal slices (total thickness 7.26 mm) in which the ventral striatum was best visualized were averaged. A single elliptical ROI was then placed bilaterally on the ventral striatum, in part using published anatomical criteria (Mai et al., 1997). The ROIs placed on the baseline integrated images for a patient were then placed on the levodopa and placebo scans from the same patient in the same position, with minor adjustments made to maximize the average activity within the ROI. The background activity was averaged from a single elliptical ROI (2055 mm^2) drawn over the cerebellum on the integrated image from 6 consecutive transaxial planes.

Binding potential extraction

Time-activity curves (TACs) were generated for each ROI. In order to reduce noise in the data, the TACs from the three putamen ROIs were averaged into a single TAC for each brain hemisphere. Thus, there were three TACs generated for each brain hemisphere: head of the caudate, putamen, and ventral striatum. RAC binding potentials (RAC BPs), defined by B_{max}/K_d, were determined using a graphical approach using the cerebellar TAC as an input function. Two alternate kinetic models were applied to the data to extract the RAC BPs, the

main biological parameter of interest (Logan et al., 1996;Gunn et al., 1997). The models make different assumptions and have different sensitivities to noise (which is non-negligible in high resolution PET data, especially if there is patient motion), and at the time of analysis it was unknown whether one model might introduce bias in the results.

5.2.8 Statistical analysis

All values are reported as mean ± SEM unless otherwise stated. Parametric and non-parametric tests were conducted as appropriate. The change in RAC BP in response to levodopa ($RAC\ BP_{baseline}$ - $RAC\ BP_{levodopa}$) was assessed using a linear multiple regression model that included age and $RAC\ BP_{baseline}$ as regressors. The change in RAC BP in response to placebo ($RAC\ BP_{baseline}$ - $RAC\ BP_{placebo}$) was explored using analyses of covariance (ANCOVA) including age, group and $RAC\ BP_{baseline}$ as covariates. Additional covariates were also explored (see results). Where ANOVA was significant, pairwise comparisons were performed using Bonferroni corrected t-tests. ERV_{obj} and ERV_{subj} were used as covariates in the ANCOVA, as well as treated as separate continuous independent variables in multiple regression analyses, in order to determine whether probability or ERV was a better predictor of placebo-induced DA release. The effect of expectation (i.e. Group) on the clinical response to placebo ($mUPDRS_{baseline}$ - $mUPDRS_{placebo}$) was investigated using an ANCOVA also adjusted for age and $mUPDRS_{baseline}$. Associations between individual percentage changes in clinical scores (mUPDRS) and subjective responses to levodopa and placebo were interrogated with the Spearman rank correlation statistic. A multiple regression analysis adjusted for age and $RAC_{baseline}$ was also carried out to investigate the correlation between the DA response to levodopa and to placebo (i.e. $RAC_{baseline} - RAC_{levodopa}$ versus $RAC_{baseline} - RAC_{placebo}$).

5.3 Results

5.3.1 Subjects

The patient characteristics are outlined in Table 5.1. The mean ± SD levodopa dose (calculated as immediate release equivalents) for all subjects was 917.08 ± 432.07 mg, and the mean ± SD DA receptor agonist dose (presented in bromocriptine equivalents) was 12.85 ± 14.75 mg. Four subjects were taking low doses of antidepressants (selective serotonin re-uptake inhibitors), but were not depressed at the time of scanning (mean BDI score = 7.5). One subject was taking amantadine.

Group	n	M:F	Mean age (yrs)	Mean disease duration (yrs)	Mean levodopa dose (mg)	Mean agonist dose (mg)
A	8	7:1	65.75 ± 4.86	11.5 ± 5.4	866.25 ± 383.1	9.00 ± 13.6
B	7	5:2	64.17 ± 5.8	9.6 ± 2.8	670 ± 487.9	10.00 ± 14.14
C	7	6:1	59.85 ± 8.27	9.0 ± 3.2	1061.79 ± 246.9	22.64 ± 17.43
D	8	7:1	59.57 ± 9.49	9.5 ± 3.1	1057.5 ± 512.6	10.62 ± 12.58

Table 5.1 Clinical characteristics of the PD patients in each group. Results are presented as mean ± SD. The mean levodopa dose is presented in immediate release equivalents. For individuals taking controlled-release tablets the dose was converted according to levodopa IR mg = levodopa CR x 0.66. The mean agonist dose is presented in bromocriptine equivalents according to the following formula: bromocriptine mg = ropinirole mg x 2, pergolide mg x 10, and pramipexole mg x 10.

The UPDRS III OFF score on Day 1 was 20.93 ± 1.82, and 20.76 ± 2.02 on Day 2. The baseline UPDRS III OFF scores from Day 1 and Day 2 were highly correlated ($\rho = 0.8651$, $p < 0.0001$, Spearman). We were unable to assess portions of the UPDRS on the lower limbs for three patients as one had a cast, another had a prosthesis, and another had a sprained ankle. Full UPDRS III scores were therefore normalized by dividing the score by the total of the sections that were able to be tested, which varied for each patient (a total UPDRS score of 92 for two patients, and 88 for the other). The mUPDRS score was normalized by dividing by 48 rather than by 56 as tremor and rigidity in that limb could not be assessed.

5.3.2 Response to levodopa

Objective levodopa response

A multiple regression analysis including age and $mUPDRS_{baseline}$ as regressors indicated modest but highly significant improvement in the mUPDRS motor score following open levodopa, from the baseline ("off" state = 14.4 ± 1.4, versus "on" state = 8.9 ± 0.9, 32% improvement; $r = 0.77$, $p = 0.0000$) (Figure 5.1A). For these purposes, a clinically meaningful change in UPDRS scores was arbitrarily defined as 25% improvement and greater. According to this criterion, 18 patients demonstrated clinical improvement on the mUPDRS (53.73 % ± 0.04), 10 had no change (0.29% ± 0.04), and one got worse (-50%).

Subjective reporting

All but three patients reported feeling benefit from levodopa. Twelve patients reported "mild" benefit, 8 reported "moderate" benefit, and 6 reported "strong" benefit (Figure 5.2B). Surprisingly, there was no correlation between the patients' subjective reporting of symptom improvement and the measured clinical response ($\rho = 0.058$, $p = 0.76$, nonparametric Spearman correlation).

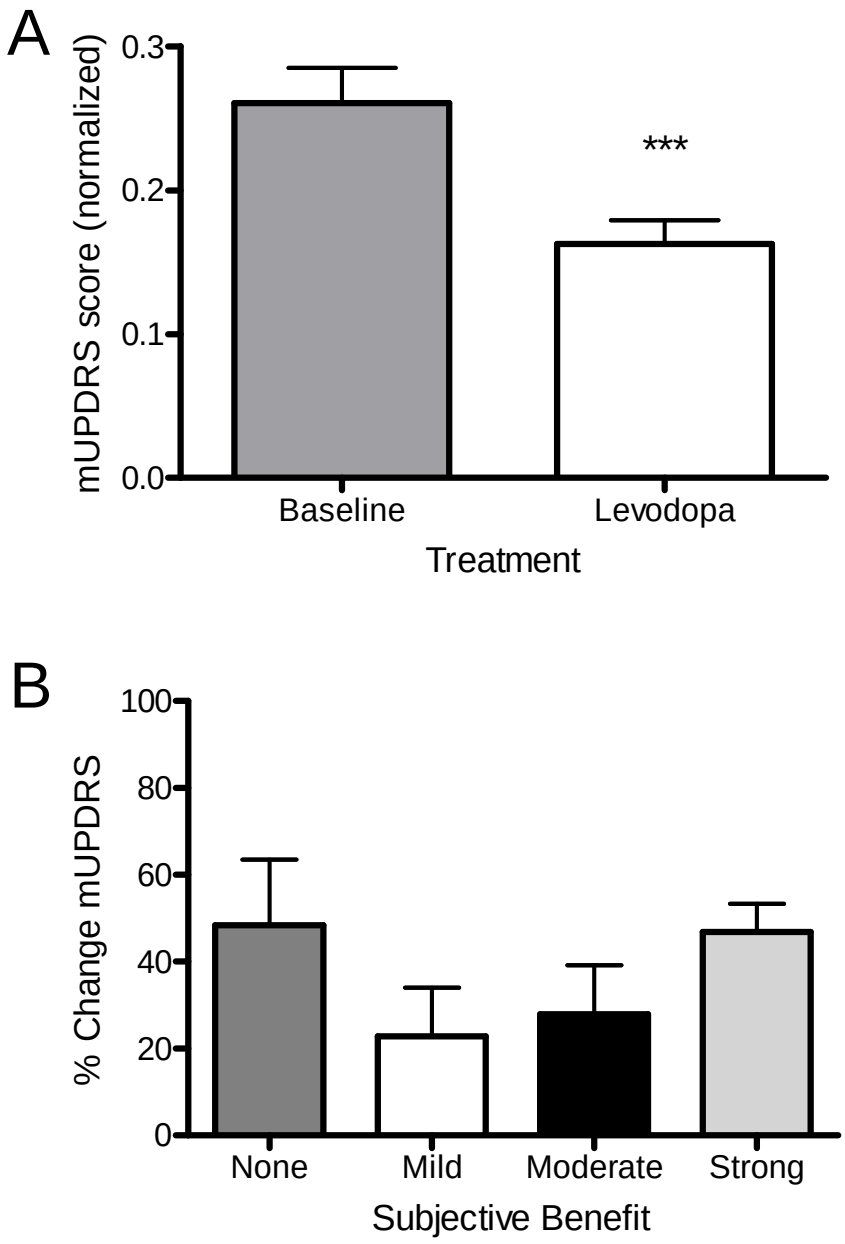


Figure 5.2 Changes in mUPDRS scores in response to oral levodopa in 30 PD patients. A) Y-axis displays Normalized mUPDRS scores at baseline (grey bar) and following oral levodopa (open bar). Levodopa administration resulted in a significant reduction (32%) in mUPDRS scores indicating clinical improvement in PD symptoms ($p = 0.0000$). B) Percentage change in mUPDRS scores ($mUPDRS_{baseline} - mUPDRS_{levodopa} / mUPDRS_{baseline} \times 100\%$) shown as a function of the patients' subjective self-reports (no benefit, mild, moderate or strong benefit). No significant association was seen between objective and subjective reports, and although there is a trend for increasing clinical benefit and increased benefit as measured by self-report, there were no significant differences observed between groups. The analysis was repeated omitting the three patients who reported no benefit from levodopa and the results were still not significant.

[^{11}C] Raclopride PET results

As can be seen in Figure 5.3, a significant 7.8 ± 1.7 % reduction in RAC BP (RAC $BP_{baseline}$ - RAC $BP_{levodopa}$) was seen in the putamen in response to levodopa (BP values of 3.69 ± 0.16 pre-treatment to 3.39 ± 0.16 post-treatment, p = 0.02). No differences were detected in the caudate nucleus or ventral striatum (absolute RAC BP values, 2.85 ± 0.12 to 2.80 ± 0.13 in the caudate, and 3.25 ± 0.13 to 3.19 ± 0.13 in the ventral striatum).

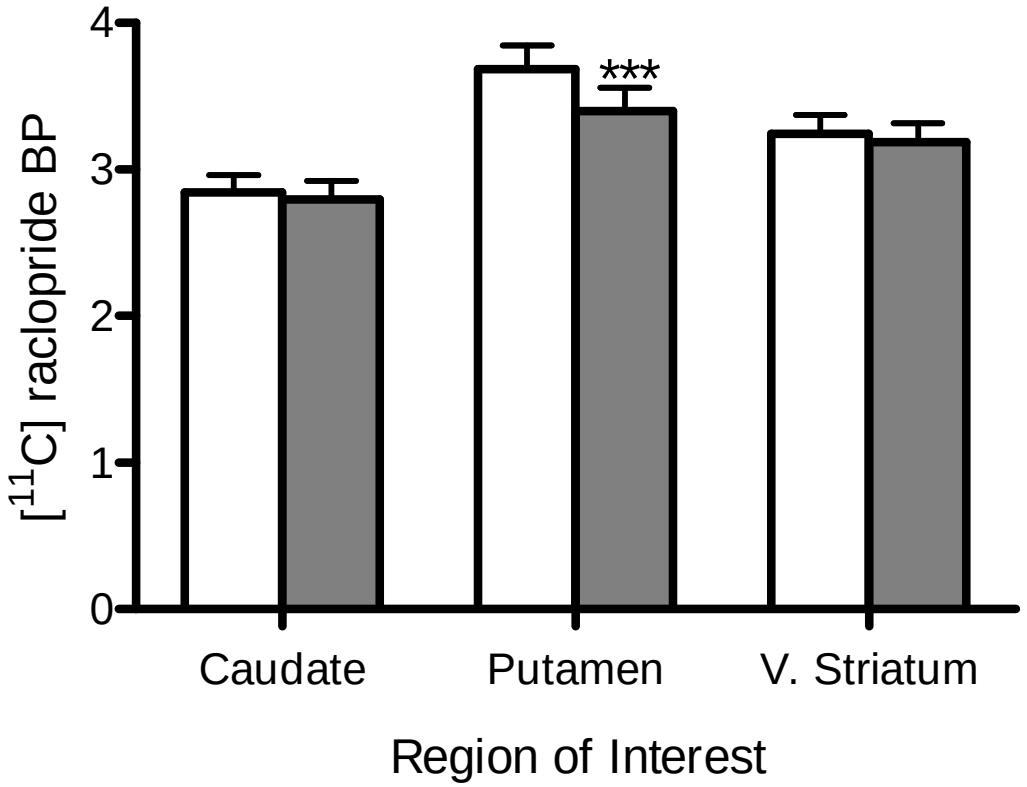


Figure 5.3 RAC BPs measured at baseline (white bars) and following oral levodopa (grey bars) in 30 PD subjects in the different striatal subregions (caudate, putamen and ventral striatum). Levodopa administration resulted in a significant decrease in RAC BP in the putamen, indicating DA release (p = 0.02). Values are presented as mean ± SEM.

