AMPHETAMINE-INDUCED DOPAMINE RELEASE IN PARKINSON'S DISEASE
PATIENTS WITH DEPRESSION MEASURED USING HIGH-RESOLUTION POSITRON
EMISSION TOMOGRAPHY WITH [^{11}C]RACLOPRIDE

by

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ABSTRACT

Depression affects around 40% of patients with Parkinson's disease (PD), which is nearly double the amount of severe depression seen in comparably disabled patients with other chronic illness. Mesocorticolimbic dopamine (DA) depletion has been implicated in the pathogenesis of depression, and thus may contribute to the high incidence of depression in PD. Amphetamine (AMPH)-induced striatal DA release was compared between depressed (n=3) and non-depressed (n=6) patients with PD using positron emission tomography. DA release was estimated by displacement of the D2/D3 receptor radiotracer [^{11}C]raclopride (RAC). Subjects completed three scans over two days within a three month period. The first scan was a baseline scan, followed by either blinded d-AMPH (0.3 mg/kg p.o.) or placebo administration, counterbalanced across subjects. Emission data were acquired for 60 minutes using a high resolution research tomograph, scans were reconstructed using Ordinary Poisson-OSEM3D including attenuation, scatter and random correction, and inter-frame realignment was performed to correct for motion. RAC binding potentials were estimated using regions of interest and a graphical tissue approach with the cerebellum as a reference region. Amphetamine induced DA release was observed at a significant level in the ventral striatum and with a strong trend in the caudate. Although amphetamine induced DA release has been observed in other studies, these PD patients exhibit a different amphetamine induced profile in which caudate and ventral DA release was greater than putamen DA release. No significant placebo effect was measured in this study but visual inspection suggests depressed patients may have a decrease in endogenous DA release with placebo. Other than this effect no difference was observed between depressed and non-depressed PD subjects. Ongoing work will attempt to extend these findings to a larger sample.

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ΔBP_{AMPH}, change of binding potential between amphetamine and baseline, ΔBP_{drug}, change of binding potential between amphetamine and placebo; ΔBP_{PB}, change of binding potential between placebo and baseline; $\Delta AIRS$, change of AIRS between amphetamine and placebo; 5HT, serotonin; AIRS, amphetamine interview rating scale; AMP, amphetamine; ANOVA, analysis of variance; AST, associative striatum; BL, baseline; BP, binding potential; CAU, caudate; CAUamp, D2 receptor occupancy of caudate following amphetamine; CON, control; CSF, cerebral spinal fluid; DA, dopamine; DAT, dopamine transporter; DEP, depressed; IBZM, iodobenzamide, GPi, internal globus pallidus; MADRS, Montgomery-Asberg Depression Rating Scale; MAO-A, monoamine oxidase A; MDN, medial dorsal nucleus; MPFC, medial prefrontal cortex; OPFC, orbital prefrontal cortex; PB, placebo; PET, positron emission tomography; Pre-DCAU, precommisural dorsal caudate; pre-DPU, precomissural dorsal putamen; post-DCA, post commissural caudate; PUT, putamen; PUTamp, D2 receptor occupancy of putamen following amphetamine; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; PAG, periaqeuductal grey, PD, Parkinson's Disease; UPDRS, Unified Parkinson's Disease Rating Scale; VA, ventral anterior nucleus, VEN, ventral striatum; VP, ventral pallidum; VMAT, vesicular monoamine transporter; VTA, ventral tegmental area

ACKNOWLEDGEMENTS

INTRODUCTION

1.1 Depression

According to Health Canada, almost 10% of Canadians fulfill the criteria for major depression at some point in their lifetime. Major depressive disorder is characterized by symptoms of decreased mood, anhedonia, sleep disturbances, changes in appetite, decreased energy, increased psychomotor agitation/retardation, difficulties sustaining concentration and recurrent thoughts of death. However, depression does not conform to a set group of symptoms, and thus is a heterogeneous disorder. Subpopulations can be identified based on clustering of certain symptoms. Depression is often present in other mental illnesses including bipolar disorder, post-traumatic stress disorders and schizoaffective disorder. Generally, patients suffering from major depression experience depressive symptoms for approximately 60% of the time if they have had one major depressive episode, suggesting that depression is a chronic disorder (Judd et al., 1998). Other outstanding features of depression include significantly increased incidence in women, significant comorbidity of substance abuse, anxiety disorders, cardiovascular disease and diabetes (Mineka et al., 1998; Volkow, 2004; Fenton and Stover, 2006).

Numerous theories have been proposed for the etiology of depression. One theory suggests that exposure to significant life events (i.e. humiliation, entrapment, loss and danger) predisposes one to developing depression (Kendler et al., 2003). However, not all those exposed to these stressors develop depression suggesting that there is some diathesis that influences susceptibility. Cognitive vulnerability has been suggested to be one such diathesis. Abramson and colleagues (Abramson et al., 1989) proposed the hopelessness theory in which negative inferences about causes, consequences and the self following life events lead to depression. In Beck's theory (Beck, 1987) dysfunctional attitudes involving themes of loss, inadequacy, failure and

worthlessness serve as the diathesis. However, these cognitive patterns do not explain how depressive symptoms are manifested physiologically. A proposed route is based on findings in the immune system. Depression has been associated with increased innate immune response, in which immune cells are marked by an abnormal expression of proinflammatory cytokines (Irwin and Miller, 2007). Patients with medical conditions resulting in elevated inflammatory responses and conditions requiring cytokine therapies are more prone to depressive symptoms. As well, these cytokines have known effects on neuroendocrine, neurotransmitter and information processing pathways related to depression. However, abnormal immune response may not be relevant to all forms of depression.

The prominent theory of depression that has held sway over the years is the monoamine hypothesis, which states that decreased levels of modulatory monoamines (serotonin, norepinephrine, dopamine) increases the susceptibility to depression and potentially changes the response to stress. It is difficult to determine the direction of causation since many studies look through cross-sections of populations or under acute laboratory conditions rather than studying them longitudinally. The monoamine hypothesis has been further advanced by (Meyer et al., 2006) with their study on the metabolic enzyme monoamine oxidase type A (MAO-A), which they found to be elevated across brain regions. In effect, the elevated metabolism results in decreased levels of monoamines if no appropriate counteractive mechanisms are taken at the synapses. These counteractive measures include down regulating reuptake transporters to increase the duration of action of the monoamine in the synapse and to prevent subsequent intracellular degradation by MAO-A. Depressive symptoms arise when counteractive measures are inappropriate or insufficient. For instance, although depressed patients had decreased dopamine transporter binding, those who exhibited psychomotor retardation had lesser down regulation of dopamine transporter and elevated D2 receptors (Meyer et al., 2001; Meyer et al.,

2006). Likewise, depressed patients have lower serotonin (5HT) transporter density but those who had greater dysfunctional attitudes had higher levels of serotonin transporter (i.e. less downregulation) and $5HT_2$ receptors (Meyer et al., 2003; Meyer et al., 2004).

The monoamine of focus and underlying the current treatment practices is serotonin. Serotonin reuptake inhibitors have become the pharmacotherapy of choice over the past decades and have recently included modifications that concomitantly block norepinephrine reuptake. Yet one monoamine that has received limited attention is dopamine, despite decreased levels of the DA metabolite homovanillic acid in cerebral spinal fluid (CSF) of patients with unipolar depression and melancholia (Roy et al., 1985). However, recent investigations into dopamine such as the aforementioned studies by Meyer and colleagues bring greater weight to its role in depression. In order to understand how dopamine can contribute to more than just psychomotor symptoms, it is important to understand how dopamine modulates the neural circuitry affected in depression.

1.2 Fronto-subcortical Circuits

COTICO-STRIATO-THALAMIC CONNECTIONS

Frontal-subcortical circuits interlink the prefrontal cortex, the striatum, and the thalamic nuclei to govern executive, sensorimotor and emotional-affective processing (Alexander et al., 1986; Haber, 2003). These circuits have been divided into parallel networks that interact with each other, and are also subject to modulation originating in the amygdala, hippocampus, hypothalamus and periaqueductal grey.

One area of particular interest with regards to depression is the ventral striatum. The ventral striatum is principally involved within the emotional-affective processing circuit. Studies involving intracranial self stimulation or localized cocaine administration in the ventral striatum

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have implicated it as part of the brain reward systems (Wise and Hoffman, 1992; McKinzie et al., 1999). The striatum itself is composed of the caudate and putamen. The most ventral-anterior portion of the caudate and putamen, along with the olfactory tubercle, is collectively referred to as the ventral striatum. Within the ventral striatum, the principle structure is the nucleus accumbens, which can be further divided into the core and shell, based on binding to different proteins (i.e. calbindin, calretinin) (Brauer et al., 2000; Prensa et al., 2003).

Afferents to the ventral striatum include the medial and orbital prefrontal cortex (MPFC and OPFC), which are thought to control reactivity to emotion and sensitivity to reward (Carmichael and Price, 1995) (Figure 1). The orbital network receives visual, gustatory/visceral, and somatic sensory signals. The MPFC network involves limbic structures and cortical structures that have been found to have altered metabolism in depressed patients (Mayberg, 1997) . Projections to the ventral striatum from these networks are subsequently routed to the globus pallidus and substantia nigra, which in turn project to thalamic nuclei that have reciprocal connections with these prefrontal networks. It is of interest to note that the amygdala, which has been greatly implicated in depression, has extensive connections with both the medial-orbital prefrontal cortex and the ventral striatum (Drevets, 2003).
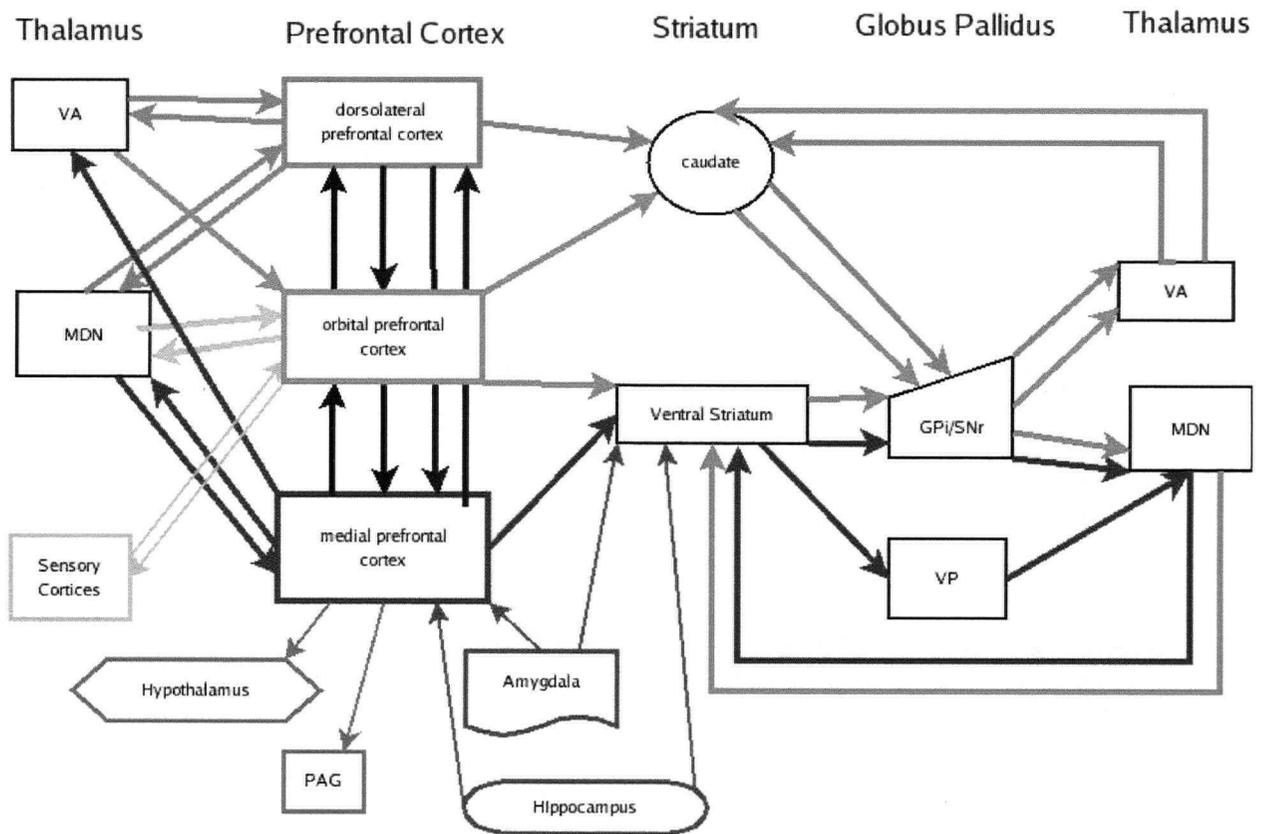
Figure 1 Frontal-subcortical circuits. Parallel circuits involving emotional (red), associative (green) and sensorimotor (blue) loops have reciprocal connections with one another. In general the prefrontal cortex is linked to basal ganglia structures, which innervate thalamic nuclei that feed back to the prefrontal cortex. GPi, lobus pallidus interna; MDN, medial dorsal nucleus; PAG, periaqueductal grey; SNr, substantia nigra pars reticulata; VA, ventral anterior nucleus of thalamus; VP, ventral pallidum.