Finally, no significant correlation was found between RAC $BP_{baseline}$ - RAC $BP_{levodopa}$ and the clinical response to levodopa ($mUPDRS_{baseline}$ – $mUPDRS_{levodopa}$).

5.3.3 Placebo effect

Objective measurements

Changes in mUPDRS following placebo administration are shown in Figure 5.4. An ANCOVA using age and $mUPDRS_{baseline}$ as covariates found no significant difference between groups. Consistent with the variability in placebo responses in PD, a wide range of clinical placebo effects was observed in all groups, including both positive and negative placebo effects (Figure 5.4B). Separate pairwise comparisons conducted in each group revealed a significant improvement in motor scores following placebo administration in Group C only ($p = 0.03$, Wilcoxon paired test, two-tailed), corresponding to a 20% change. Nine patients met the criteria for demonstrating clinical improvement as measured by mUPDRS scores ($mUPDRS_{baseline} - mUPDRS_{placebo}$), and were distributed in all four groups (4 in Group A, 1 in each of Groups B and D, and 3 in Group C). 7 patients demonstrated a worsening of motor symptoms (defined as a deterioration of $\geq 25\%$ in mUPDRS scores). 13 patients showed no change in motor signs in response to placebo.

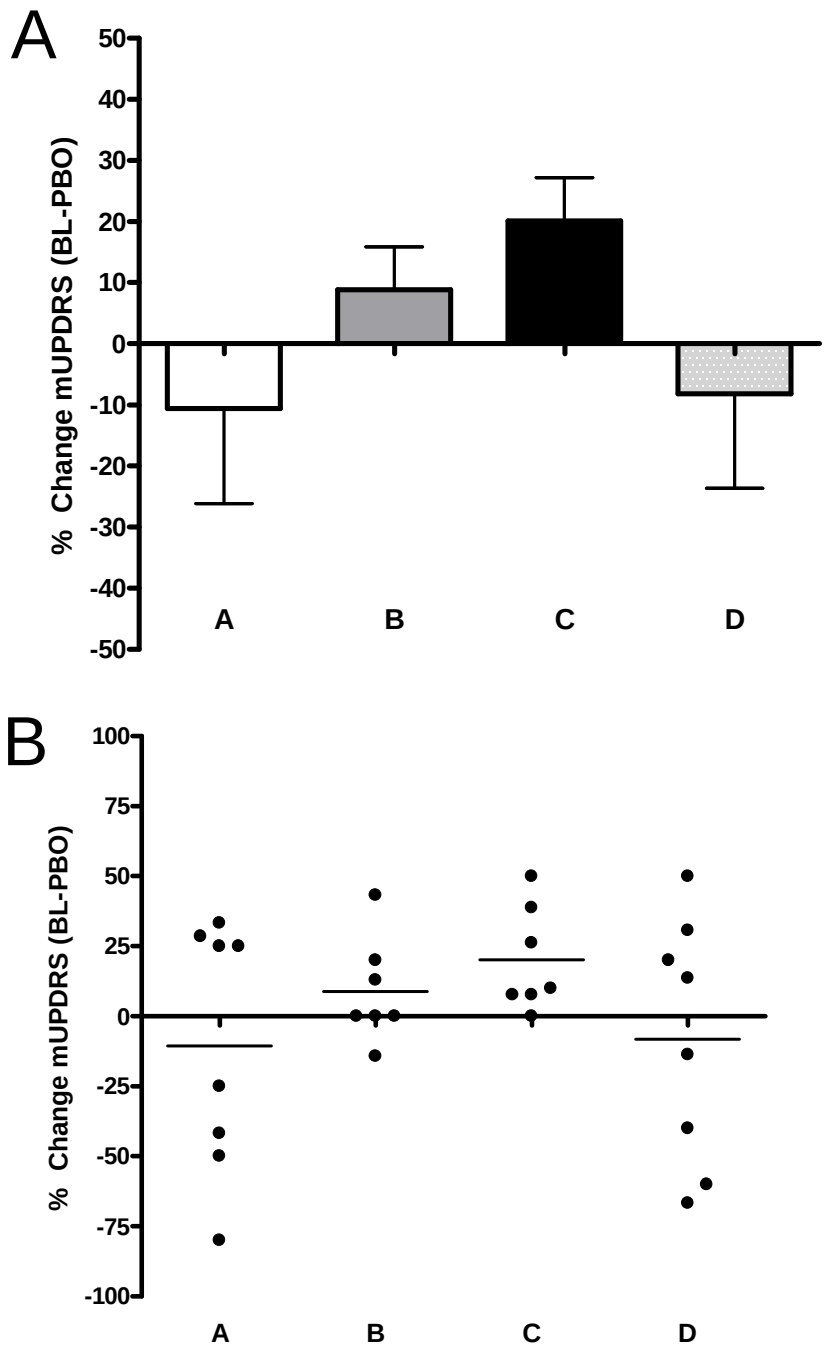


Figure 5.4. Clinical placebo effects as a function of group (A-D). The same data are presented as a bar graph (A) and a vertical scatter plot (B) to illustrate the variability in placebo effects. Y-axes represent the percent change in mUPDRS ($mUPDRS_{baseline} - mUPDRS_{placebo} / mUPDRS_{baseline} \times 100\%$). (A) Although there is a trend for an improvement in motor symptoms with increasing expectation, the main effect of Group was not statistically significant, although patients in Group C demonstrated a modest significant improvement ($p = 0.031$,Wilcoxon paired test, two-tailed). (B) Horizontal lines indicate the mean for each group. It can be seen that despite the verbal instructions, there was a high degree of variability in the clinical response to placebo within each group, where patients improved, worsened or exhibited no change.

Subjective reporting

Self-reports indicated that 13 patients felt no benefit from the placebo, 14 reported "mild," 2 reported "moderate," and one reported "strong" benefit. Interestingly, those reporting benefit were found in all four groups. No correlation was seen between the objective changes in motor function and subjective reporting following placebo administration (Figure 5.5).

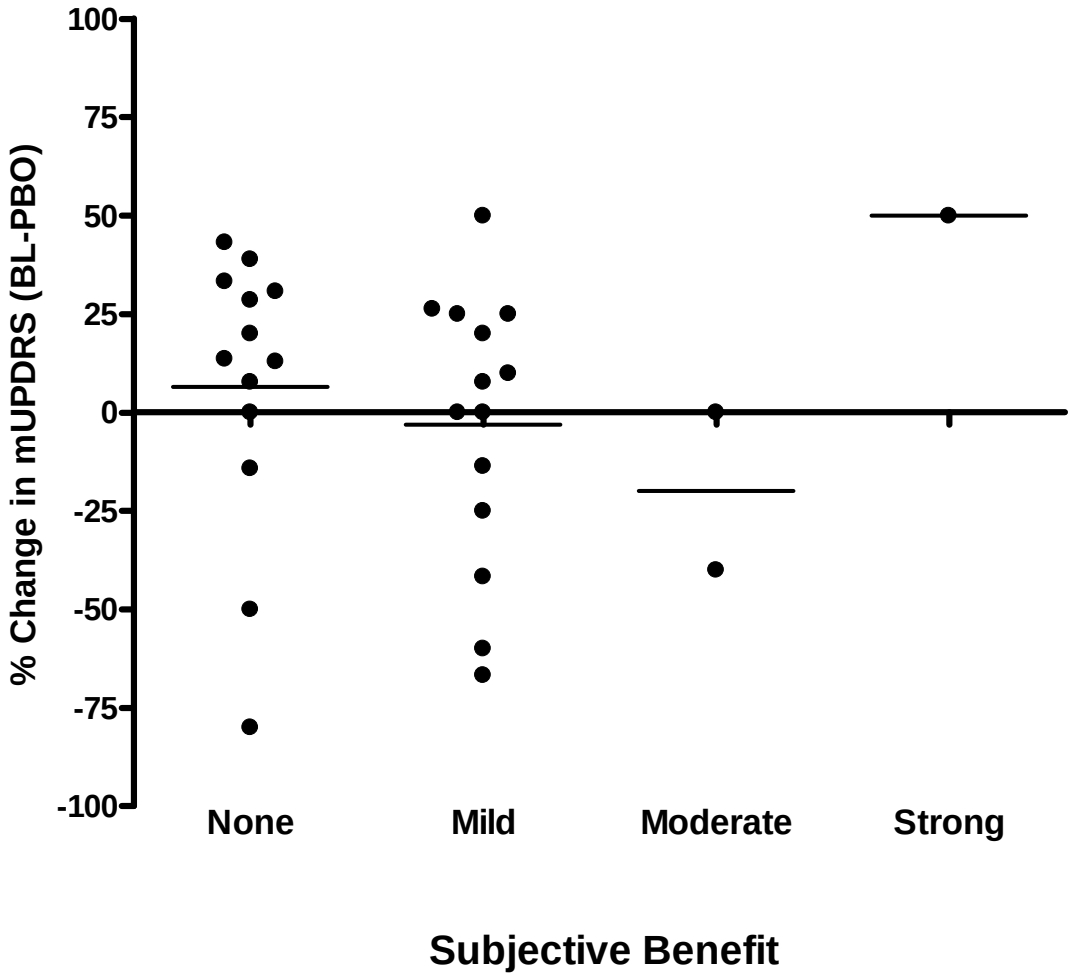


Figure 5.5 Percentage change in mUPDRS scores ($mUPDRS_{baseline} - mUPDRS_{placebo} / mUPDRS_{baseline} \times 100\%$) in response to placebo as a function of patient subjective self-report. Horizontal lines indicate the mean for each group. Despite the verbal instructions, the perceived benefit felt by the patients did not correlate with changes in motor scores as assessed by a blinded examiner.

[^{11}C] Raclopride PET results

Changes in RAC BP in response to placebo are shown in Figure 5.6. Increases and decreases in RAC BP were detected in all striatal subregions, across all groups (Figure 5.5 Panel D). There was a significant main effect of group in the putamen ($p = 0.028$) and in the ventral striatum ($p = 0.005$), where patients in Group C (75% expectation) demonstrated the greatest reduction in RAC BP, indicating that they released significantly more DA. Furthermore, the pattern of RAC BP changes was similar in all regions of the striatum, demonstrating a peak in DA release at 75%, and essentially no change at the other levels of expectation. Interestingly, age had a significant impact on the degree of change in RAC BP in response to placebo in all groups except for Group C, and in the ventral striatum in particular. However, disease duration (years of disease since symptom onset) or disease severity (as measures by absolute baseline UPDRS) did not.

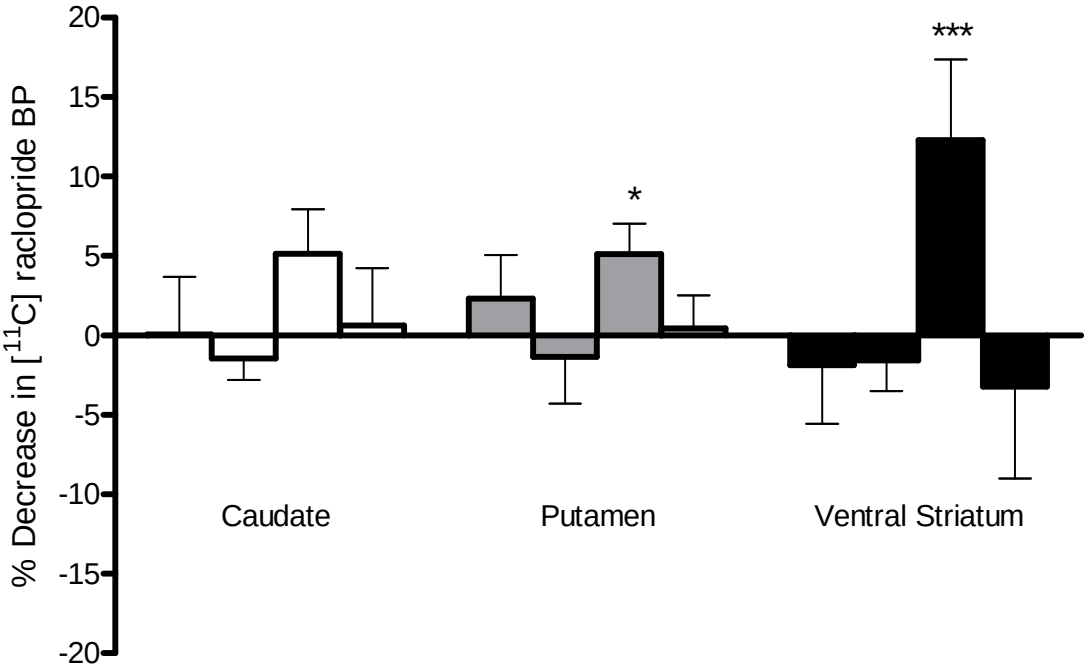


Figure 5.6 Mean + SEM percent change in RAC BP ((RAC $BP_{baseline}$ –RAC $BP_{placebo}$)/ RAC $BP_{baseline}$ x 100%) as a function of group (x-axis) in each striatal subregion. Within each subregion, the expectation increases from left to right (i.e. Group A, B, C, D). An increase in percent change in RAC BP indicates an increase in DA release. A highly significant increase in DA release in response to placebo was seen in Group C in the ventral striatum ($p = 0.005$) and putamen ($p = 0.028$), with a non-significant trend in the caudate ($p = 0.15$), with the same pattern in all three subregions.

The ANCOVA on RAC $BP_{baseline}$–RAC $BP_{placebo}$ in the ventral striatum and putamen was repeated using different covariates in order to attempt to define the model which best fit the data, however none improved the result. No significant results were found in the caudate. The covariates and the resulting p-values are shown in Table 5.2 below.

Standard Model	Additional covariate	Meaning	p-value Putamen	p-value Ventral Striatum
Age + Baseline			0.028	0.005
Age + Baseline	$mUPDRS_{baseline}$ – $mUPDRS_{levodopa}$	Magnitude of reward (objective)	0.045	0.005
Age + Baseline	ERV_{obj}	Value (p x objective magnitude)	0.065	0.009
Age + Baseline	ERV_{sub}	Value (p x subjective magnitude)	0.041	0.017
Age + Baseline	Levodopa subjective benefit	Magnitude of reward (subjective)	0.036	0.007
Age + Baseline	Placebo subjective benefit	Responder / Non Responder	0.035	0.007
Age + Baseline	Baseline full UPDRS	Disease severity	0.035	0.003

Table 5.2 Covariates used in the ANCOVAs for RAC $BP_{baseline}$– RAC $BP_{placebo}$ (dependent variable) vs. Group (independent variable) in the putamen and ventral striatum and the corresponding p-values. The 'Meaning' column indicates the theoretical meaning of the variable as defined by reward literature.

Variability in placebo effects

A considerable amount of variability in placebo-induced DA release was seen within each group. Patients demonstrated a wide range of placebo-induced changes in RAC BP, including both increases and decreases (Figure 5.7). Two data points that demonstrated substantial decreases (> than 20%) in response to both levodopa and placebo were suspected of being outliers, and when the analysis was repeated omitting these data the results were essentially unchanged.

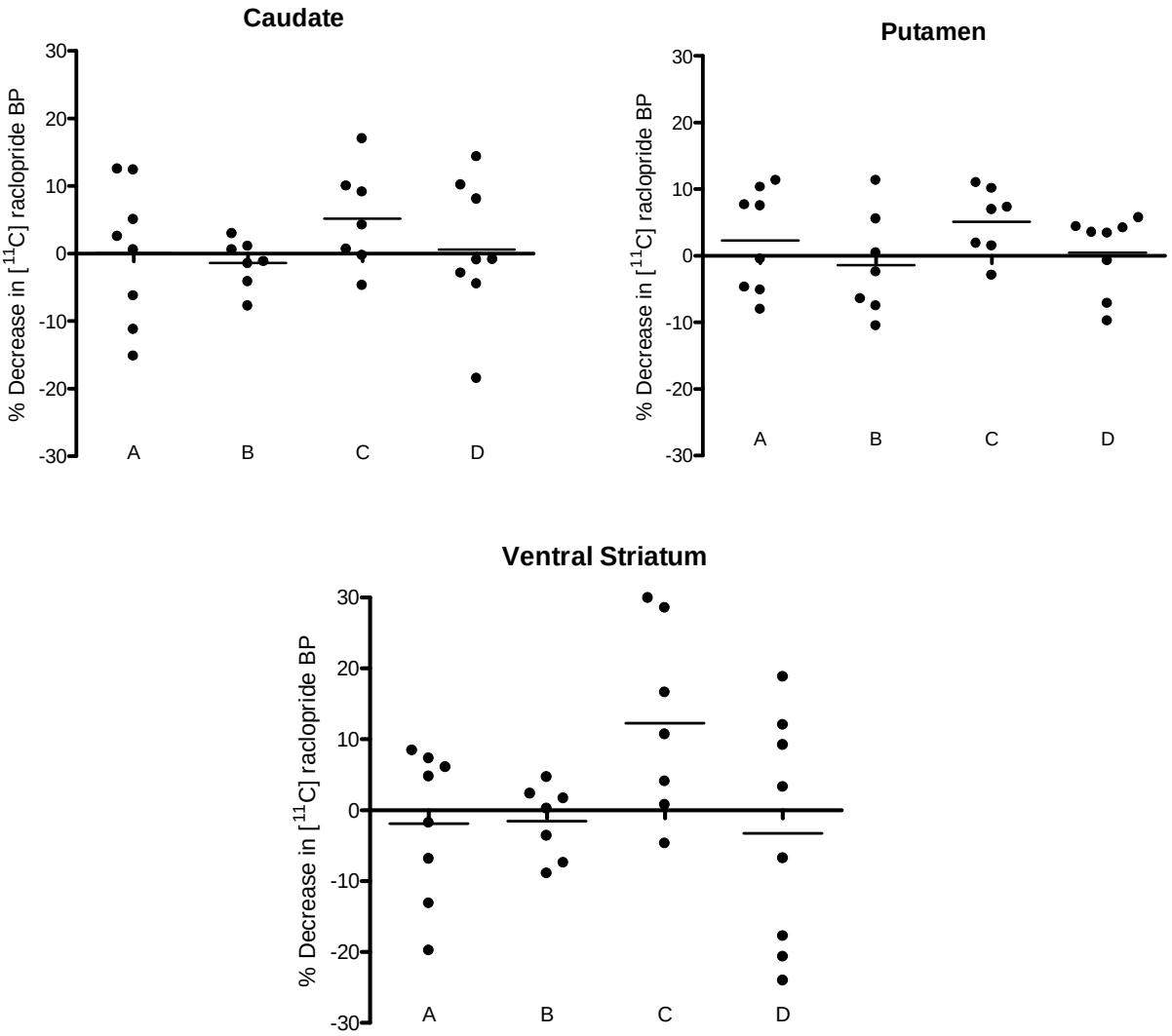


Figure 5.7 Vertical scatterplots indicating the individual variability in the percent decrease in RAC BP ((RAC $BP_{baseline}$ –RAC $BP_{placebo}$)/ RAC $BP_{baseline}$ x 100%) in response to placebo as a function of Group (A-D, x-axes). Values were calculated in the same manner as in Figure 5.5. A positive percent change in RAC BP from baseline indicates DA release, and a negative change suggests a decrease in DA below baseline. Positive and negative responses can be seen in all groups, in all striatal subregions.

In order to attempt to account for this high variability in changes in RAC binding, patients were divided into those who reported feeling benefit from placebo ("mild" and above, placebo responders) and those who did not (placebo non-responders) (Figure 5.8). No significant difference was found between responders and non-responders in any of the regions in the striatum, although the difference was most pronounced in Group C in the ventral striatum.

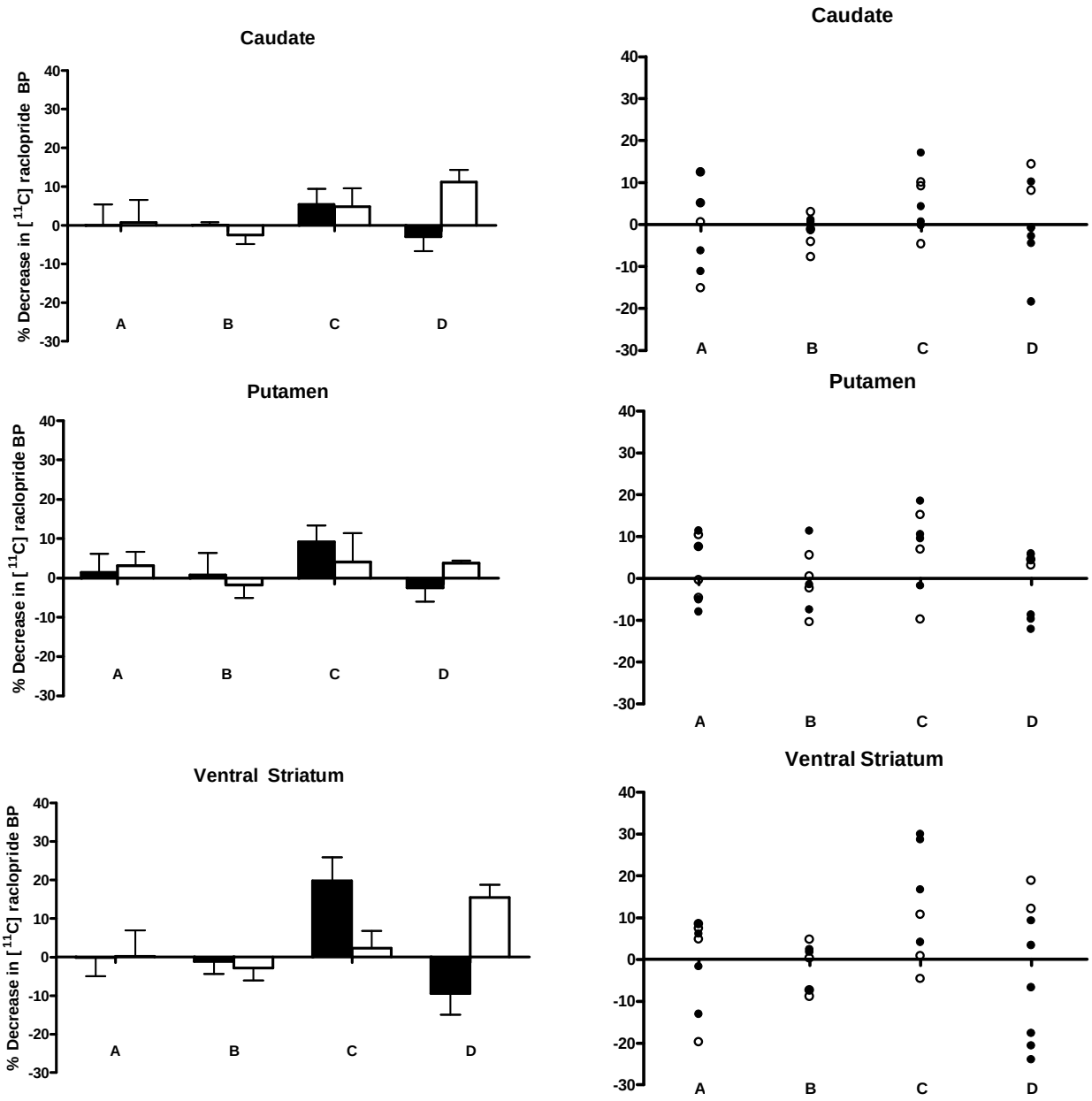


Figure 5.8 Mean + SEM percent decrease in RAC BP ((RAC $BP_{baseline}$–RAC $BP_{placebo}$)/ RAC $BP_{baseline}$ x 100%) in each striatal subregion in response to placebo in placebo responders (black) and non-responders (open), as determined by subjective self-report. The same data are presented on both the left and right sides, but displayed differently. Panels on the left show mean (+ SEM) change in RAC BP and suggest increased DA release in placebo responders only in Group C in all subregions, although this difference did not reach significance. Panels on the right indicate the individual values for responders and non-responders within each group. It can be seen that there was no correlation between subjective measures of clinical improvement and DA release in response to placebo.