Abnormalities within these prefrontal areas, their afferents and their efferents have been demonstrated in depression using neuroimaging technology. Response to antidepressant treatment in depression measured by brain glucose metabolism is associated with decreases in limbic and striatal regions (subgenual cingulate, insula, hippocampus and pallidum) and increases in brainstem and dorsal cortical (prefrontal, parietal, anterior and posterior cingulate) (Mayberg, 1997). However, for some regions including the basal ganglia, thalamus, amygdala and midbrain-pons no consensus exists as to a consistent pattern of changes (Mayberg, 1997). This could be attributed to numerous factors including heterogeneity of depressive profiles (symptoms, severity, family history, comorbidity), transient mood fluctuations during study, study design or data analysis techniques (Mayberg, 2003). Lesion and neurodegenerative studies provide further insight. In early frontotemporal dementia, which predominately affects the medial and orbital prefrontal cortex at this stage, apathy is found in greater than 90% of patients (Levy and Dubois, 2006). If one were to assume that lesions to any component of the circuit would lead to apathy then such should be the case with the ventral striatum. However, there are rare reports of ventral striatal lesions alone inducing apathy, but increasing the lesions to include dorsal striatal regions induce severe apathy. Nonetheless, ventral striatal lesions in monkeys result in pathological changes of reward processing. Although apathy can serve as one of the core features of depression according to the diagnostic and statistics manual (IV), patient populations like Parkinson's disease exhibit greater incidence of apathy but those with highest level of apathy are not more likely to have depression than those with the low level of apathy (Pluck and Brown, 2002; Kirsch-Darrow et al., 2006). However, it may be that depressed patients presenting with apathy may have greater abnormalities in frontal-subcortical circuit structures.

1.3. Dopamine

Dopamine plays a significant modulatory role in these frontal-subcortical circuits, particularly the striatum. As well, there is mounting evidence that dopamine is implicated in the manifestation of depression.

DOPAMINE IN FRONTAL-SUBCORTICAL CIRCUITS

In the mesencephalon, the substantia nigra pars compacta (SNc) DA neurons are located in the ventral tegmentum. Medial and rostromedial to the nigral cells is the ventral tegmental area (VTA) while the retrorubral nucleus (RRN) is located caudally to the decussation of the superior cerebellar peduncles.

One predominant dopaminergic projection system is the mesostriatal pathway (Figure 2). The midbrain neurons and the striatum can be segregated into three areas representing limbic, associative and sensorimotor modalities. The VTA neurons project to the ventral striatum (limbic), the dorsal SNc (substantia nigra par compacta) projects to precomissural caudate and putamen (associative) and the ventral SNc projects to dorsolateral putamen (sensorimotor). The striatal areas have reciprocal connections to these midbrain neurons as well as non-reciprocal connections to adjacent midbrain neurons. The ventral striatum sends efferents to the dorsal SNc, the precomissural caudate and putamen send efferents to SNc, and the dorsolateral striatum sends efferents to the substantia nigra pars reticulata. These non-reciprocal projects permit all incoming information to be transmitted from limbic, to associative and then to sensorimotor areas (Haber, 2003).
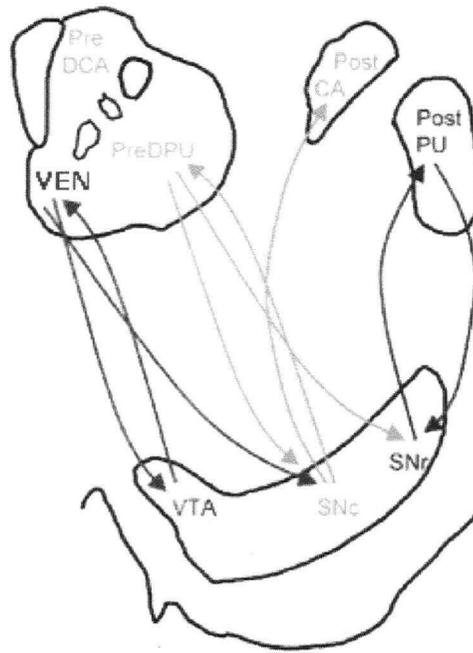
Figure 2 Simplified representation of strionigralstriatal subcircuits. The upper left structure represents precommisural striatum, the upper right represents post-commissural striatum and the bottom represents the ventral tegmental area (VTA) and substantia nigra (SN). Color-coding represents functional modalities of limbic (red), associative (green) and sensorimotor (blue). Arrows going to the striatum from the VTA/SN are dopaminergic while arrows going to the VTA/SN are inhibitory γ aminobutyric acid (GABA) releasing neurons. Each striatal subdivision sends reciprocal GABAergic projections to VTA/SN areas from where they receive input. Furthermore, the ventral striatum (VEN) sends reciprocal connections to dopaminergic output neurons targeting association striatum, which in turns sends reciprocal connections to dopaminergic output targeting sensorimotor striatum. In effect this relays information from limbic to cognitive to sensorimotor areas of the striatum. Pre-DCAU, precommisural dorsal caudate; Pre-DPU, precommisural dorsal putamen; Post-DCA, post commissural caudate; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulate. (Adapted by permission from Macmillan Publishers Ltd: Journal of Cerebral Blood Flow and Metabolism; Martinez et al., 2003; © 2003).

A second dopaminergic projection system is the mesolimbocortical pathway. Mesencephalic DA neurons terminate on limbic structures including the ventral striatum, lateral septal nucleus, amygdala, piriform, ventral entorhinal, prefrontal cortex, anterior cingulate cortex, habenula and locus coeruleus (Bjorklund and Lindvall, 1984). As with striatal targets, these prefrontal cortical areas have reciprocal and non-reciprocal connections to the SN and VTA. Of interest is that both the prefrontal cortex and midbrain DA neurons are responsive to stress, which has been identified as a factor that not only contributes to the development of depression but one that can also influence the course and outcome (Moghaddam, 2002).

The most recently proposed dopaminergic system is the thalamic pathway. It originates from the hypothalamus, periaqueductal grey matter, ventral mesencephalon and the lateral parabrachial nucleus that subsequently project into the thalamus. This was reflected in immunolabeling of the human and macaque monkey thalamus by dopaminergic markers tyrosine hydroxlase, dopamine and dopamine transporter (Sanchez-Gonzalez et al., 2005).

DOPAMINE IN DEPRESSION

Although commonly neglected, dopamine (DA) has been evidenced to play a role in the monoamine hypothesis of depression. First off, it has been well established that depressed patients and animal models have reduced amounts of homovanillic acid, a DA metabolite, in their cerebral spinal fluid (CSF) (Traskman-Bendz et al., 1984; Reddy et al., 1992). Agents that deplete dopamine from synaptic vesicles, like reserpine, along with drugs that block D_2 receptors have been reported to induce depressive symptoms (Willner, 1983). Furthermore, anti-depressants and electroconvulsive shock enhance mesolimbic dopamine functioning. DA

agonists (pramipexole) have been found to be as effective as selective serotonin reuptake inhibitors (fluoxetine) in severely depressed patients (Corrigan et al., 2000).

Neuroimaging data suggest that depressed patients exhibit increased striatal D_2 receptors, decreased presynaptic dopamine function and decreased dopamine transporter expression; however, discrepancies remain to be resolved. With regards to D2 receptors, two [^{123}I] iodobenzamide (IBZM) studies have found increased D2 receptor binding in depressed patients compared to controls (D'Haenen H and Bossuyt, 1994; Shah et al., 1997), whereas two other [^{123}I]IBZM studies have found no change (Klimke et al., 1999; Parsey et al., 2001). The disagreement of these studies can be resolved by studies that had found elevated D2 receptors in depressed patients with psychomotor retardation (Ebert et al., 1996; Meyer et al., 2006). The D2 receptor binding inversely reflects dopamine levels; therefore, the elevated levels of D2 receptors in depressed with psychomotor retardation suggest decreased levels of dopamine. With regards to dopamine transporter, studies have found decreased dopamine transporter (DAT) levels in the amygdala and the striatum of depressed individuals (Meyer et al., 2001; Neumeister et al., 2001; Klimek et al., 2002). As with D2 receptors the DAT binding was inversely correlated with motor performance (Meyer et al., 2001). In contrast, other studies have identified elevated levels of DAT in depressed patients compared to control subjects (Laasonen-Balk et al., 1999; Laasonen-Balk et al., 2001). Note that at the dopaminergic synapse an increase in synaptic DA levels might be expected to lead to an increase in dopamine transporter while the opposite would be true for low DA levels (Gordon et al., 1996). Current research indicates that the role of DAT may not be separate from the expression of D2 receptors. Increased expression of D2 autoreceptors has been found to increase the expression of DAT through protein-protein interactions (Lee et al., 2007).

Two studies by Martinot and colleagues have demonstrated decreased [^{18}F]-dopa uptake in depressed patients. Statistical parametric mapping found decreases in the left caudate nucleus (Martinot et al., 2001) and bilateral putamen and nucleus accumbens, and left parahippocampus and dorsal brainstem for subjects with affective flattening and psychomotor retardation. Subjects with impulsivity had a different profile with decreased uptake in the anterior cingulate and hippocampus and increased uptake in the right parahippocampal gyrus (Bragulat et al., 2007). Again these findings are in line with decreased dopamine levels, since decreased uptake leaves less dopamine to be released from synapses and subsequently metabolized to homovanillic acid.

As well the dopaminergic system has been implicated in the placebo effect in depressed patients. A meta-analysis of 19 anti-depressant studies evaluated that 75% of the treatment benefited was due to the placebo effect. A metabolic PET study in depressed patients has shown consistent engagement of the prefrontal and cingulate cortex during placebo, areas that receives prominent dopaminergic innervations (Mayberg et al., 2002). The placebo effect in this study demonstrated a parallel metabolic profile to active drug. However, in a study of electroencephalographic activity, alterations in prefrontal activity differed between placebo responders and medication responders (Leuchter et al., 2002). Expectations of benefit contribute to the placebo effect. The metabolic study of depressed patients treated with fluoxetine or placebo responders showed increased metabolism in the ventral striatum and orbital frontal cortex at one week. With antidepressant placebo effect in depression affecting dopaminergic targets and that increases of striatal dopamine have been implicated in placebo (de la Fuente-Fernandez et al., 2002; Boileau et al., 2007), one could speculate that dopamine influences the antidepressant placebo effect. This could be verified by examining changes of dopamine sensitive radiotracers in an antidepressant versus placebo study.

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The sum of these pharmaceutical and neuroimaging studies strongly suggests that any insult to the dopaminergic system would increase the probability of depressive symptoms.