Expected reward value

Although the study was designed to use probability as the independent variable and investigate its effect on placebo-induced DA release, we additionally sought to examine ERV_{obj} and ERV_{subj} as independent variables in order to assess whether probability alone or ERV better predicted the degree of placebo-induced DA release in the each region of the striatum. When used as a continuous independent variable in a multiple regression analysis with age and $RAC_{baseline}$ as regressors, a significant effect was seen in the putamen for ERV_{obj} ($r = 0.61$, $p = 0.0058$, Figure 5.9). This was not seen using ERV_{sub} as the independent variable ($r = 0.496$, $p = 0.0579$). Both ERV_{obj} and ERV_{sub} provided significant results in the ventral striatum ($r = 0.58$, $p = 0.012$ and $r = 0.60$, $p = 0.007$, respectively) but did not increase the highly significant main effect of Group in this region (see Table 5.2). Other independent variables were also investigated, and the p-values for the putamen and ventral striatum are shown in Table 5.3. No significant effects were seen in the caudate using any of the regressions. No correlation was found between ERV_{obj} alone and Group when adjusted for age.

Standard Model	Independent variable	Meaning	p-value, Putamen	p-value, Ventral Striatum
Age + $RAC_{baseline}$	ERV_{obj}	Value (p x objective magnitude)	0.0058	0.012
Age + $RAC_{baseline}$	ERV_{subj}	Value (p x subjective magnitude)	0.0579	0.0071
Age + $RAC_{baseline}$	Levodopa subjective benefit	Magnitude of reward (subjective)	0.068	0.0156
Age + $RAC_{baseline}$	Placebo subjective benefit	Responder / Non Responder	0.0554	0.0081

Table 5.3 P-values for placebo-induced DA release ($RAC_{baseline} - RAC_{placebo}$ as the dependent variable) regressed against different independent variables in the putamen and ventral striatum. Multiple regression analyses were conducted using different independent variables and corrected for age and $RAC_{baseline}$ BP. Only ERV_{obj} as an independent variable produced a significant effect in the putamen. Whereas all of the variables produced statistically significant results in the ventral striatum, the overall significance in this region was higher when Group alone was used as a factor in the ANCOVAs (Table 5.2).

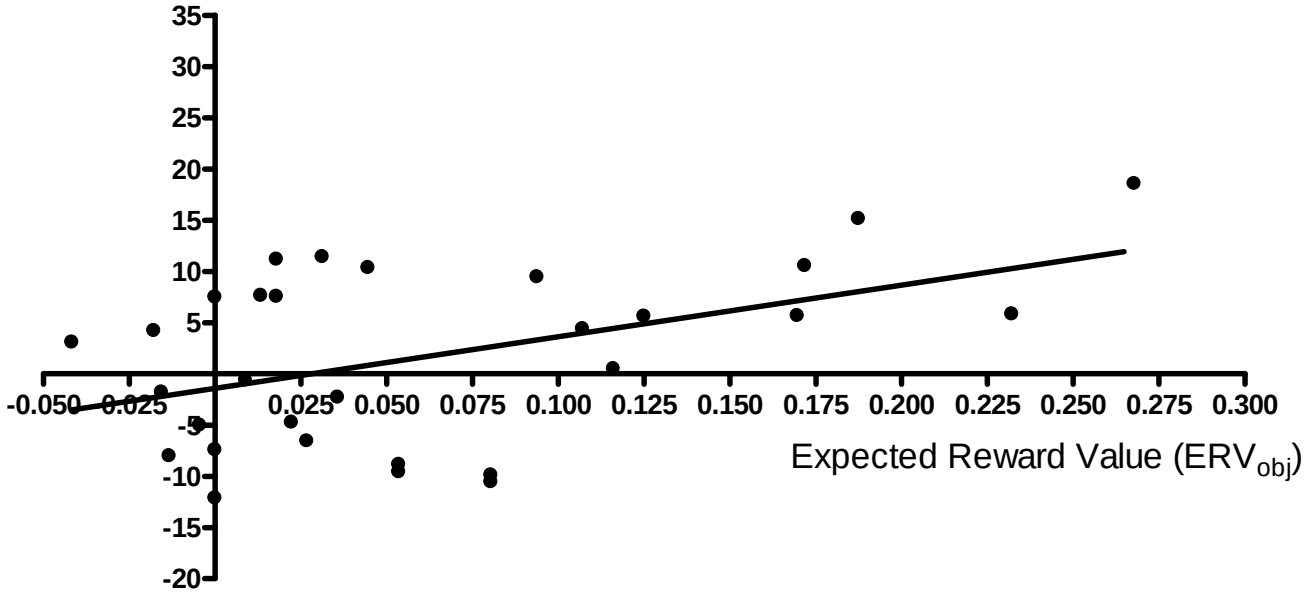


Figure 5.9 Placebo-induced percent decrease in RAC BP (($RAC_{baseline} - RAC_{placebo}$) / $RAC_{baseline}$), y-axis) in the putamen plotted against ERV_{obj} for all subjects (n=30). ERV_{obj}, and not probability alone, better predicted the degree of placebo-induced change in RAC BP in this region ($r = 0.61$, $p = 0.0058$ versus $p = 0.028$) when adjusted for age and $RAC_{baseline}$.

Correlations between [^{11}C] raclopride BP and objective scores

Although the patterns were similar (i.e. greatest improvement in Group C), there was no significant correlation observed between the changes in mUPDRS scores ($mUPDRS_{baseline} - mUPDRS_{placebo}$) and the changes in RAC BP ($RAC_{baseline} - RAC_{placebo}$) in response to placebo in any subregion.

Correlations between levodopa response and placebo response

Highly significant positive correlations were found between changes in RAC BP in response to levodopa and in response to placebo in all three striatal subregions (caudate, $r = 0.67$, $p = 0.0013$; putamen, $r = 0.65$, $p = 0.0022$; ventral striatum, $r = 0.74$, $p = 0.0001$) (Figure 5.10).

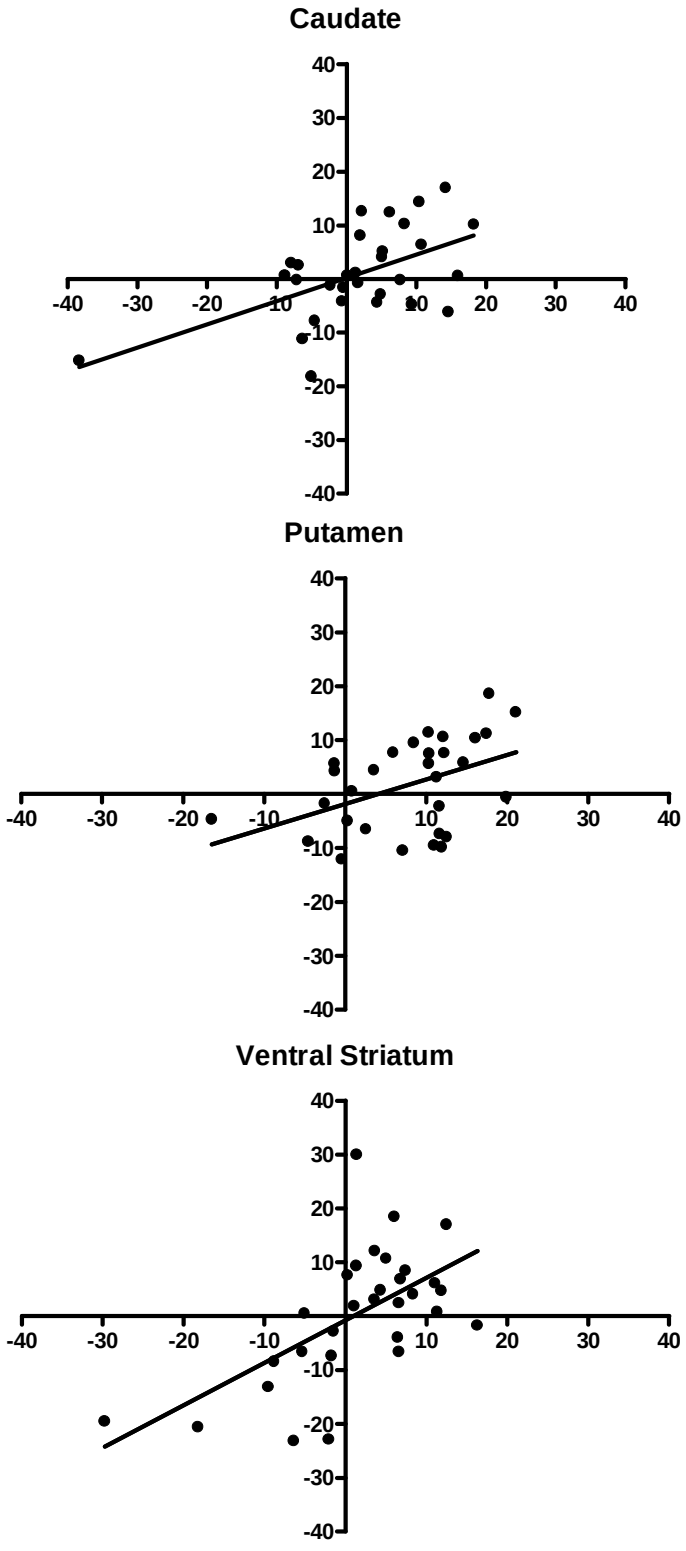


Figure 5.10 Correlations of the percent decrease in RAC BP in response to levodopa (x-axes) and to placebo (y-axes). Significant correlations were found in all striatal subregions (caudate, r = 0.65, p = 0.0022; putamen, r = 0.65, p = 0.0022), with the strongest in the ventral striatum (r = 0.74, p = 0.0001).

5.4 Discussion

The present study was conducted to investigate whether the degree of DA released in response to a placebo in mild to moderate PD patients can be modulated by the degree of likelihood of improvement. Based on the role of DA in reward signaling, we hypothesized that placebo-induced DA release would follow either a linear monotonic or an inverted-U-shaped dose response curve as a function of the patients' strength of expectation of symptom improvement. The results presented here indicate that placebo-induced DA release can be modulated by the strength of expectation, and that a dissociation exists between dorsal and ventral striatal placebo-induced DA release in PD. Whereas the degree of DA release in the putamen is related in a monotonic fashion to the expected reward value (i.e. the potential degree of clinical improvement to the active medication multiplied by the probability of receiving it), the DA release in the ventral striatum is associated with the uncertainty or saliency of benefit. Thus, the mechanism of the placebo effect in PD involves two separate components to the anticipation of benefit that are mediated by different portions of the DA system: the expectation of benefit itself which is scaled to reflect the value of the drug response to the patient and is mediated by nigrostriatal DA release in the putamen, and the uncertainty of benefit that is mediated by mesolimbic DA release in the ventral striatum. We additionally measured DA release in response to an oral levodopa challenge in the patients, and found an approximate 8% decrease in RAC BP from baseline levels in the putamen, which is in line with published results in PD patients of similar disease severity (Pavese et al., 2006;de la Fuente-Fernandez et al., 2001a). A significant reduction in RAC BP was not detected in the caudate nucleus or the ventral striatum in response to levodopa, which is in accordance with previous studies and consistent with the putamen being the primary site of levodopa-induced increases in synaptic DA in PD (de la Fuente-Fernandez et al., 2001a;de la Fuente-Fernandez et al., 2004;Pavese et al.,

2006). This indicates that the PET data are robust and reliable, and that the analysis methods employed were sound.

5.4.1 Placebo-induced dopamine release and strength of expectation

The reward hypothesis of the placebo effect proposes that the expectation of improvement in disease symptoms that is stimulated by a placebo is analogous to the expectation of reward, and thus recruits reward circuitry in the brain, specifically, the mesolimbic DA system (de la Fuente-Fernandez and Stoessl, 2002). In the context of the placebo effect in a patient living with a chronic illness, the reward is symptom relief. During the expectation of rewards, midbrain DA neurons demonstrate two distinct modes of activity: a phasic response at the presentation of a reward-predicting cue that encodes both the probability and the expected magnitude of the reward (Fiorillo et al., 2003;Tobler et al., 2005), and a sustained, tonic activity during the interval between the cue and the reward delivery which signals the uncertainty (or variance) associated with the probability distribution (Fiorillo et al., 2003). The phasic response increases in a monotonic fashion with increasing probability or value, such that the greater the likelihood of reward, the greater the DA cell firing. In extensively trained non-human primates, the tonic response follows an inverted-U shaped dose-response curve that is maximal at peak uncertainty, which occurs at a probability of 50% (Fiorillo et al., 2003). This finding was supported in functional magnetic resonance imaging (fMRI) studies in healthy human subjects that demonstrated sustained BOLD responses in the midbrain that covaried with activity in the ventral striatum and reflected reward uncertainty (Dreher et al., 2006;Aron et al., 2004). Our results indicate that in PD patients, the peak DA release in the ventral striatum in response to placebo occurs when the probability of reward is 75%, yet is absent at 25%, 50% and 100% (Figure 5.6). The finding that Group D, who were told that they were receiving active medication, demonstrated no change in DA release (a slight decrease, if anything) indicates that

the uncertainty of receiving clinical benefit likely contributes to the increase in DA release in the ventral striatum.