1.4. Parkinson's disease

SYMPTOMS, ETIOLOGY, PATHOGENESIS AND TREATMENT

One method to investigate the role of dopamine in depression is by using a natural population of individuals who suffer from decreased levels dopamine and following the consequences. Parkinson's disease provides such an opportunity. The selective destruction of DA producing cells of the substantia nigra underlies Idiopathic Parkinson's Disease (PD). This progressive neurodegenerative disorder typically presents with symptoms of minor motor impairment but eventually progresses to severe immobility, as well as mood and cognitive dysfunction. According to a world health report, 305,000 are diagnosed each year and PD usually affects those over 50. The diagnosis of Parkinson's disease is based on the presence of two typical features: tremor, cogwheel rigidity, bradykinesia and postural instability. Non-motor symptoms associated with PD include dementia, psychosis, anxiety, insomnia, autonomic dysfunction and mood disorders (Snyder and Adler, 2007). About 10% of PD can be explained by genetic mutations in genes including involving mitochondrial dysfunction (PINK, DJ-1, parkin), ubiquitin proteasome system failure (ubiquitin C-terminal hydrolase), α-synuclein aggregation and abnormal cell signaling (LRRK2) (Hardy et al., 2006). Therefore, most PD remains idiopathic. Yet various environmental risk factors have been investigated including occupation and exposure to certain compounds that have been shown to be inhibitors of mitrochondrial complex I proteasomal function (Sherer et al., 2002). Post-mortem studies have established greater loss of ventrolateral nigral dopaminergic projections to the putamen than dorsomedial projections to the head of the caudate (Kish et al., 1988). Neuroimaging studies of PD subjects confirm this with observations that [^{18}F]-dopa uptake is reduced in the posterodorsal putamen on

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the contralateral side of the "asymptomatic" limb and is later followed by diminishing levels in the anterior and ventral putamen as well as the dorsal caudate (Morrish et al., 1995). Meanwhile, in early PD compensatory mechanisms included increased DA activity in nigral-pallidal and mesofrontal dopaminergic projections (Rakshi et al., 1999; Whone et al., 2003). Treatment of PD primarily consists of dopamine replacement with the dopamine precursor l-dopa. Dopamine agonists such as ropinirole, pramipexole, bromocriptine, and pergolide may serve as preliminary therapy in early PD and as adjunctive therapy in more advanced PD. Other drugs may be introduced to ameliorate symptoms as the disease progress. For example, a catechol-O-methyl transferase inhibitor entacapone can be used to alleviate the wearing off phenomenon whereas the pharmaceutical amantadine can be used to ameliorate dyskinesias (Horstink et al., 2006).

DEPRESSION IN PARKINSON'S DISEASE

Depression is a major complication in PD, affecting ~40% of patients (Tandberg et al., 1996). This is nearly double the amount of severe depression seen in comparably disabled patients with other chronic illness (Ehmann et al., 1990). While depression may in part be an understandable reaction to living with a chronic and potentially debilitating illness, it appears likely that in many patients there is an underlying neurochemical substrate (Leentjens et al., 2003), Indeed, in a recent retrospective study depression was found to be strongly associated with subsequent risk for PD. The hazard ratio 3.13 +/- 1.53 was controlled for age, sex and socioeconomic status (Schuurman et al., 2002; Leentjens et al., 2003). Detection bias was removed by concurrently investigating epilepsy, multiple sclerosis, stroke and diabetes.

Following a diagnosis of Parkinson's disease, it is more difficult to establish a diagnosis of depression since many PD symptoms overlap with depression, including sleep disturbance, fatigue, psychomotor slowing, difficulty concentrating, diminished sexual functioning,

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withdrawing from social activities and severe melancholic depression countenance (bradykinetic movements and flat faces). However, the depressive features of PD depressed patients are different than the profile of primary depression. PD depressed patients have higher rates of anxiety, pessimism, irrationality, suicidal ideation without suicidal behavior and little guilt, feelings of failure or self-blame. Unfortunately, PD patients with depression experience greater cognitive decline, deterioration in diurnal activities and increased PD motor symptoms than those without depression (McDonald et al., 2003). These cognitive deficits are exacerbated if depression is present earlier in the course of PD. Conversely, cognitive deficits can lead to increased risk of developing depression.

On a neurological level, PD patients exhibit dopaminergic loss across all midbrain neurons (German et al., 1989) and to a greater extent in the ventral tegmental area (VTA) of patients with a history of depression (Brown and Gershon, 1993). However, depressed PD patients also have greater degeneration of serotonin cells in the dorsal raphe nucleus (Paulus and Jellinger, 1991)and noradrenergic cells of the locus coeruleus (Chan-Palay and Asan, 1989). Studies have also found that CSF levels of the serotonin metabolite 5-hydroxyindoleacetic acid is reduced in PD depression and are negatively correlated with depression symptom severity (Mayeux, 1990). With neuroimaging a metabolic study has shown decreased activity in the caudate, anterior temporal cortex and orbital-inferior frontal cortex in depressed PD compared to non-depressed PD subjects (Mayberg et al., 1990). A recent PET study of PD depressed patients using the dopamine/norepinephrine transporter ligand [^{11}C] RTI-32 showed loss of binding in both noradrenergic regions such as the locus coeruleus and thalamus, as well as dopaminergic regions such as the amygdala, ventral striatum and anterior cingulate cortex (Remy et al., 2005).

Although dopamine sensitive neuroimaging studies have investigated changes in PD or in depression, this study provides an opportunity to study the combination of these two conditions. Interpreting neuroimaging is highly dependent on using appropriate models but the ability to investigate these questions in vivo with humans avoids the complications and confounds of post-mortem or animal studies. Furthermore, using neurotransmitter sensitive radiotracers like [^{11}C]raclopride allows us to look beyond metabolic differences and help us understand the effects of antidepressant therapies.

The purpose of this thesis was to determine the role of dopamine in the depression of Parkinson's disease. The working hypothesis of this thesis was that depression in PD is associated with reduced dopamine release in the ventral striatum compared to non-depressed PD patients. It would follow that depression severity will be inversely correlated with DA release in the ventral striatum. To test this hypothesis amphetamine induced dopamine release will be estimated using positron emission tomography (PET) with the selective D2/D3 DA receptor radiotracer [^{11}C]raclopride. Amphetamine through its dopamine reuptake transporter blocking action has become an ideal method to investigate in vivo dopamine release (Laruelle, 2000).

METHODS

2.1 Subjects

Thirteen subjects with mild to moderate idiopathic Parkinson's disease were recruited through the Movement Disorders clinic at the Pacific Parkinson's Research Centre, University of British Columbia (UBC). Inclusion criteria included a prospective diagnosis of idiopathic Parkinson's Disease as outlined by the United Kingdom Parkinson's Disease Society Brain Bank criteria (Hughes et al., 1992). Subjects were considered depressed if they scored 16 or greater on the Beck Depression Inventory (Beck et al., 1996). Exclusion criteria included atypical parkinsonism, head trauma, significant other neurological disease, other psychiatric Axis I diagnosis, patients deemed at risk for suicide, history of cardiac disease or uncontrolled hypertension, current or lifetime history of alcohol or substance abuse/dependence, lifetime exposure to illicit psychostimulants, glaucoma or hyperthyroisdim. Label and non-label use of anti-depressants (i.e. insomnia, tremor) was an exclusion criterion for non-depressed patients. Depressed patients were permitted use of antidepressants, except for those with a predominately catecholinergic action (e.g. bupropion). The UBC clinical research ethics board approved this study and all subjects completed consent forms.

2.2 Study Design

This study used positron emission tomography (PET) to measure amphetamine (AMP) induced dopamine release using the selective D2/D3 receptor antagonist radiotracer [^{11}C]raclopride. Dextro-amphetamine sulfate (Glaxosmithkline) was selected to stimulate DA release because it has been safely and successfully been used in several other PET studies. The tracer [^{11}C]raclopride was used because of its ability to be displaced by endogenous dopamine (Morris and Yoder, 2007). Subjects stopped all levodopa/carbidopa and DA agonists the night prior to

the PET scans and resumed medication after PET scans were complete for the day. Depressed subjects were asked to delay antidepressant treatment if newly diagnosed of depression; otherwise, they only suspended the anti-parkinsonian medication. Subjects completed three scans over two days within a three-month period. The first scan was a baseline scan, followed by either blinded AMP (0.3 mg/kg p.o.) or placebo administration, counterbalanced across subjects. At least one day between the second and third scans was provided to ensure complete washout of amphetamine and scans were completed in three months to ensure no drift in baseline [^{11}C]raclopride binding potential. The subjective response to AMP or placebo was measured using an Amphetamine Interview Rating Scale (AIRS) after the PET scans. AIRS is composed of 6 subscales including activation, euphoria, depressive affect, dysphoria, somatic symptoms and sleepiness (Van Kammen and Murphy, 1975). The difference of scores between amphetamine and placebo for each subscale was used as a measure. On the day of the PET scan a Mini-Mental State Examination to screen for dementia (Folstein et al., 1975), Montgomery Asberg Depression Rating Scale (MADRS) to assess depression severity (Montgomery and Asberg, 1979), and UPDRS (III) to determine PD severity were completed (2003). Participants scoring lower than 14 on the MADRS were excluded from the study (Leentjens et al., 2000). Heart rate and blood pressure were measured at baseline, at 30 minutes following administration of amphetamine or placebo and for every 15 minutes thereafter up to 4 hours.

2.3 Image acquisition

PET images were acquired on a high-resolution high sensitivity research tomograph (HRRT, Siemens, Knoxville) with a resolution of 2.5x2.5x2.5 mm FWHM (full width at half-maximum), 6% sensitivity and 25 cm axial field of view. Subjects were placed in a supine position with an orientation parallel to the inferior orbitomedial plane such that the cerebellum was present in all scans. To reduce patient motion subjects were fitted with a customized thermoplastic mask.

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Additionally, a neoprene cap with motion detector markers was used and all subjects underwent a 15 min transmission scan using ^{68}Ge rods to correct for attenuation. An average of 366.3 +/-3.8 MBq of [11C]raclopride for each scan was injected intravenously over 1 minute using a Harvard pump and emission data were collected in 3D mode for 60 minutes in frames of increasing duration (4x1min, 3x2min, 8x5min, 1x10min).

2.4 Image Analysis

Emission data were reconstructed using Ordinary Poisson-3D ordered subject expectation maximization (OSEM) including detector normalization, attenuation, scatter and random correction. To correct for motion between frames automatic image registration (AIR 3.0) was used to coregister all emission scan frames to the averaged image of the last half hour of the baseline scan (Woods et al., 1993). During this process all remaining correction factors (i.e. decay time, dead time, branching factor) were integrated into the raw emission image.

PET data were analyzed using a regions of interest (ROI) based approach. Slice selection and ROIs placement were performed on the average image of the last half hour of the corrected and realigned emission frames. ROIs were placed on the dorsal striatum (one ellipse on the caudate, two circles on the anterior putamen and one ellipse on the posterior putamen), ventral striatum (one ellipse) and cerebellum (one ellipse). ROIs were placed on averaged images of 9 transaxial slices (3 planes/average image) for the dorsal striatum (total volume putamen=1310mm^3, caudate=668mm^3), 6 coronal slices for the ventral striatum (total volume 232mm^3) and 6 transaxial slices for the cerebellum (total volume 15125mm^3). Selection of dorsal striatum transaxial slices started dorsally where the head of caudate and the putamen were clearly visible and inferiorly where the caudate can still be delineated and the posterior putamen continues to project laterally. From these boundaries the first and last slices were compared and 9 contiguous

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slices exhibiting the best quality and highest activity were selected. Cerebellar slices were selected from where the cavity between the temporal lobe and cerebellum were clearly visible bilaterally. Coronal slice selection started where the head of caudate and the putamen are clearly separated by the internal capsule superiorly, but not inferiorly, and continued for 5 more slices in the anterior direction. The merged caudate and putamen should be visible through all 6 slices. Slice selection was only performed on the baseline scan of each subject since realignment was assumed to correct any positional differences between scans.