However, if DA release is driven by uncertainty, why should it be maximal at 75% rather than 50% probability? One possibility is that due to the loss of midbrain DA neurons, the reward-processing circuitry is abnormal in PD patients. Several studies have indicated dysfunctional reward processing in PD patients, where patients are either impaired on the task itself (Knowlton et al., 1996;Brand et al., 2004;Frank et al., 2004), or demonstrate different patterns of brain activity while performing the task (i.e. compensatory mechanisms) (Kunig et al., 2000;Goerendt et al., 2004;Mattox et al., 2006;Schott et al., 2007;Sawamoto et al., 2008). Un-medicated PD patients have been shown to have a tendency to overestimate aversive outcomes and underestimate the expected value of predicted rewards (Frank et al., 2004;Schott et al., 2007). Frank et al. (2004) used a trial-and-error probabilistic learning task and demonstrated that when off medication, PD patients with mild to moderate disease displayed an enhanced ability to learn by avoiding negative feedback. This bias reversed while on medication, where patients exhibited enhanced positive-feedback learning, even beyond that of healthy controls. If this is indeed the case, we would expect that the probability of reward distribution curve in PD patients might be shifted to the right, such that a 75% probability of reward might be interpreted by an un-medicated PD patient as equivalent to a 50% probability of reward in healthy controls.

Another possibility involves the success of the expectancy manipulation. Clearly the verbal instructions influenced the degree of DA release in the ventral striatum and putamen, but it remains unknown what the expected subjective probability of the patients actually was. The subjective probability is represented by an individual's interpretation of an objective probability, and does not always occur consciously. For example, even among healthy individuals, a 50% chance of rain might be interpreted as closer to 25% by one person, but closer to 75% by

another. Although we gave the patients clear verbal instructions explicitly outlining their probability of receiving levodopa, we did not formally measure how they interpreted that probability. There is evidence to suggest that PD patients have deficits in learning tasks that involve probabilistic classification, such as a task that involves weather prediction (Knowlton et al., 1996). These results were corroborated by an fMRI study where PD patients of mild disease severity, elderly adults and healthy young controls performed a reward-prediction task, and it was found that unlike the young controls, PD patients and the elderly subjects displayed no activation in the midbrain and ventral striatum during reward anticipation, but a robust activation in these regions when they received the reward (Schott et al., 2007). The authors attributed this lack of response to a deficiency in learning the predictive value of reward cues. Thus, it is possible that the verbal instruction given to the PD patients was not interpreted by the brain in the same manner as in healthy controls, and thus would be reflected in a different brain DA response.

An alternate (although not mutually exclusive) hypothesis for the role of DA in reward that is highly relevant to the placebo effect is the incentive motivation hypothesis of DA function (Mogenson and Phillips, 1978;Fibiger and Phillips, 1986;Ikemoto and Panksepp, 1999). Incentive salience is a learning process whereby a "liked" stimulus is transformed into a "wanted" stimulus following exposure to that stimulus when the organism is in a particular motivational state (for example, water when thirsty or food when hungry). The attribution of incentive value to a rewarding stimulus is mediated by mesolimbic DA, and occurs independently of any conscious experience of the affective quality of the stimulus (Berridge and Robinson, 1998). The incentive motivation hypothesis links the concept of incentive salience to behaviour, proposing that DA activity reflects the incentive motivational state of an organism which is triggered by stimuli which have gained incentive salience. It is based on findings in the animal literature showing that DA efflux in the NAC (as measured by microdialysis) increases

upon the presentation of a "wanted" stimulus but decreases with satiety, reflecting devaluation of the stimulus (Vacca et al., 2007;Ahn and Phillips, 1999;Balleine and Dickinson, 1998). This has been demonstrated in using RAC PET in humans, where DA release in the ventral striatum has been shown in response to amphetamine-related self reports of "drug wanting" (Leyton et al., 2002). In PD patients one would assume that there is a basal level of motivation to be in a symptom-free state. These patients may take several doses of medication a day, often cycling through periods of good and poor mobility ("on" and "off" periods). According to this framework, in everyday life levodopa pills would almost certainly acquire incentive salience through repeated associations with symptom improvement, and thus be capable of activating incentive motivational processes. In short, the patients "want" their medication and are highly motivated to have their symptoms alleviated. These concepts are highly relevant to the placebo effect, particularly in patients with a chronic illness, where the desire for symptom relief is strong. It is interesting to note that patients with chronic, clinical pain demonstrate more prominent placebo analgesia effects than healthy laboratory volunteers participating in pain experiments (BEECHER, 1960). The desire-motivational theory for the mechanism of the placebo effect argues that the placebo response is most likely to occur when individuals have a goal that can be fulfilled by confirmation of the placebo expectation, i.e. the placebo effect is consistent with their desire for symptom improvement (Price et al., 2008). Experimental results demonstrate a role for the desire for an effect across a variety of symptom domains, including those related to positive (approach or appetitive) and negative (avoidance) goals (Geers et al., 2005). In the current study, all of the patients were experienced with levodopa and were given active levodopa on Day 1, and therefore had experience with their response to it before they were given the placebo on the second day. Thus, the patients would have a clear expectation and motivation for symptom improvement in response to placebo, which almost certainly would have acquired the same incentive value as the real levodopa given on Day 1. It is not

unreasonable to postulate that the mesolimbic DA response to stimuli that have acquired incentive motivational properties could also be modulated by probability. This could explain the strong positive correlation between the DA release in response to levodopa and the DA release in response to placebo that was seen in the ventral striatum. The peak DA release in the ventral striatum at 75% could therefore not represent uncertainty of reward per se, but rather the motivational value of the drug to the patient which was modulated by their probability of receiving it. While the expected reward value might be maximal when probability is 100%, the motivational stimulus or salience might be less compared with a lower probability, precisely because the outcome is deemed as certain. One interesting alternative idea that is consistent with incentive motivation is that the decreases in DA release seen in half of the patients in Group D (Figure 5.7) could represent the "devaluation" of the levodopa stimulus; some patients expressed disappointment when they were told that they had been randomized to Group D and were receiving levodopa, since they had already received it on Day 1, and take it multiple times every day. To these select patients, the placebo was more interesting (or, rewarding) as the outcome was uncertain and was thus of greater value than the levodopa.

To our knowledge, this is the first RAC PET study that specifically manipulates probability of reward in a between-group design and measures DA release in humans. Other studies have demonstrated striatal DA release in response to monetary reward (Koepp et al., 1998;Pappata et al., 2002), drug rewards (Leyton et al., 2002;Martinez et al., 2003;Drevets et al., 2001;Brody et al., 2004;Oswald et al., 2005;Volkow et al., 2001) and primary rewards (Small et al., 2003) but the probability in each of these studies was kept constant. Zald et al. (2004) examined DA release in response to reward using both fixed and variable schedules of reinforcement, with a reward rate of only 25%. DA release was higher in the variable schedule of reinforcement condition in the left medial caudate only, but there were simultaneous decreases in DA release in the putamen and other areas of the caudate and no change in the ventral striatum

(Zald et al., 2004). The present results also indicate that a reward probability of 25% might be too low to detect DA release using RAC PET.

5.4.2 Expected reward value and dopamine release in the dorsal striatum

The second major finding of this study was that although the probability of receiving medication significantly predicted the degree of placebo-induced DA release in the putamen of PD patients, the degree of DA release was better predicted by the ERV_{obj}. Thus, while DA release in the putamen was maximal at a probability of 75% the *p* value of the ANCOVA declined from 0.028 when ERV_{obj} was ignored to a non-significant value of 0.065 once this factor was accounted for. ERV_{obj} was significantly and linearly correlated with DA release in both ventral striatum and putamen, but did not modify the relationship between probability and DA release in the ventral striatum. Thus, DA release appears to have been predominantly driven by probability (uncertainty) in the ventral striatum, but by ERV_{obj} in the putamen.

Neuroeconomists have adopted theoretical concepts from microeconomics and utility theory and applied them to the functioning of the DA system in reward and decision making (Glimcher 2003). The ERV has emerged as a parameter that integrates the probability of obtaining a reward and the reward magnitude. Midbrain DA neurons and the regions they innervate, including the striatum and the medial prefrontal cortex, have been shown to play a critical role in the computation of ERV (Tobler et al., 2005;Knutson et al., 2005;Tobler et al., 2007;Rolls et al., 2008). When ERV_{obj} was used as a continuous independent variable and not just probability alone, we found a highly significant, monotonic increase in putaminal DA release with increasing ERV_{obj} ($r = 0.61$, $p = 0.0058$). Thus, the product of the probability of therapeutic benefit and the actual clinical benefit that was produced by levodopa on Day 1 determined the amount of DA release elicited by the placebo on Day 2. This makes conceptual sense if one considers the situation in which a patient has a poor response to levodopa on Day 1, and is then randomized to a high probability of active drug on Day 2, and told that they are being

given levodopa again. The placebo effect produced in this situation (i.e. DA release) is likely to be lower than for someone who had a strong response to levodopa. This finding suggests that the placebo effect in PD involves a scaled degree of DA release to the putamen that reflects the "motor value" of the active medication to the patient. In other words, probability alone is insufficient to account for the degree of placebo-induced DA release in the putamen, and one must incorporate a scaling factor that represents the actual symptomatic benefit that the patient experiences in response to the active medication. This is consistent with the findings in depression and pain that indicate that the placebo effect "goes where it is most needed," and emulates the biochemical effect of the treatment from which it is yoked (Benedetti et al., 2005).

Interestingly, this relationship was not seen using ERV_{subj}, suggesting that the clinical response plays a more important role in the placebo effect seen in the putamen than the subjective feeling of benefit felt by the patient. The lack of correlation seen between the objective clinical response and the subjective feeling of benefit could explain this, and it is interesting to note that the striatum has been shown to respond to novelty in the absence of conscious awareness (Berns et al., 1997). However, it is also possible that the self-reporting of subjective benefit in response to placebo did not always reflect the benefit experienced by the subjects, as many patients commented that it was difficult for them to assess their motor symptoms while lying still in the PET scanner.

Although the placebo-induced DA release in the ventral striatum was also correlated with the ERV_{obj} and ERV_{subj} , these factors had no significant additional impact on the relationship between probability and DA release. Three fMRI studies that explicitly probed ERV failed to find correlations between ERV and ventral striatal BOLD signals (Knutson et al., 2005;Dreher et al., 2006;Rolls et al., 2008), although in one study some evidence of a positive correlation with ERV was found in a more posterior and dorsal part of the ventral striatum which could be the putamen (Rolls et al., 2008). Further support for a dissociation between the computation of ERV

and uncertainty in the striatum comes from an fMRI study in healthy controls demonstrating that separate regions of the striatum coded distinct aspects of ERV and uncertainty: activity in the medial and posterior striatum correlated with ERV but not with uncertainty, and the ventral striatum BOLD responses correlated with uncertainty and reaction time, which was interpreted as coding the increasing motivation associated with increasing reward (Tobler et al., 2007).

5.4.3 Placebo responders and non-responders

Our previous study demonstrated that an injection of saline in PD patients caused a reduction of RAC BP in the dorsal striatum that was greater in patients who reported feeling benefit (placebo responders). In the current study, we found this to be the case only in Group C, and in fact the opposite relationship was seen in Group D. It is interesting to note that in the previous study, the strength of expectation of benefit was also 75%, as patients were aware that they would receive apomorphine for three of the PET scans and placebo for one (de la Fuente-Fernandez et al., 2001b). As previously noted, the self-reporting of symptomatic benefit in these studies was of questionable utility, as the patients were lying in the PET scanner when the medication (or placebo) took effect. It is possible that the symptomatic benefit produced by the placebo would be more noticeable if the subjects could engage in the movements they would do in everyday life, such as walking. Anecdotally, once upright and out of the scanner, many patients did comment that they could then feel the medication working. This could also explain the failure to observe a correlation between the objective and subjective measures in response to levodopa.