ROIs were transposed onto the frames to generate time-activity curves of [^{11}C]raclopride kinetics. Using these time-activity curves binding potentials (BP) were estimated using a graphical tissue approach (Logan et al. 1996) with the cerebellum as a reference region. The cerebellum was used as the non-specific tissue reference input because it is nearly devoid of D2/D3 receptors and [^{11}C]raclopride binding in the cerebellum is uninfluenced by DA antagonists (Hall et al., 1996). Binding potentials of the right and left side of each ROI were averaged and the 3 putamen ROI values were averaged. The reduction of [^{11}C]raclopride binding potential between interventions were expressed in terms of relative reduction of BP:

$$\Delta BP_{PB} = (baseline\ BP - placebo\ BP)/baseline\ BP$$

$$\Delta BP_{AMP} = (baseline\ BP - amphetamine\ BP)/baseline\ BP$$

$$\Delta BP_{drug} = (placebo\ BP - amphetamine\ BP)/baseline\ BP$$

2.5 Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Chicago). Following descriptive statistics to identify the distributional properties of the variables (range, means, standard deviations), inferential statistics were performed. Cardiovascular measures (heart rate, systolic blood pressure, diastolic blood pressure) were compared between

placebo and amphetamine conditions with a two-way (Time X Condition) repeated measures analysis of variance (ANOVA). Subjective measures of AIRS subscales between drug conditions were compared using a two-way (Group X Condition) repeated measures ANOVA. Binding potentials derived from ROIs during baseline, placebo and amphetamine were examined using a two-way (Group X Condition) repeated measures analysis of covariance (ANCOVA). Baseline BP was the covariate for all ANCOVAs. Reductions of binding potential following amphetamine or placebo were compared with another two-way (Group X Condition) repeated measures ANCOVA. Another set of two-way repeated measures ANCOVAs with ROIs as the repeated measure (Group X ROI) were performed as in (Martinez et al., 2003). Relationships between all continuous variables were determined using Pearson product moment correlation coefficients. A two-tailed probability of $p < 0.05$ was selected as significant. For all analyses data from left-sided and right-sided ROIs were averaged.

RESULTS

3.1 Subjects

The following results include data from 6 PD subjects without depression and 4 depressed PD subjects. Originally 8 non-depressed PD patients and 5 depressed patients were recruited. Two non-depressed patients were excluded due to high blood pressure, one depressed subject due to tracer delivery failure and one depressed subject due to claustrophobia. Note that tracer failure occurred during a placebo scan for one non-depressed patient and an amphetamine scan for one depressed patient. All subjects were on some form of anti-parkinson medication. One non-depressed subject was taking clonazepam for sleep and all depressed subjects were on anti-depressant medication (Table 1). Disease severity ranged from Hoehn & Yahr stage 1.5-2.5, Unified Parkinson's Disease Rating Scale (III) of ($25+/-3$) with disease duration ranging from 5-13 years. Patients were not matched by age and disease severity due to small sample size.

Table 1 Profile of Parkinson disease patients participating in this study.

Gender	Age	Duration	UPDRS	MADRS	PD meds	Hypnotic	Antidepressants
Non-DEP							
M	47	7	21	2	levo/carb CR 600mg pramipexole 4.5mg		
F	63	13	37	2	levo/carb CR 800mg bromocriptine 10mg		
F	47	5	7	2	pramipexole 2.25mg		
M	63	6	24	0	levo/carb CR 800mg		
M	74	13	38	0	levo/carb CR 450mg levo/carb IR 100mg bromocriptine 37.5mg amantadine 100mg		
M	64	8	16	2	levo/carb CR 900mg levo/carb IR 100mg	clonazepam 1mg	
DEP							
F	47	6	26	20	pramipexole 1.5mg	clonazepam 0.5mg	citalopram 20mg
M	28	5	25	21	levo/carb 1200mg amantadine 200mg	zopiclone 7.5mg	citalopram 40mg
M	61	5	22	15	levo/carb 700mg	zopiclone 5mg	venlafaxine 5mg
M	74	11	31	17	levo/carb CR 1400mg trihexyphenidyl 2mg		mirtazapine 15mg

levo/carb IR ,levodopa/carbidopa immediate release; levo/carb CR, levodopa/carbidopa controlled release; PD, Parkinson's Disease, UPDRS-Unified Parkinson's Disease Rating Scale; MADRS, Montgomery-Asberg Depression Rating Scale; DEP, depressed

3.2 Cardiovascular Effects

A two-way repeated measures analysis of variance found no significant difference in heart rate (Figure 1), systolic (Figure 2) and diastolic blood pressure (Figure 3) across time and between the placebo and oral d-amphetamine conditions. However, comparing only measures at baseline and 3hrs resulted in a significant interaction between time and drug condition for heart rate ($F_{1,16}$=5.23, p=0.04) and diastolic blood pressure ($F_{1,16}$=8.26, p=0.01). In contrast to a previous study on the pharmacokinetics of oral d-amphetamine (Angrist 1997), there was no significant increase compared to baseline of cardiovascular measures 2hr after receiving amphetamine, rather the peak cardiovascular effect occurred at 3hrs. Furthermore, results suggest that cardiovascular measures decreased at 2hrs in both placebo and amphetamine conditions. This could reflect subjects resting during the PET scan, which occurred between 60-150 min following drug administration.
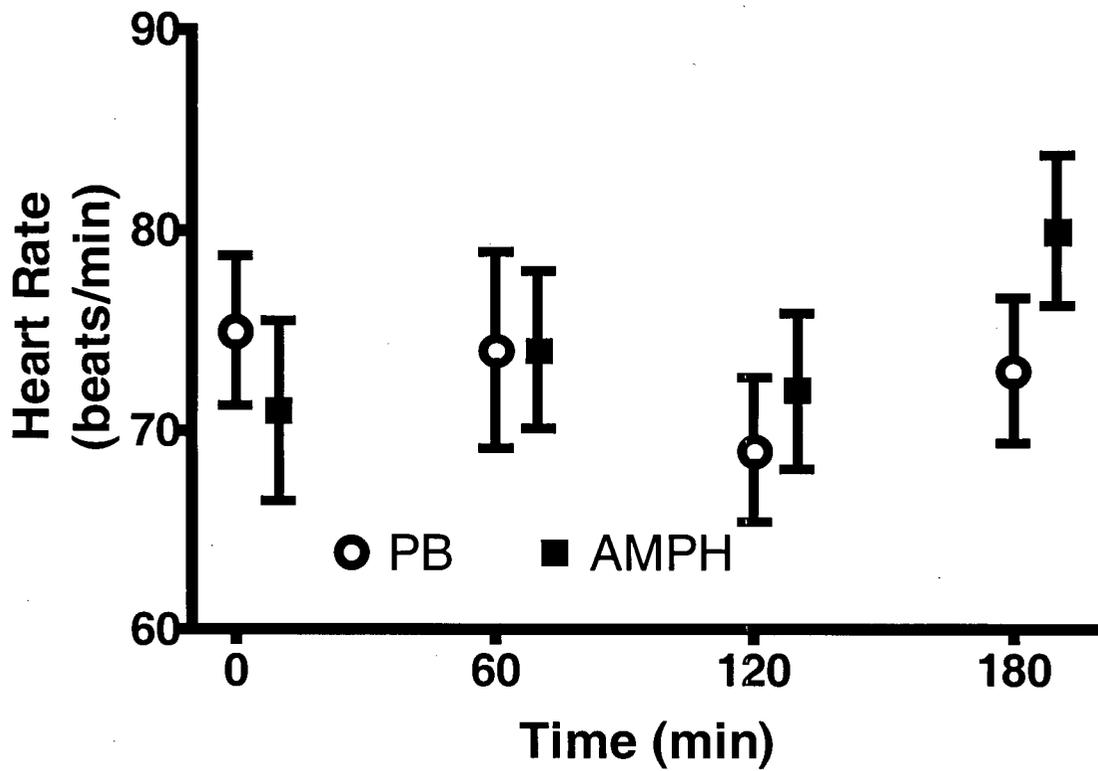
Figure 3 Mean (± SEM) heart rate of all Parkinson's disease subjects following placebo or

0.3mg/kg amphetamine.
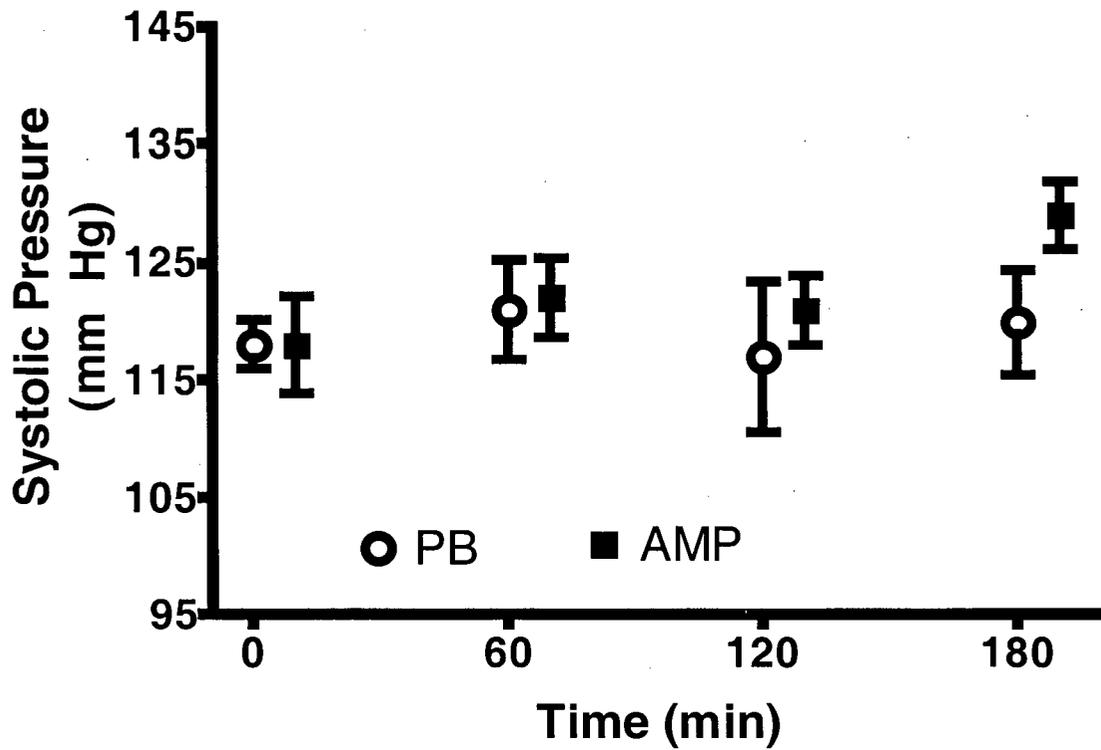
Figure 4 Mean (± SEM) systolic pressure of all Parkinson's disease subjects following placebo or 0.3mg/kg amphetamine.
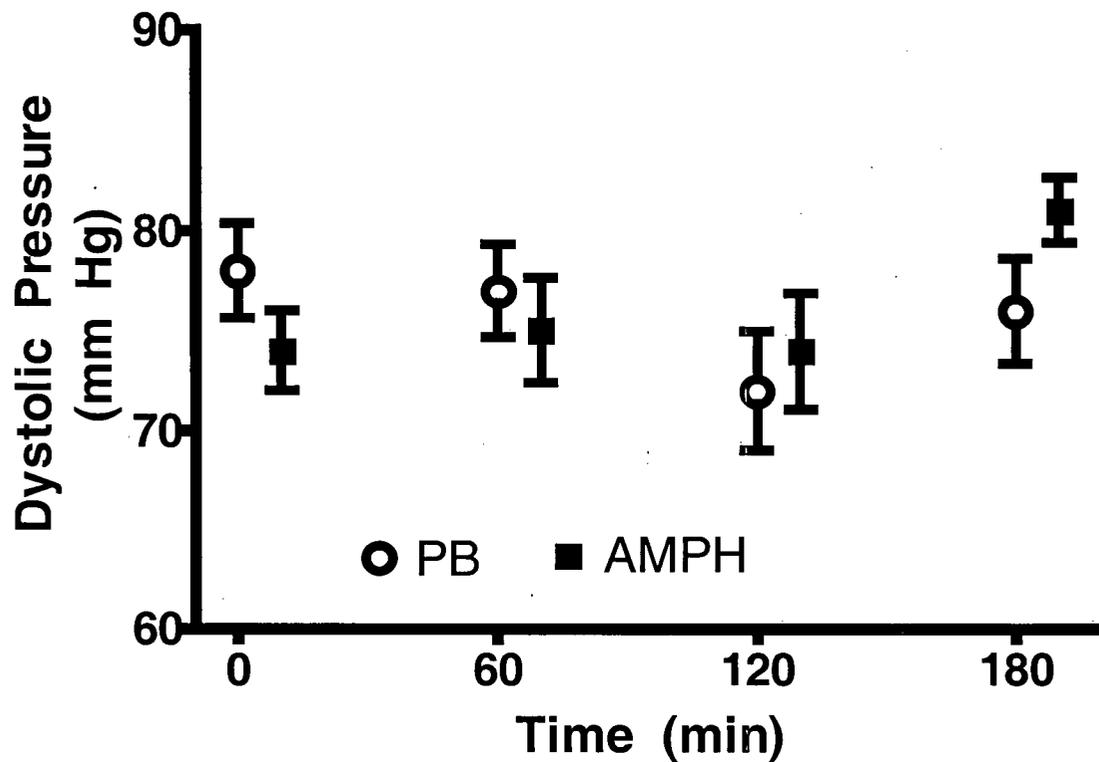
Figure 5 Mean (± SEM) diastolic pressure of all Parkinson's disease subjects following placebo or 0.3mg/kg amphetamine.