Another possibility is that due to the temporal resolution of PET, it is likely that RAC displacement measures the cumulative effects of both tonic and phasic DA release. DA neuron firing at the time of reward delivery has been shown to reflect the discrepancy between the expected outcome and the actual outcome (the reward prediction error) (Mirenowicz and Schultz, 1994;Schultz et al., 1997;Schultz, 1998). It is therefore also possible that in non-

responders, the belief that they got placebo could have created a negative prediction error, leading to a depression in phasic DA cell firing and a decrease in DA release, which would lead to an increased RAC signal overall. The contribution of this negative DA response would likely be largest in Group D, since the expectation was 100%, those who did not perceive benefit would have a greater (more negative) reward prediction error. However, as only two patients were defined as non-responders in that group because the majority of them believed that they were receiving active medication, we would expect to see the maximal contrast between the DA response in responders and non-responders therefore in Group C. This is indeed what was observed (Figure 5.8).

5.4.4 Dopamine release and clinical outcomes

In this study, we did not observe a correlation between DA release and the changes in mUPDRS scores in response to either levodopa or placebo. This is not entirely surprising for the latter, as the DA release is associated with expectation, and would not necessarily lead to clinical improvement, although this would clearly be desirable. It should be noted that the overall pattern of clinical improvement paralleled the pattern of DA release in response to placebo, however the correlation was not significant. Improvements in rigidity and bradykinesia, but not in tremor or axial symptoms, have been shown to be correlated with DA release in the putamen of PD patients in response to levodopa as measured by RAC PET (Pavese et al., 2006). Although in this study, the patients were of greater disease duration (12 years) and severity (the mean Hoehn and Yahr stage in the "off" state was 2.8) and the full UPDRS III was used after the scan was completed. The absence of correlations in this study could be due to the fact that we used an abridged version of the UPDRS and therefore may have missed some symptoms that improved in response to either levodopa or placebo.

5.4.5 Levodopa response and placebo response

One interesting observation was the strong positive correlation between the DA release in response to levodopa and the DA release in response to placebo that occurred in all striatal subregions. This finding suggests that the DA system is capable of responding the same way to placebo as it does to levodopa, and may serve as an index of the responsivity of the patient, or, the capacity for the patient to respond to medication. The notion that the brain response to placebo mimics the response from the active treatment from which it is yoked has been put forth as a mechanism underlying the placebo effect in depression and pain (Benedetti et al., 2005;Mayberg et al., 2002;Petrovic et al., 2002). Furthermore, expectation has been shown to amplify the brain response to methylphenidate in cocaine abusers when they are expecting to receive the active drug but instead receive placebo, demonstrating that expectation alone can emulate the same drug-induced brain effects (Volkow et al., 2003).

5.4.6 Individual variability of the placebo effect in Parkinson's disease

We found a high degree of inter-subject variability in response to placebo, in all three measures of the placebo effect. This is consistent with the literature which shows the mean prevalence of placebo responses ranging from 17 – 70%, depending on the criteria used to define a placebo responder (Shetty et al., 1999;Goetz et al., 2000;Goetz et al., 2002). We found that age correlated positively with placebo-induced DA release in every group except group C, where there was a flat relationship between age and DA release. Therefore, controlling for age had a strong effect on the results, unmasking the main effect of Group on placebo-induced DA release. This may suggest that older people don't encode probability the same way as younger individuals, which was seen in part in Schott and colleagues (2008). However, a positive correlation with age and propensity to demonstrate placebo effects has been shown in two clinical studies in PD patients (Goetz et al., 2002;Goetz et al., 2008). In this study, disease duration was not correlated with the presence of a placebo effect (and was not correlated to age), nor was gender.

The high variability we detected could occur as a result of differences in the many variable psychological and social factors that contribute to a patient's expectations which are impossible to control for in an experiment such as this. Personal experience is likely to be the largest determinant of expectations, and include medication experiences (i.e. the response to medication, which is variable itself in PD), medical history and experiences with physicians and the health-care setting, the severity and duration of the disease, as well as the knowledge and insight into their own disease and its symptoms. Personality factors could include the degree of motivation for improvement, anxiety level, tendency toward optimism or pessimism, and the propensity for novelty-seeking or risk-taking behaviour. There are also several factors on the experimenter side that could contribute to the high variability seen in this study. Primarily, the UPDRS is essentially a subjective scale, and is intrinsically variable. We attempted to control for this by using the same blinded rater for all assessments within the same patient, as well as throughout the study. However, due to the long-term nature of the study it was practically difficult to use the same examiner, and three separate individuals performed this role (although always the same rater within subject). We must also account for the possibility that the nurse coordinator and I, who spent the majority of time with the patients, could have subconsciously communicated subtle signs to the patients which could have altered their expectations or perceptions, by virtue of the fact that we were not blind to the study design. The placebo effect is often a product of the entire treatment milieu and psychosocial context surrounding the patient, and although we attempted to be as consistent as possible over the course of the study, each patient was different and thus would have had a different experience, resulting in different responses to placebo.

5.4.7 Sources of error

Aside from the sources of variation mentioned in the previous section, there were other sources of error in this study that could have contributed additional variability to the results.

Although we tried to recruit patients of moderate disease severity, the baseline UPDRS motor scores of some patients were quite low (e.g. 5, and the mean was approximately 21). This was puzzling to us, for each patient was seen in the clinic prior to recruitment to ensure that they fit the criteria. This could reflect a related phenomenon in which the very act of participating in a study may be rewarding (so-called Hawthorne effect), or could simply represent the well-known cycling of "good" days and "bad" days in PD. The finding that placebo-induced DA release was not predicted by disease severity might indicate that this may not be as problematic.

In order to obtain the clinical measure of the placebo response, we chose to conduct the mUPDRS at the midpoint of each scan, which could have impacted the PET data in two negative ways: (1) it could degrade the scans with motion artifacts, and (2) movement itself has been shown to induce striatal release of DA (Ouchi et al., 2002;Goerendt et al., 2003). Although we can reliably and accurately correct the data for inter-frame motion (as described in Section 4.5), we are not able to correct the scans for intra-frame motion, which was the case in some instances.

A third source of error which likely contributed to the discrepancy in subjective self-report ratings was the variable time to "on" that is seen with oral levodopa (Pavese et al., 2006). The patients were administered immediate-release levodopa exactly one-hour prior to radiotracer injection, which gave them 40 minutes outside of the scanner before positioning on the bed when they were mobile and could feel the effects of the medication. Due to individual variation in the time it takes the medication to take effect, some patients did not perceive benefit prior to positioning. We attempted to control for this in part by at least restricting subjects to a non-protein diet prior to scanning to prevent competition from other amino acids at the blood-brain-barrier.

5.5 Conclusion

This study advances the understanding of not only the mechanism of the placebo effect in Parkinson's, but also the fundamental mechanisms underlying reward processing in the human brain. We found that the expectation of symptom improvement that is stimulated by a placebo activates two distinct components of reward processing in PD: an uncertainty or saliency-based element that results in DA release from mesolimbic neurons to the ventral striatum, and DA release from nigrostriatal neurons to the putamen that is scaled to reflect the expected reward value of the potential clinical benefit. Both of these responses are modulated by the likelihood of receiving active treatment, reinforcing previous studies indicating that expectations are central in driving the placebo effect. These results show that conscious, cognitive expectancies are able to modulate the activity of midbrain DA neurons in a manner that integrates the patient's past experience into the neural representation of reward value, while simultaneously maintaining a state of uncertainty about the outcome. It lends further support to the reward hypothesis of the placebo effect, advancing the idea that the expectation of therapeutic benefit is analogous to the expectation of reward. However, the present results further refine this hypothesis to include a role for expected reward value, which acts to "personalize" the placebo effect by scaling the degree of DA release with the clinical efficacy of the active treatment that is experienced by the patient. These findings have important implications for the design of clinical trials, as we have shown that the probability of receiving active treatment can influence DA system activity and impact the clinical outcome.

The present results also provide important insights into the functioning of reward circuitry in the human brain in response to probability. This is the first study to measure DA release in humans in response to different probabilities of reward, and to demonstrate separate neural DA responses that encode different aspects of reward expectation. We have shown that reward probability affects both dorsal and ventral DA release, but in different ways. Ventral

striatal DA release seems to be more fundamentally associated with uncertainty and/or the incentive motivational aspects of reward expectation, whereas dorsal striatal DA release encodes the expected value of the reward, thereby incorporating the magnitude of reward into the DA signals. These findings provide a solid foundation for future investigations of how the different parameters associated with reward expectation are coded for in the human brain by the DA system and its afferent structures.

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APPENDICES

The University of British Columbia
Office of Research Services
Clinical Research Ethics Board – Room 210,
828 West 10th Avenue, Vancouver, BC V5Z 1L8

ETHICS CERTIFICATE OF EXPEDITED APPROVAL: RENEWAL WITH AMENDMENTS TO THE STUDY

PRINCIPAL INVESTIGATOR:	**DEPARTMENT:**	**UBC CREB NUMBER:**
A. Jon Stoessl		H03-70185

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

Institution	Site
Vancouver Coastal Health (VCHRI/VCHA)	Vancouver General Hospital
Vancouver Coastal Health (VCHRI/VCHA)	UBC Hospital

Other locations where the research will be conducted:
N/A

CO-INVESTIGATOR(S):
Vesna Sossi
Thomas J. Ruth
Rodrigo Mercado
Sarah Christine Lidstone
Christopher Honey

SPONSORING AGENCIES:
Canadian Institutes of Health Research (CIHR) - "Expectation, the Placebo Effect and Dopamine Release in Parkinson's Disease:Expectation and the Placebo Effect in Parkinson's Disease Patients With Subthalamic Deep Brain Stimulation. "
Unfunded Research - "Expectation and the Placebo Effect in Parkinson's Disease Patients with Subthalamic Deep Brain Stimulation"

PROJECT TITLE:
Expectation, The Placebo Effect and Dopamine Release in Parkinson's Disease Patients with Subthalamic Deep Brain Stimulation.

The current UBC CREB approval for this study expires: February 18, 2009

AMENDMENT(S):			**AMENDMENT APPROVAL DATE:**
Document Name	**Version**	**Date**	**February 18, 2008**
Addition of Study Team Member DD 14 January 2008			

CERTIFICATION:
In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.
The Chair of the UBC Clinical Research Ethics Board has reviewed the documentation for the above named project. The research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved for renewal by the UBC Clinical Research Ethics Board.

Approval of the Clinical Research Ethics Board by:

Dr. Bonita Sawatzky,

APPENDIX B: Full Unified Parkinson's disease Rating Scale (III)

Project: _____ _____

Date & Time _____ _____

Examiner: _____ _____

Subject ID: _____

Comments: _____

Speech

0 = Normal
1 = Slight loss of expression, diction, volume.
2 = Monotone, slurred but understandable; moderately impaired
3= Marked impairment, difficult to understand
4= Unintelligible

Facial Expression

0 = Normal
1 = Slight hypomimia, could be poker face
2 = Slight but definitely abnormal diminution in facial expression
3= Moderate hypomimia; lips parted some of time
4= Marked or fixed face with severe or complete loss of facial expression; lips parted ¼ inch or more

Tremor at Rest

0 = Absent
1 = Slight and infrequently present
2 = Mild in amplitude and persistent. Or moderate in amplitude, but only intermittently present
3= Moderate in amplitude and present most of the time
4= Marked in amplitude and present most of the time

Action/Postural Tremor Of Hands

0 = Absent
1 = Slight; present with action
2 = Moderate in amplitude , present with action
3= Moderate in amplitude, present with action and posture holding
4= Marked in amplitude; interferes with feeding

Rigidity

Judged on passive movement of major joints with patient in sitting position; ignore cogwheeling

0 = Absent
1 = Slight, or detectable only when activated by mirror or other movements.
2 = Mild or moderate
3= Marked, but full range of motion easily achieved.
4= Severe, range of motion achieved with difficulty.

Finger Taps

0 = Normal
1 = Mild slowing and/or reduction in amplitude
2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement.
3= Severely impaired. Frequent hesitation en initiating movements or arrests in ongoing movement.
4= Can barely perform the task

Hand Movements

0 = Normal
1 = Mild slowing and/or reduction in amplitude
2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement
3= Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.
4= Can barely perform the task.

Rapid Alternating Movement of Hands

0 = Normal
1 = Mild slowing and/or reduction in amplitude
2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement
3= Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.
4= Can barely perform the task.

APPENDIX B: Full Unified Parkinson's disease Rating Scale (III)

Leg Agility

0 = Normal
1 = Mild slowing and/or reduction in amplitude
2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement
3= Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.
4= Can barely perform the task.