3.3 Amphetamine Interview Rating Scale (AIRS)

Most patients exhibited minimal negative side effects to amphetamine. On the AIRS, two subjects noted increased dizziness that was not present during baseline. There was subjective experiences of drug "wanting" (n=2) and there was spontaneous reporting of insomnia (n=1) and large impulsive purchases after leaving the hospital (n=2). One subject experienced a "blinding" headache, vomiting and fever the evening afterwards. This patient had headaches of this nature in the past but none this severe and no previous history of migraines. No significant differences were found between amphetamine and placebo across the different AIRS subscales (Figure 6). However, there was a strong trend of increased activation ($F_{1,8}$=5.26, p=0.051) and euphoria ($F_{1,8}$=3.52, p=0.10). As well, there was a significant difference between depressed and non-depressed patients in depressive symptoms between drug conditions ($F_{1,8}$=6.78, p=0.03).
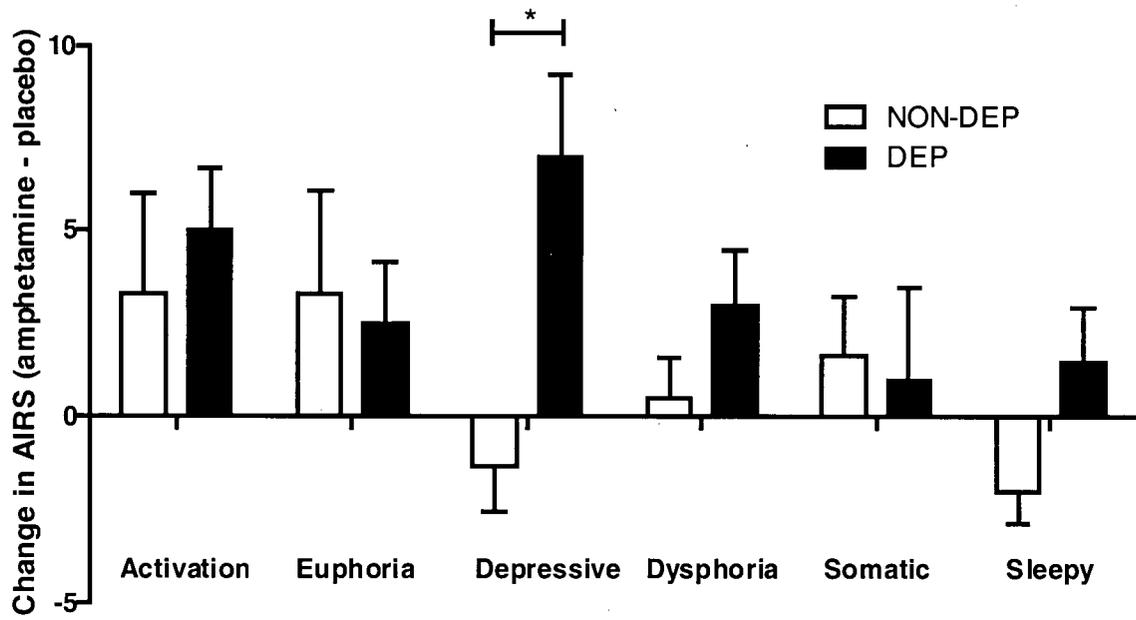
Figure 6 Amphetamine Interview Rating Score Subscales. Values are the mean ± SEM of the

AIRS subscale score of amphetamine compared to placebo. *p<0.05

3.4 Comparison between Scan Conditions

The binding potentials referred to herein are the [^{11}C]raclopride D2 receptor binding potentials derived from a Logan graphical analysis using PET based ROI analysis. Representative parametric maps are presented in Figure 7. With the [^{11}C]raclopride binding potential as the dependent variable and scan condition as the repeated measure, there was no significant difference between depressed and non-depressed patients. However, there was a difference observed between scan conditions significantly in the ventral striatum ($F_{2,16}=6.24$, $p=0.01$) and caudate ($F_{2,16}=3.80$, $p=0.05$). Using a Bonferroni correction, BP following amphetamine was significantly lower than baseline ($p=0.04$) and placebo ($p=0.05$) in the ventral striatum, and lower than placebo ($p=0.04$) in the caudate (Figure 8).
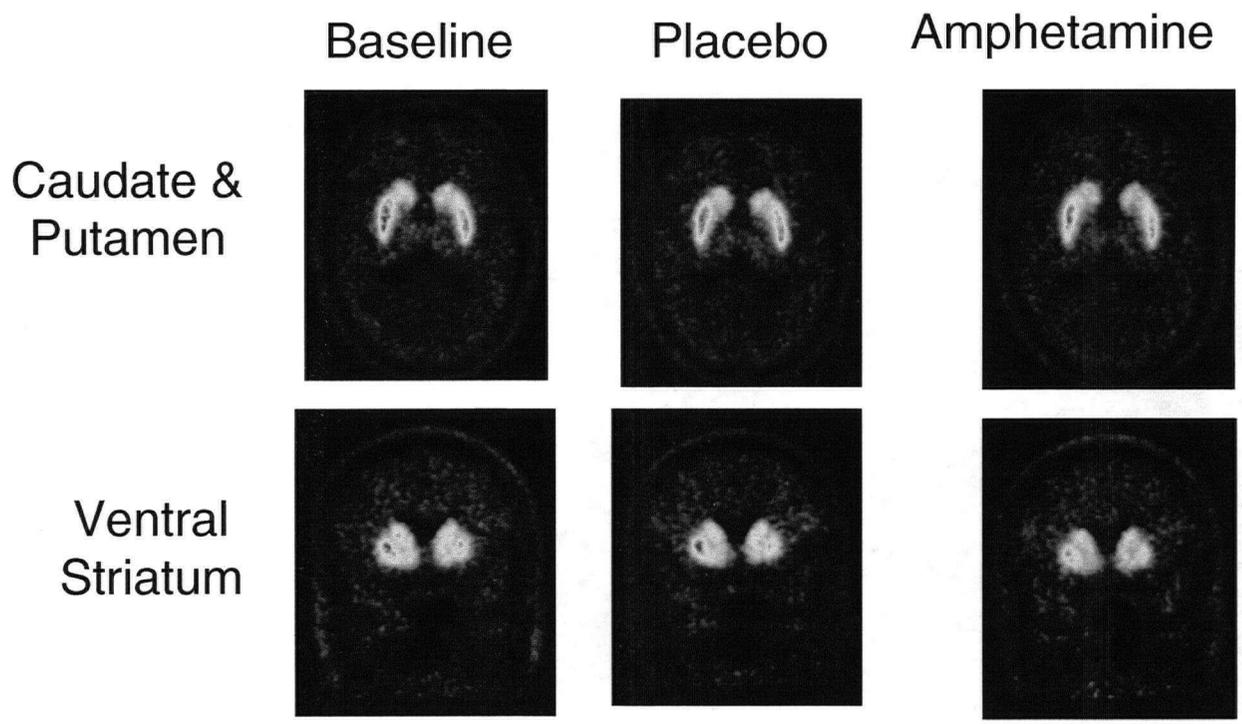
Figure 7 Parametric maps of a representative subject for each scan condition
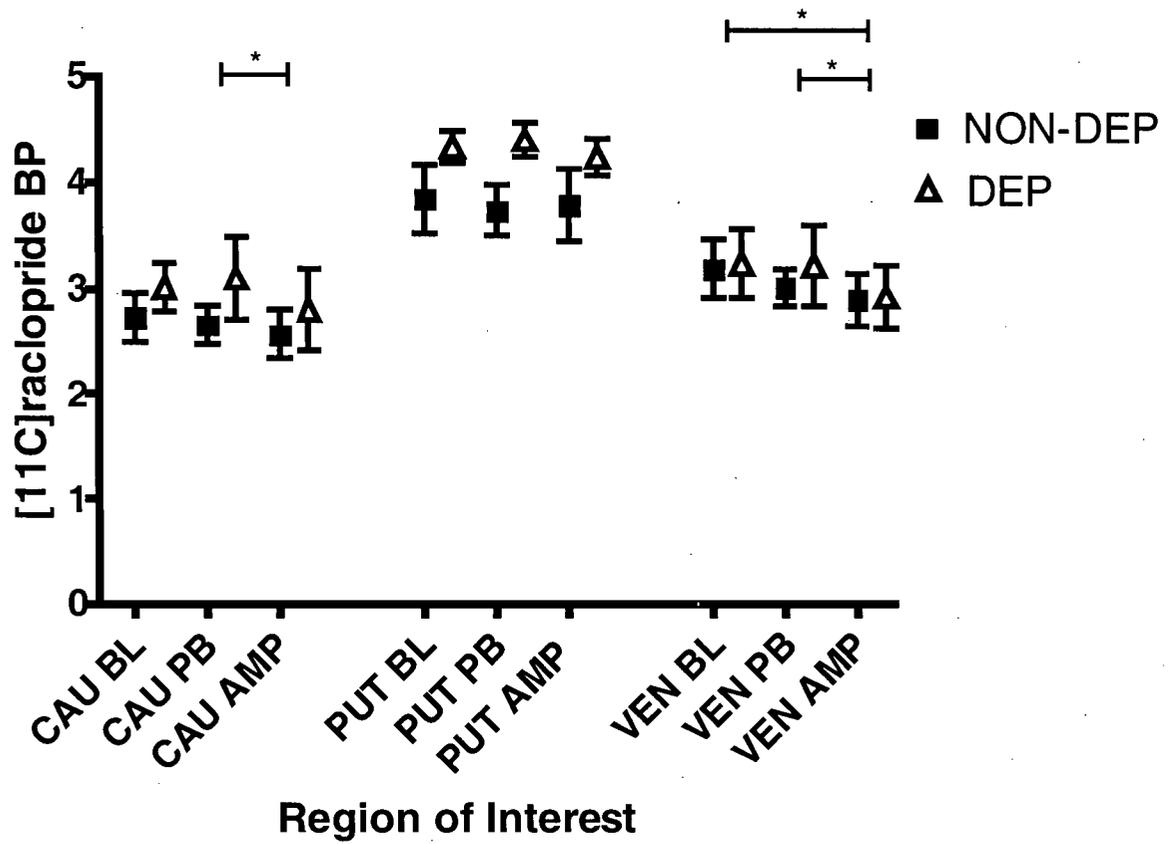
Figure 8 Mean (± SEM) [^{11}C]raclopride binding potential (BP) in the striatum of non-depressed and depressed subjects. Amphetamine (AMP), baseline (BL), caudate (CAU), depressed (DEP), placebo (PB), putamen (PUT), ventral striatum (VEN), * $p < 0.05$

When change in binding of D2 receptors (assumed to reflect occupancy by endogenous dopamine) was used a dependent variable, DA release relative to baseline following placebo (ΔBP_{PB}) was significantly less than DA release following amphetamine (ΔBP_{AMPH}) in the caudate ($F_{1,8}=9.17$, p=0.02), and ventral striatum ($F_{1,8}=8.05$, p=0.02). When baseline BP was added as a covariate differences became non significant in the caudate ($F_{1,7} = 1.27$, p=0.30) and ventral striatum ($F_{1,7} = 1.68$, p=0.24) (Figure 9). No significant differences were found between depressed and non-depressed patients under any scan condition.
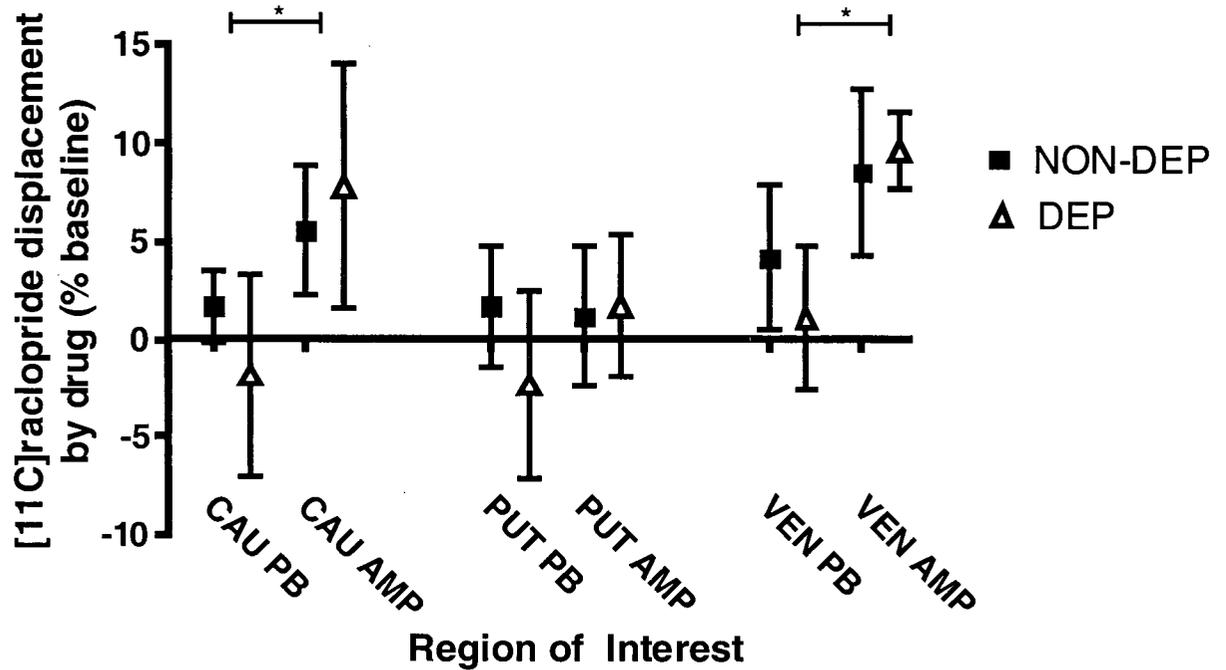
Figure 9 Mean (± SEM) percentage change of binding potential from baseline or placebo organized by region of interest. Higher values are reflective of greater DA release. Amphetamine (AMP), caudate (CAU), depressed (DEP), placebo (PB), putamen (PUT), ventral striatum (VEN).

3.4 Comparison between regions of interest

Next, differences between the regions of interest were examined to determine the D2 receptor and DA release profile of Parkinson's disease patients. Using regions of interest as the repeated factor in a repeated measures ANOVA a significant difference between ROIs was found with D2 receptor availability under baseline ($F_{2,16}$= 39.69, p<0.001), placebo ($F_{2,16}$= 66.98, p<0.001) and amphetamine ($F_{1.1,8.8}$= 50.56, p<0.001) conditions (Figure 10). Post-hoc Bonferroni corrected comparisons were significant with putamen BP>caudate BP (p<0.001), caudate BP> ventral striatum BP (p=0.005) and putamen BP>ventral striatum BP (p=0.002) in the baseline condition. The same pattern was found in the placebo condition with putamen BP>caudate BP (p<0.001), caudate BP> ventral striatum BP (p=0.005) and putamen BP>ventral striatum BP (p<0.001), and in the amphetamine condition with putamen BP>caudate BP (p<0.001), caudate BP> ventral striatum BP (p=0.004) and putamen BP>ventral striatum BP (p<0.001). No significant differences between groups were calculated under any scan condition.
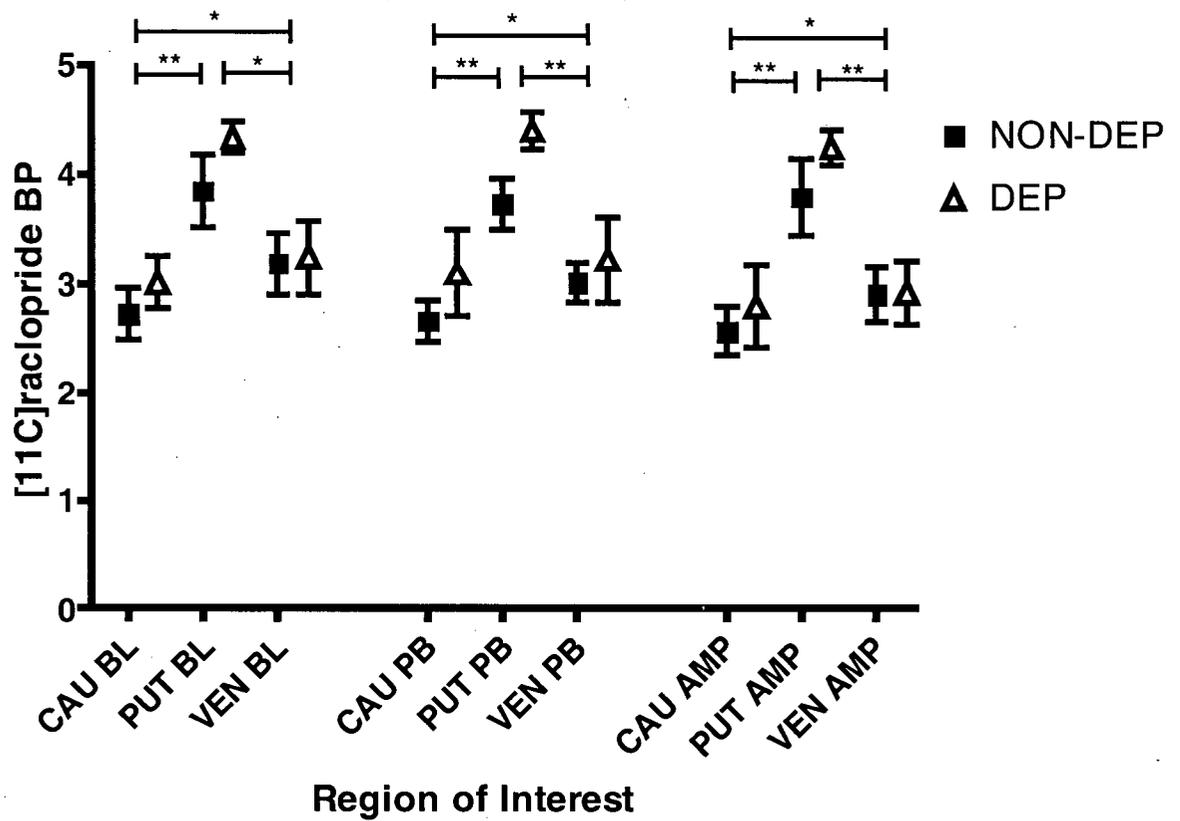
Figure 10 Mean (± SEM) [^{11}C]raclopride binding potential of depressed and non-depressed subjects organized by intervention condition. Amphetamine (AMP), baseline (BL), caudate (CAU), depressed (DEP), placebo (PB), putamen (PUT), ventral striatum (VEN), *$p<0.01$, **$p<0.001$

With DA occupancy of D2 receptors as the dependent variable, there was a significant difference between regions of interest for ΔBP_{AMPH} ($F_{2,16}=5.33$, $p=0.02$). There was a significant difference in the ventral striatum to be greater than in the putamen ($p=0.04$) and a strong trend for the caudate to be greater than the putamen ($p=0.07$) (Figure 11). However, no significant differences were found for ΔBP_{PB} between ROIs. Neither were there significant differences observed between the depressed and non-depressed groups for the different ROIs.
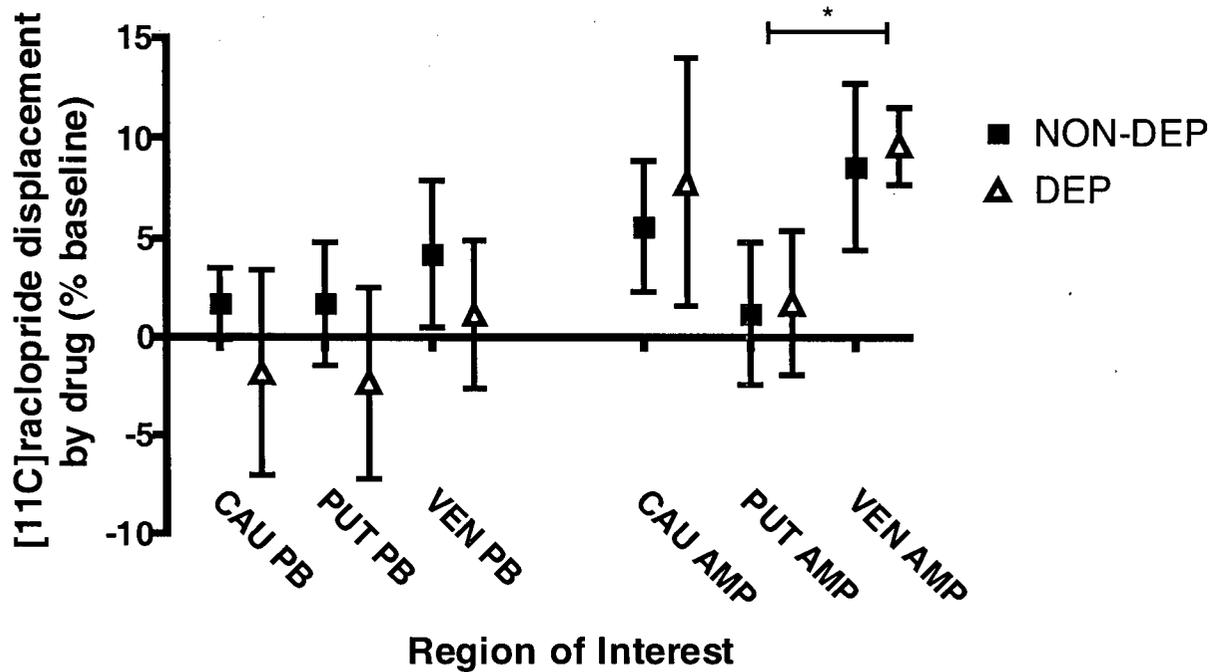
Figure 11 Mean (± SEM) percentage change of binding potential from baseline or placebo organized by intervention condition. Higher values are reflective of greater DA release. Amphetamine (AMP), baseline (BL), caudate (CAU), depressed (DEP), placebo (PB), putamen (PUT), ventral striatum (VEN).