Arising From Chair

0 = Normal.
1 = Slow; or may need more than one attempt.
2 = Pushes self up from arms of seat.
3= Tends to fall back and may have to try more than one time, but can get up without help.
4= Unable to arise without help.

Posture

0 = Normal erect
1 = Not quite erect, slightly stooped posture; could be normal for older person.
2 = Moderately stooped posture, definitely abnormal; can be slightly leaning to one side.
3= Severely stooped posture with kyphosis; can be moderately leaning to one side.
4= Marked flexion with extreme abnormality of posture

Gait

0 = Normal
1 = Walks slowly, may shuffle with short steps, but no festination (hastening steps) or propulsion
2 = Walks with difficulty, but requires little or no assistance, may have some festination, short steps, or propulsion.
3= Severe disturbance of gait, requiring assistance.
4= Cannot walk at all, even with assistance.

Postural Stability

0 = Normal
1 = Retropulsion, but recovers unaided.
2 = Absence of postural response; would fall if not caught by examiner.
3= Very unstable, tends to lose balance spontaneously.
4= Unable to stand without assistance.

Body Bradykinesia and Hypokinesia

0 = None
1 = Minimal slowness, giving movement a deliberate character; could be normal for some persons. Possibly reduce amplitude.
2 = Mild degree of slowness and poverty of movement which is definitely abnormal. Alternatively, some reduced amplitude.
3= Moderate dergree of slowness, poverty or small amplitude of movement.
4= Marked slowness, poverty or small amplitude of movement.

Modified Hoehn and Yahr Staging:

Stage	
0	▪ No signs of disease
1	▪ Unilateral disease
1.5	▪ Unilateral plus axial involvement
2	▪ Bilateral disease ▪ No impairment of balance (0/4)
2.5	▪ Bilateral disease: mild ▪ Recovery on pull test (1/4)
3	▪ Bilateral disease: mild to moderate ▪ Some postural instability; Physically independent (2/4)
4	▪ Severe disability ▪ Still able to walk or stand unassisted (3/4)
5	▪ Wheelchair bound or bedridden unless unaided

APPENDIX C: Modified Unified Parkinson's disease Rating Scale (III)

Project: _____ Wearing Off _____

Date & Time _____ Dyskinesias _____

Scan no. (circle) 1 2 3 4 Last Meds Taken _____

Examiner: _____

Name: _____

DOB & Age _____

Tremor at Rest NO HEAD

0 = Absent
1 = Slight and infrequently present
2 = Mild in amplitude and persistent. Or moderate in amplitude, but only intermittently present
3= Moderate in amplitude and present most of the time
4= Marked in amplitude and present most of the time

Rigidity NO HEAD

Judged on passive movement of major joints with patient in sitting position; ignore cogwheeling

0 = Absent
1 = Slight, or detectable only when activated by mirror or other movements.
2 = Mild or moderate
3= Marked, but full range of motion easily achieved.
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3= Severely impaired. Frequent hesitation en initiating movements or arrests in ongoing movement.
4= Can barely perform the task

Hand Movements

0 = Normal
1 = Mild slowing and/or reduction in amplitude
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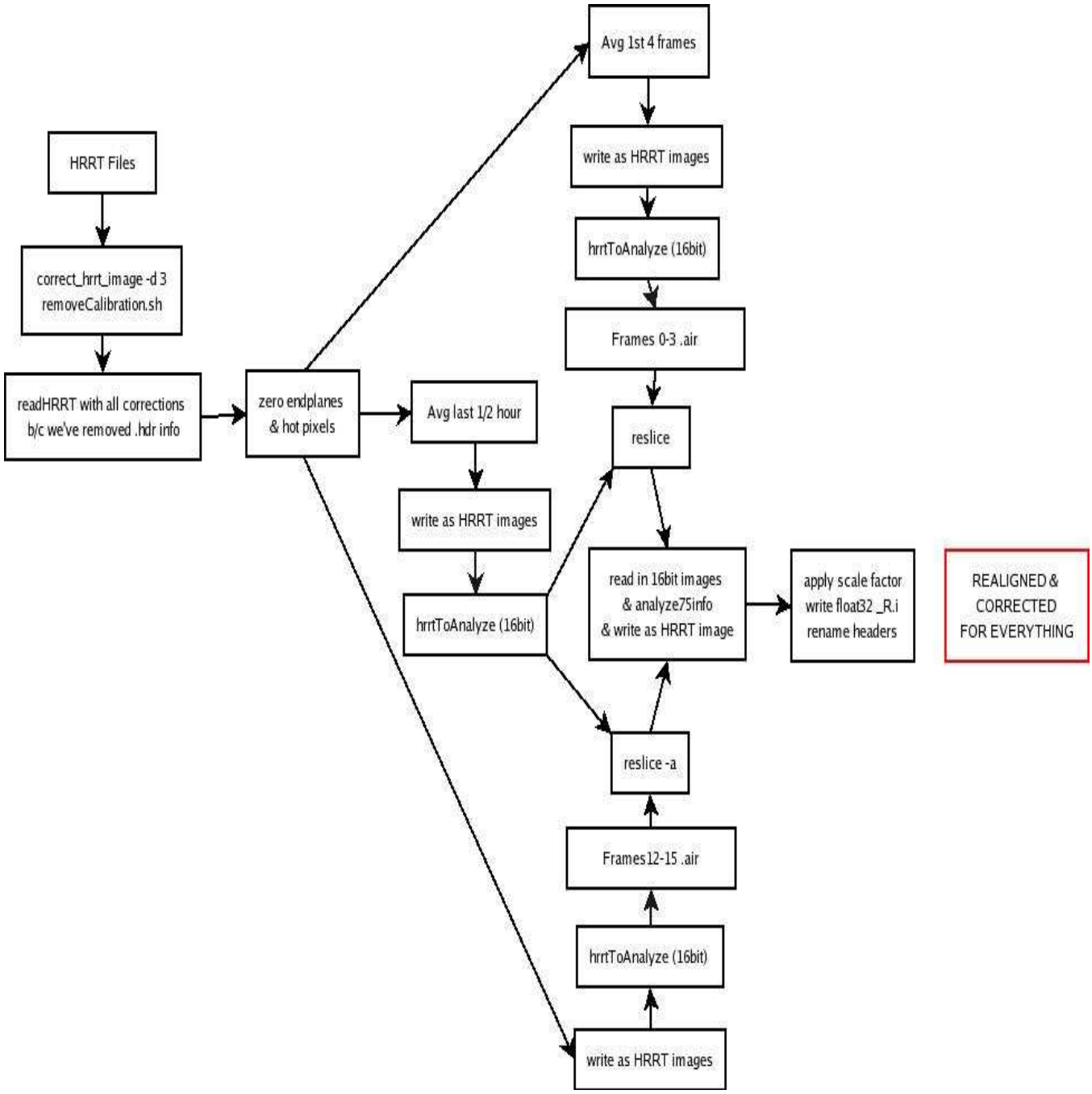
Rapid Alternating Movement of Hands

0 = Normal
1 = Mild slowing and/or reduction in amplitude
2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement
3= Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.
4= Can barely perform the task.

APPENDIX D: Frame-to-frame realignment protocol flow chart



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828 West 10th Avenue, Vancouver, BC V5Z 1L8

ETHICS CERTIFICATE OF EXPEDITED APPROVAL: RENEWAL WITH AMENDMENTS TO THE STUDY

PRINCIPAL INVESTIGATOR:	**DEPARTMENT:**	**UBC CREB NUMBER:**
A. Jon Stoessl		H05-70124

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

Institution	Site
Vancouver Coastal Health (VCHRI/VCHA)	UBC Hospital

Other locations where the research will be conducted:
N/A

CO-INVESTIGATOR(S):
Vesna Sossi
Anthony G. Phillips
Thomas J. Ruth
Sarah Christine Lidstone
Martin J. McKeown

SPONSORING AGENCIES:
Canadian Institutes of Health Research (CIHR) - "Manipulation of Expectation Using Pharmacological Treatment; CIHR Grant "Expectation, the Placebo Effect and Dopamine Release in Parkinson's Disease""

PROJECT TITLE:
Manipulation of Expectation Using Pharmacological Treatment; CIHR Grant "Expectation, the Placebo Effect and Dopamine Release in Parkinson's Disease"

The current UBC CREB approval for this study expires: March 26, 2009

AMENDMENT(S):	**AMENDMENT APPROVAL DATE:**
Change of Study Team Member	**March 26, 2008**

CERTIFICATION:
In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.
The Chair of the UBC Clinical Research Ethics Board has reviewed the documentation for the above named project. The research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved for renewal by the UBC Clinical Research Ethics Board.

Approval of the Clinical Research Ethics Board by:

[redacted]

Dr. James McCormack,
Associate Chair

APPENDIX F: Subject Self-Reporting Form

Date: _____

Examiner: _____

Name: _____

Group: _____

Scan 1

How did you feel during this scan?

Worse than usual	Average	Better than usual

Comments:

Scan 2

Did you notice any benefit following the medication?

worse	none	mild	moderate	strong

How did it compare with your usual medication?

Comments:

Scan 3

Did you notice any benefit following the medication?

worse	none	mild	moderate	strong

How did it compare with your usual medication?

Comments:

THE UNIVERSITY OF BRITISH COLUMBIA

Pacific Parkinson's Research Centre
Faculty of Medicine
Vancouver Coastal Health Authority
Purdy Pavilion, 2221 Wesbrook Mall
Vancouver, B.C. Canada V6T 2B5

Tel: 604-822-7660
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CONSENT FORM

Title of project:	**Manipulation of expectation using pharmacological treatment**
Institute:	University of British Columbia
Principal Investigator:	Dr. A. Jon Stoessl
Co-Investigators:	T. Ruth, A. Phillips, S. Lidstone
Phone:	
24 hour contact (pager):	[redacted]

You are invited to participate in a research study sponsored by the Canadian Institutes of Health Reaserch (CIHR) and conducted by the Pacific Parkinson's Research Centre at the Heath Sciences Centre Hospital – UBC site. It is important that you read and understand the following general principles that apply to all participants in our studies:

a) Participation is entirely voluntary.
b) You will receive no direct benefit from participation in the study although knowledge may be gained that will benefit others.
c) You may withdraw from the study at any time without jeopardizing your access to future health care or losing good will.
d) We do not have information for pregnant women or lactating mothers. Therefore, you should not be participating in this study if there is a possibility that you are pregnant or breastfeeding. If you are a woman of childbearing age and have not experienced menopause or had a surgical procedure to prevent pregnancy, you will be asked to complete a urine pregnancy test prior to participating in this study.

The nature or the study, risks, inconveniences, discomforts, and other pertinent information about the study are discussed below. Please feel free to ask any questions you may have of those discussing the project with you.

We are conducting a study that will examine how a person's thoughts and feelings about treatment for Parkinson's disease affect their Parkinson's disease symptoms. You have been invited to participate in this study because you have been diagnosed with idiopathic Parkinson's disease. In other studies, we have discovered that a person's expectations about their treatment can contribute to their response to that treatment.

In studies of depression and pain it has been observed that belief in a device or drug results in a significant improvement in symptoms. Recent studies of brain functioning have also shown that use of suggestion or

belief in a device or drug by people with Parkinson's Disease has resulted in increased dopamine, the chemical (neurotransmitter) responsible for sending messages from one nerve cell to another, and therefore to a reduction of symptoms. This study will further evaluate those observations.

The purpose of this study is to examine the different factors that contribute to a person's response to the treatment of their Parkinson's disease. The study requires the use of some deception, and as a result the full purpose of the study cannot be revealed to you at this time. However, nothing that has been described above about the purpose is false. We have simply omitted some details. These will be described to you once the study has been completed. At that time, we will fully debrief you about the background, purpose and methods that were used during the experiment and answer any questions that you may have.

This study will be entirely conducted at UBC Hospital. 40 subjects will be invited to participate. You must have been diagnosed with idiopathic Parkinson's disease, and your symptoms are considered to be 'moderate.' In order to participate in this study, you must be taking levodopa/carbidopa (Sinemet), although the additional use of any dopamine agonists is also permitted.

You should not participate in this study if:

- You have atypical parkinsonism, dementia, or significant other neurological disease (i.e. stroke);
- You are pregnant or breastfeeding;
- You are unable to tolerate staying off anti-parkinsonian medication for at least 12 hours;
- You have significant memory problems;
- You have a history of significant depression or are currently depressed;
- You have a history of drug abuse or any significant psychiatric history or symptoms.