3.5 Pearson Correlation analysis

Pearson correlation analysis was also performed on all measured values including all [^{11}C]raclopride BP and DA occupancy values across conditions and regions of interest, amphetamine interview rating scale, and subcategories (activation, depression, euphoria, dysphoria, physical symptoms, sleepiness), age, disease duration, UPDRDS and MADRS. First, ΔAIRS negatively correlated with the age of subjects ($r^2=0.74$, p=0.001; Figure 12). The AIRS subcategories of depressive symptoms ($r^2=0.65$, p=0.005) and dysphoria ($r^2=0.50$, p=0.02) increased with amphetamine-induced DA occupancy relative to placebo in the putamen (Figure 13). In parallel, depressive symptoms ($r^2=0.52$, p=0.02) and dysphoria ($r^2=0.49$, p=0.03) correlated positively in the ventral striatum (Figure 14). Lastly, amphetamine-induced ($r^2=0.74$, p=0.005) and placebo-induced ($r^2=0.65$, p=0.001) DA occupancy of the caudate and the putamen were positively correlated with one another (Figure 15).
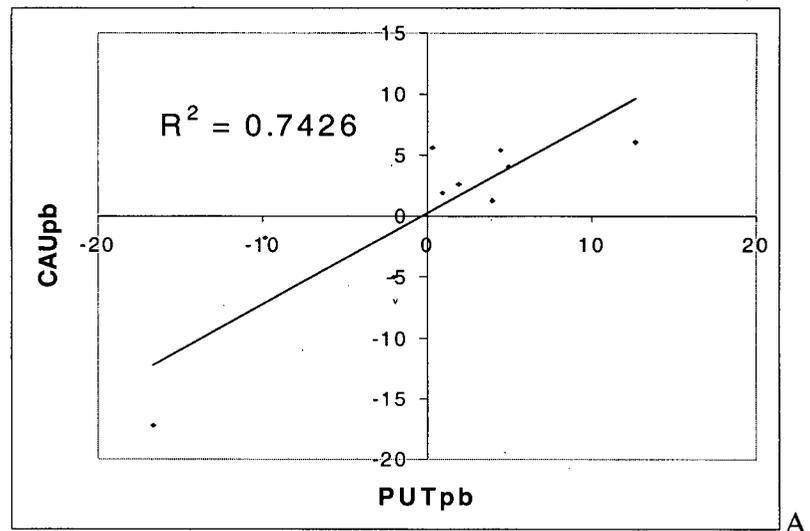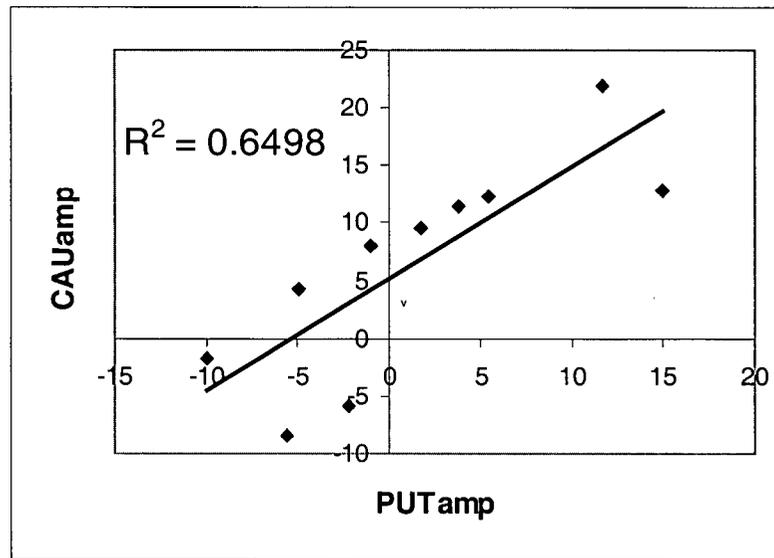
Figure 12 D2 receptor occupancy of the putamen (PUT) and caudate (CAU) positively correlate under (A) placebo (pb) and (B) amphetamine (amp) conditions.
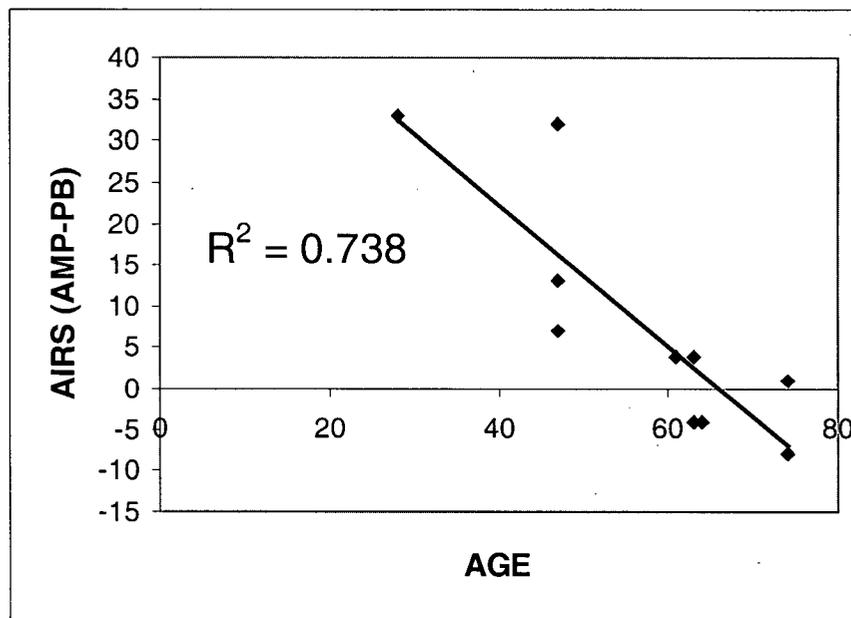
Figure 13 Changes in amphetamine interview rating scores (AIRS) between placebo and amphetamine conditions decrease with age.
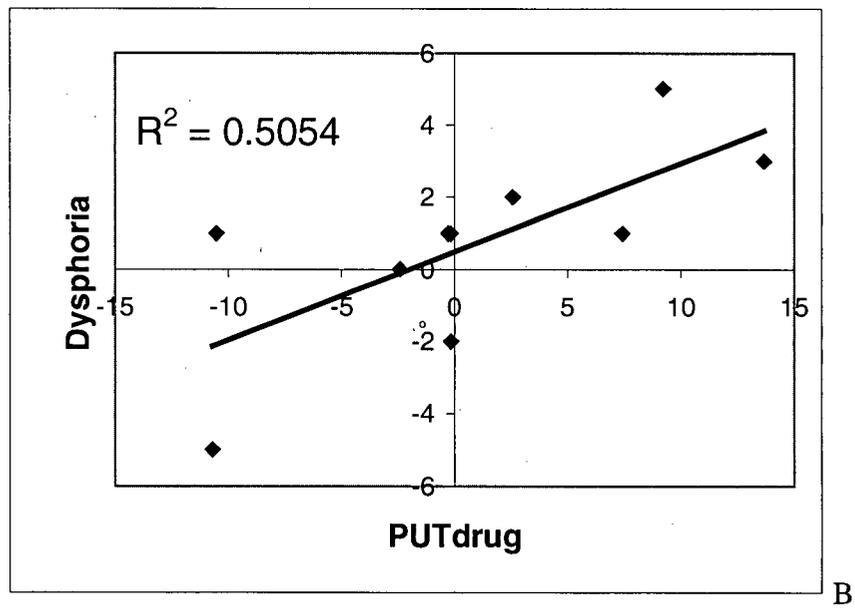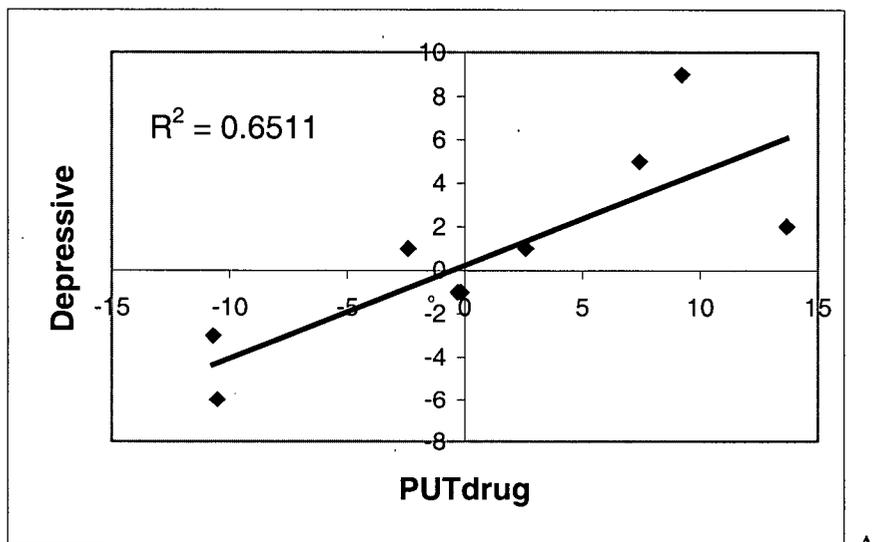
Figure 14 Between placebo and amphetamine scans the increased (A) depressive and (B) dysphoria symptoms were positively correlated with D2 receptor occupancy in the putamen (PUTdrug).
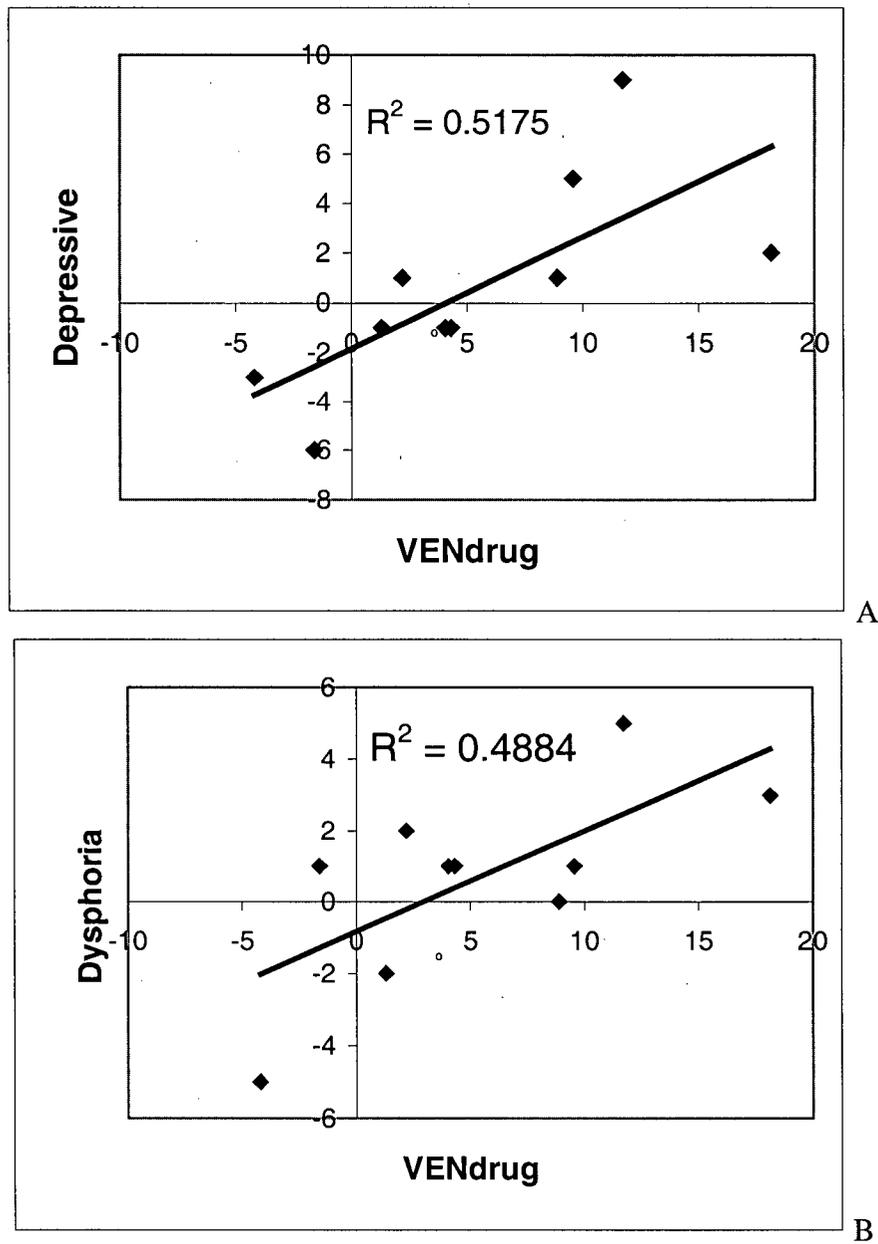
Figure 15 Between placebo and amphetamine scans the increased (A) depressive and (B) dysphoria symptoms were positively correlated with D2 receptor occupancy in the ventral striatum (VENdrug).