We will be using a brain imaging technique called Positron Emission Tomography (PET). PET involves injecting a very small amount of a known medication attached to a very small amount of radiation and following the medication via the scanner, which in turn gives us information about how nerve cells in the brain are working. The PET scanner is similar to a CT scanner in size and shape. While PET scanning is not used as a diagnostic imaging tool for movement disorders in Canada and is considered by Canadian regulatory agencies to be an investigative device, it is considered the optimal way to examine the behaviour of living nerve cells.

The experiment will consist of 3 PET scans over the course of two consecutive days—2 scans on Day 1 and one scan on Day 2. The first scan (Scan 1) will be a baseline scan, in which you won't receive any medication. The second scan (Scan 2) will be the medication scan, in which you will receive an oral dose of levodopa/carbidopa (Sinemet). The third PET scan (Scan 3) will take place the following morning. You will receive either the same Sinemet dose you received for Scan 2, or you will receive a placebo. A placebo is an inactive substance that looks identical to the medication used in this study (i.e. Sinemet), but contains no active ingredients and is therefore completely inert.

Placebos are routinely used in medical research to test how effective a particular drug is. In the case of this study, you will be randomly assigned (by chance, like the flip of a coin) to one of four groups which will determine your likelihood of receiving Sinemet. For example, subjects in Group A will have a 25% chance of receiving Sinemet, and so a 75% chance of receiving placebo. Subjects in Group B will have a 50% chance of receiving Sinemet, and a 50% chance of receiving placebo. Subjects in Group C will have a 75% chance of receiving Sinemet, and a 25% chance of receiving placebo.

Finally, subjects in Group D will have a 100% chance of receiving Sinemet (no chance of receiving placebo). Your chance of being in Group A is the same as your chance of being in Group B, C or D.

You will be told which group you have been assigned to, and so you will be made fully aware of your chances of receiving Sinemet or placebo, however you will not be told what you actually received during the scan until after the experiment has been completed.

Prior to receiving medication (levodopa/carbidopa on day 1 & levodopa/carbidopa or placebo on day 2) you will also be given 20 mg of the medication Domperidone. This is to prevent any possibility of nausea from taking levodopa/carbidopa.

This study requires a time commitment of 1.5 days. You will be compensated for parking and travel expenses and provided with a mid-day meal and light snacks while here. Receipts will be needed for compensation.

This study also requires that you have an MRI (magnetic resonance imaging) scan. This scan, which should not take more than ½ an hour may or may not happen during the 2 days you are completing your PET scans. The MRI scan will provide us with information about thickness, size and shape of parts of your brain and it will be correlated with the PET data.

You should not have an MRI if:

- You have any of the following: a cardiac pacemaker,wires, debrillator, artifical heart valve, brain aneurysm clip, electrical bone/nerve stimulator, implanted drug infusion pump, implanted metal joints,screws, plates, or rods, coil/catheter/filter in any blood vessel, imbedded shrapnel, bullets or metal fragments, non-removable dentures, braces, retainers
- You have had surgery, tattoos or ear pearcings in the last 6 weeks
- You have a joint injection the last 4 weeks
- You have been a metal worker or machinist

If you cannot complete the MRI scan, this will not prevent you from participating in the study.

Each night prior to the PET scans; we will ask that you not take any Sinemet after 10:00 PM. If you are taking Sinemet CR, or any of the anti-parkinsonian medications such as bromocriptine, Permax, Requip, or Mirapex we will ask that you not take the drug after 6:00 PM the day before the visit. On each of the days of your scans we will ask you to limit your fluids and eat a light breakfast before coming to University Hospital.

You will be met at UBC Hospital on the morning of the scan and we will review the consent form. In order to determine if you are eligible for the study, you will first be asked to complete two questionnaires that will take approximately 15 minutes. Following this, you will meet with the doctor and will be asked to undergo a 15-minute neurological assessment.

We will then go to the PET suite to setup for scanning, which is located in our Nuclear Medicine Department at UBC Hospital. You will be asked to lie on your back on a padded bed. You may be asked to wear a swim cap that has motion markers attached to it. This will allow us to record any head movement during the scan. A thermoplastic mask with eye and ear holes will be shaped over your forehead and cheekbones. It will harden and help keep your head still while you are being scanned and can be used for all 3 scans.

At the start of each day a needle will be used to introduce a fine plastic tube into a vein in your arm (intravenous, IV). This will remain in place for the remainder of the day for the injection of the tracers. A 15-minute pre-scan (similar to a CT scan) will be performed prior to tracer injection. This allows us to

account for the effect of the brain and skull on our ability to measure radioactivity from the injected tracers.

After the pre-scan, the first raclopride scan will begin and you will be scanned for 60 minutes. Raclopride is a medication that is used to treat psychiatric disorders. Upon completion of this scan you will have a break to have a snack and stretch your legs. We will perform a second raclopride scan 1.5 hours after the first and this will last for 60 minutes as well. Upon completion of these 2 scans you will restart your regular anti-parkinsonian medications and be free to depart until the following morning.

On the second day, the third scan will be done in the same manner as for the first day, and at the end of the scan you will resume your medications and be free to depart. However, following the end of the scheduled scan, you may be asked, if you are willing, to be scanned on another PET scanner so that we can compare the 2 scanners. This does not require any further injections and only a very small amount of radioactivity (much less than that received from the injection); it will simply extend your time here by up to a maximum of 40 minutes.

Risks, Inconveniences and Discomfort

Some people find that lying in the PET or MRI scanner for extended periods may be uncomfortable and others find that they will feel claustrophobic (a fear of closed in spaces).

There are few known risks related to MRI. The presence of certain types of loose metalwork (eg, surgical artery clips or metal bits, and pacemakers may be problematic as they become warm and are pulled to the magnet in the scanner. You will not be able to have an MRI if any of the exclusion factors listed on the previous page are present so this risk should not be an issue.

Because you will not be taking your anti-parkinsonian medication while being PET scanned you may experience a temporary return of your Parkinson's symptoms. These symptoms will invariably resolve when you restart your medications. If you are certain that you cannot tolerate this you should not participate in this study. Because your balance and movement may not be as good as usual overnight we encourage you to be cautious in your activities. If you at any time during the night you feel that cannot remain off your medication you should telephone the 24 hour contact number and restart your medications. Every effort will be made to ensure your comfort while you are being scanned.

There may be some discomfort associated with the placement of the intravenous needle during the PET scan. There is a remote risk of fainting associated with the placement of the needle, and a slight possibility of arm bleeding, inflammation or clotting of the blood vessel. Permanent damage from these complications is extremely rare. We have done over 4000 scans at UBC without complications. The risk is therefore less than 0.05%.

Raclopride is a medication that is used to treat psychiatric disorders and has a tranquilizing effect at treatment doses. Common effects at treatment doses can include restlessness, abnormal muscle contractions and a mild decrease in blood pressure. These side effects are not expected at the doses used here, which are substantially lower. We estimate the risk of any side effects to use of this tracer be less than 1.0 %

Levodopa is the best drug for the treatment of Parkinson's disease, which is approved and widely used. The dose of levodopa/carbidopa (250/25 mg) given during the procedure may be higher than your usual individual doses. The most common side effect of Sinemet is nausea. Because you already are taking this medication this side effect is not expected here.

However because there is about a 2% risk of nausea we will give you another standard medication, domperidone, shortly before taking the Sinemet. Sinemet may also cause a lowering of the blood pressure, which can cause light-headedness and, rarely, fainting. There is less than 1% possibility of this happening. You may also experience a temporary increase in dyskinesias if this is a usual symptom of your Parkinson's disease treatment. These possible side effects are temporary and will resolve in 1 to 2 hours.

Domperidone is a medication commonly given in conjunction with Sinemet or dopamine agonist therapy in order to reduce or prevent nausea or postural dizziness that may result from these medications. Because we are giving you a dosage of Sinemet that may be higher than what you usually take, we wish to minimize this risk by giving you domperidone first. Regular prescribed use of this medication might result in a lower than 1% occurrence of nervousness, dizziness, thirst, lethargy, abdominal cramps and irritability. Side effects are unlikely to occur at all with use of this medication for the short duration of this study and we calculate risk of side effects to be well below 1%.

Benefits

As stated in Section B of Page 1 of this document, you will receive no direct benefit from participation in the study. However, knowledge may be gained that will benefit others.

Confidentiality

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of Health Canada and the UBC Research Ethics Board for the purpose of monitoring the research. However, no records, which identify you by name or initials, will be allowed to leave the Investigators' offices. The information obtained from this study will eventually be used in scientific publications. Your identity will not be revealed in such publications or other reports.

Signing this consent form does not in any way limit your legal rights against the investigator or anyone else. At any time you may call the doctors involved or the study co-ordinator at [redacted]. If you have concerns about your treatment or rights as a research participant, you may call the Research Subject Information Line in the UBC Office of Research Services [redacted].

Consent to Participate

- *I have read and understood the subject information and consent form.*
- *I have been told that I will receive a dated and signed copy of this form.*
- *I have had sufficient time to consider the information provided and to ask for advice if necessary.*
- *I have had the opportunity to ask questions and have had satisfactory responses to my questions.*
- *I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.*
- *I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.*
- *I understand that I am not waiving any of my legal rights as a result of signing this consent form.*
- *I understand that that this study will not provide any direct benefit to me.*
- *I have read this form and I freely consent to participate in this study.*

****

_____ _____

Subject's Signature Printed Name Date

_____ _____

Witness Signature Printed Name Date

_____ _____

Investigator's Signature Printed Name Date

DEBRIEFING FORM

Title of project: **Manipulation of expectation using pharmacological treatment: Expectation, the Placebo Effect and Dopamine Release in Parkinson's Disease**
Institute: University of British Columbia
Principal Investigators: A. Jon Stoessl, T. Ruth, V. Sossi, A. Phillips, S. Lidstone

You have just participated in a study where it was necessary to use deception in order to achieve the goals of the research. The true purpose of this study is to better understand the role of expectation in the placebo effect in Parkinson's disease, and how expectation may affect dopamine release in the brain.

A placebo is a substance that looks, tastes, or feels similar to an active drug, but has no active ingredients and is therefore inert. The placebo effect occurs when a person takes a placebo (for example a sugar pill) which they think is a real drug, and ends up feeling better. The improvement the person feels can therefore not be due to the placebo, since it has no action in the body, but may occur because of some mechanism in the person's brain. The possibility also exists that the person feels better because the illness simply runs its course and is naturally over. Prominent placebo effects occur in a wide variety of medical conditions, including pain, depression and Parkinson's disease. Research in this and other Centres has shown that a person's expectations about the drug or placebo they are taking are critical to how the placebo effect works. For example, if a person takes a placebo but thinks that it is a painkiller, they report feeling less pain and experience a placebo effect. However, if those people are told that they are being given a substance that does nothing and are then exposed to a painful stimulus, they do not feel less pain and therefore do not experience a placebo effect.

However, how these expectations are able to trigger a placebo effect in the brain is still unknown. By observing the placebo effect in people who have been diagnosed with Parkinson's disease, our research has discovered that the expectations a person has about how they will feel after taking their medication is able to stimulate dopamine release in the brain. Since dopamine is the key chemical messenger (neurotransmitter) that is depleted in the brain in Parkinson's disease, we believe that perhaps this dopamine release is responsible for making the person feel better and for the improvement in their symptoms.

In this study, we wanted to see if the strength of a person's beliefs or expectations about whether or not they would receive levodopa is related to the dopamine levels in their brain. To achieve this, it was necessary for you to believe that you had a certain chance of receiving levodopa for Scan 3 when in fact, you actually received placebo. Since you had no active medication for this scan, we were able

to measure the changes in dopamine levels in your brain that were caused by the expectations you had, and not the levodopa.

We deeply appreciate your participation in this study, and are more than happy to answer any questions you may have about any aspect of the study. If you should have any further inquiries, feel free to contact our PET Imaging Coordinator [redacted]. If you have any concerns about the use of deception or your general rights as a research participant, you may call the Research Subject Information Line, Office of Research Services and Administration at [redacted].

APPENDIX I

CO-AUTHORSHIP STATEMENT

Much of the work conducted in Chapters 3-5 was carried out in collaboration with my research supervisor, mentors, and colleagues at the Pacific Parkinson's Research Centre, the UBC PET Program, TRIUMF, and the UBC Movement Disorders Clinic. Every attempt has been made to give credit where credit is due, either by sharing authorship in published manuscripts and abstracts, or by acknowledging assistance at the end of publications or finally, in the Acknowledgements section of this thesis. In general, I performed 70-80% of the work required to conduct these experiments and produce publishable results, including the experimental design, patient recruitment, data collection, analysis and interpretation, and all of the writing, except comments and suggestions from co-authors on the manuscripts.