DISCUSSION

This thesis was conducted to investigate the role of DA in depression in PD patients. Amphetamine was used to evoke endogenous dopamine release and was measured using the displaceable D2/D3 receptor antagonist [^{11}C]raclopride in combination with a positron emission tomograph. The data are preliminary due to the small number of depressed subjects, and are tenable. Despite this, amphetamine was shown to induce DA release in both the caudate and ventral striatum, in both non-depressed PD patients and in the depressed subjects. Although no differences were detected between groups, correlation analysis found interactions between DA release and subjective changes.

4.1 Amphetamine-induced DA release

INTERVENTION CONDITIONS

Using [^{11}C]raclopride BP values, the caudate and ventral striatum had a significantly lower binding potential in the amphetamine condition compared to placebo. Compared to placebo-induced [^{11}C]raclopride binding potential changes (ΔBP_{PB}), amphetamine decreased [^{11}C]raclopride binding potential (ΔBP_{AMPH}) in the ventral striatum and the caudate, indicating increased DA release. However, the difference for the caudate and ventral striatum was non significant when baseline BP was added as a covariate. These findings are consistent with several other studies demonstrating amphetamine induced DA release in the ventral striatum and caudate of healthy control subjects, however, in these studies DA release was also seen in the putamen (Drevets et al., 2001; Leyton et al., 2002; Martinez et al., 2003; Oswald et al., 2005; Boileau et al., 2006; Munro et al., 2006; Boileau et al., 2007). In PD patients, decreases of [^{11}C]raclopride BP have been observed in the putamen and caudate in response to oral methamphetamine (Piccini et al., 2003). This group additionally observed methamphetamine

induced decreases in [^{11}C]raclopride binding in the frontal cortex, but in the current study our analysis was limited to striatal regions of interest.

REGIONS OF INTEREST

Using [^{11}C]raclopride BP values, the putamen BP>ventral BP>caudate BP across all scan conditions. The elevated BP values could represent regional differences of D2/D3 receptors or lower endogenous dopamine levels. However, the lack of amphetamine-induced release in the putamen suggests that these receptor BP values represent reduced DA levels. Further statistical analysis of ΔBP_{AMPH} revealed DA release was greater in the ventral striatum and caudate compared to the putamen. These results are in contrast with the Martinez et al (2003) studying regional differences of amphetamine induced release in which the putamen and ventral striatum had elevated release compared to the caudate.

Thus Parkinson disease patients may exhibit an alternate dopamine release profile following amphetamine intervention. In a paper by Martinez (2003), the authors suggest that the DA release profile is reflective of striatal-nigral feedforward connections. The inhibitory GABAergic projections from the striatum to the midbrain DA neurons allow for the limbic ventral striatum to inhibit dopaminergic output to the associative/cognitive caudate, which causes disinhibition of dopaminergic output to the sensorimotor dorsolateral putamen (Haber, 2003). In consequence the ventral striatum and putamen have elevated dopamine release while the caudate does not exhibit amphetamine-induced DA release. In Parkinson's disease, dopaminergic innervation of the putamen is substantially decreased and hence the absence of amphetamine-induced DA release. A neuroimaging study using [^{11}C] dihydrotetrabenazine to measure presynaptic DA terminals found that the striatal binding reductions correlated with duration of PD symptoms (Bohnen et al., 2006). Post-mortem studies have also indicated that the ventral tegmental area (VTA), which

sends dopaminergic projections to the ventral striatum, displays loss of dopaminergic neurons (Figure 16). Therefore, in Parkinson's disease dopaminergic input to the ventral striatum is reduced, which concomitantly disinhibits dopaminergic input to the caudate. This explains why amphetamine-induced DA release is significant in both the caudate and ventral striatum but not in the putamen.
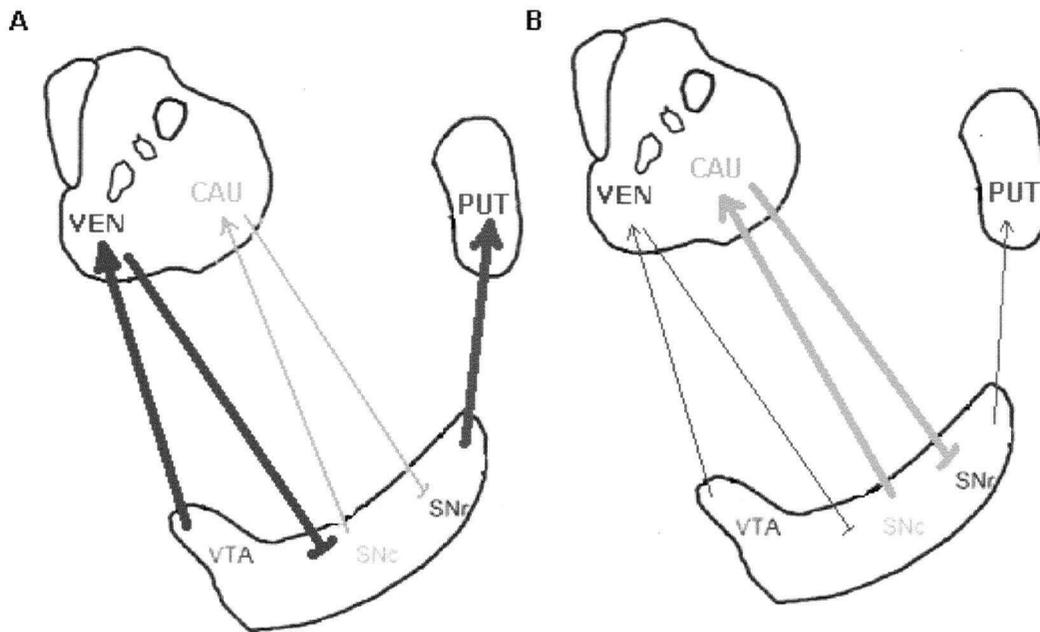
Figure 16 Strionigrostrial subcircuits in healthy normals (A) and in Parkinson's disease (B) during exposure to amphetamine. The ventral striatum (VEN) receives DA input (->) from the ventral tegmental area (VTA) and sends inhibitory (-|) efferents to the substantia nigra pars compacta (SNc). The SNc projects DA neurons to the caudate (CAU), which subsequently send non-reciprocal connections to the substantia nigra pars reticulate (SNr) which sends DA projections to the putamen (PUT). In healthy controls, amphetamine induces potent release in the VEN and PUT as indicated by the wide arrows. Non-reciprocal connections from the VEN inhibit DA output to the CAU. In Parkinson's disease, loss of DA cells in the VTA/SN results in lower amphetamine induced DA output to the PUT and VEN. However, the decreased inhibitory feedback from the VEN permits increased DA output to the CAU. (Adapted by permission from Macmillan Publishers Ltd: Journal of Cerebral Blood Flow and Metabolism; Martinez et al., 2003; © 2003).

DEPRESSED VERSUS NON-DEPRESSED

Several problems have limited the recruitment and successful scanning of patients as part of this thesis. Patient recruitment was more difficult then originally expected since cardiovascular disease, a comorbidity of depression, was part of the exclusion criteria to ensure no deleterious side effects with amphetamine. The comorbidity of anxiety in both depression and PD resulted in frequent concerns of claustrophobia. In fact one subject had to drop out due to uncontrollable anxiety during the scan. With four depressed subjects no significant effects were found between non-depressed and depressed groups in any scan condition, region of interest or D2 receptor occupancy measures. Results match those of Parsey and colleagues (Parsey et al., 2001) comparing primary depression to controls using amphetamine induced DA release measured by $[^{123}I]IBZM$. Although amphetamine led to transient improvement of symptoms, no difference was found between amphetamine-induced reductions of $[^{123}I]IBZM$ between the depressed and control group. As well there was no difference between baseline D2 receptor availability between the groups. However, patients with psychomotor retardation or affective flattening were not separated for independent analysis in that study. Note, that the results of this study may not be comparable with these PD subjects.

Although no conclusions can be drawn about the difference between groups due to the small number of depressed subjects, the four patients scanned demonstrated elevated levels of DA release in response to amphetamine relative to placebo. As well, visual inspection suggests that the depressed patients actually experienced negative placebo effects in the dorsal striatum (i.e. nocebo). If studying more subjects substantiates this trend, it has two implications. First, it will be important to distinguish whether changes due to amphetamine are relative to baseline or to placebo, since the assumptions that placebo moves in the same direction as drug, that placebo is equivalent to baseline or that placebo would have the same effect in different groups are no

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longer valid. Second depressed individuals may have earlier onset decreases of DA in response to failed predictions. In support of this latter argument is evidence from expectation/prediction studies. In one study, subjects who had minimal experience with stimulant drugs were given either placebo or methylphenidate with an expectation that they were given one or the other (i.e. expected methylphenidate and received placebo) (Volkow et al., 2006). The authors found that [^{11}C]raclopride injected immediately after placebo decreased binding potential in the ventral striatum suggesting an expected "uncertain drug effect". Electrophysiology studies in ventral striatal DA neurons of monkeys have found that cues (i.e. placebo pill) will evoke increased firing of dopamine neurons but if the expectations are not met there is a decrease in dopamine neuron firing (Schultz et al., 1997). Dopamine neuron firing has been shown to correspond to endogenous dopamine release (Tepper et al., 1991). Since subjects had 1.5 hrs before their scan, they may have realized they did not experience the "uncertain drug effect" and during their actual scan had a resultant decrease of DA release.

Visual inspection of results also suggests that depressed patients have elevated D2 receptors in the putamen. Literature regarding baseline D2 receptor density in depressed individuals compared to controls has been controversial. Two [^{123}I]IBZM studies found increased D2 receptor binding in depressed patients (D'Haenen H and Bossuyt, 1994; Shah et al., 1997), whereas two others found no change (Klimke et al., 1999; Parsey et al., 2001). One [^{123}I]IBZM and one [^{11}C]raclopride study have found increased D2 receptor binding in depressed patients with psychomotor retardation (Ebert et al., 1994; Meyer et al., 2006). The elevated levels of D2 receptors may represent a compensatory mechanism for diminished synaptic DA levels by increasing post synaptic sensitivity to the levels of DA that are present (Laruelle, 2000).

The non-significant results could be the consequence of several other factors. First, the subjects of this study had mild depression, which might be expected considering that ~45% of PD patients diagnosed in a community based study had only mild symptoms (Tandberg et al., 1996). Secondly, all depressed subjects in this study were on antidepressants. The use of anti-depressant treatment has been found to decrease dopamine receptor density in responders whether via tricylcics (Ebert et al., 1996), specific serotonin reuptake inhibitors (SSRI) (Montgomery and Andersen, 2006) or sleep deprivation (Ebert et al., 1994) in depressed patients, and the SSRI citalopram has also been reported to decrease D2 receptor binding in controls (Tiihonen et al., 1996). Although statistically non-significant the elevated D2 receptor levels would suggest that these depressed patients were non-responsive to medications. Note that not all antidepressant therapies have shown the same effect. For instance, healthy controls exposed to fluoxetine do not change D2 receptor density and an [^{123}I]IBZM study correlated clinical recovery with increased D2 receptor binding in the anterior cingulate, the surrounding frontal lobe and the striatum (Klimke et al., 1999).

4.2 Placebo-induced DA release

At the current stage of this study no significant DA release was observed in non-depressed or depressed subjects in response to placebo. However, initial inspection suggests that there could be a negative placebo induced DA decrease in depressed subjects. The placebo effect is the response of an individual to sham treatment when compared to absence of any treatment. As mentioned previously with antidepressants, the placebo response may play a significant role in the effects of active drug. The placebo effect can be attributed to mechanisms including expectation and conditioning. Expectation has been shown to elicit DA release in the ventral striatum in a placebo apomorphine administration in Parkinson's disease patients (de la Fuente-Fernandez et al., 2002). Since patients had a 50% chance of receiving amphetamine in this study,

patients receiving placebo first should have a strong placebo effect according to expectation-dopamine studies in monkeys (Fiorillo et al., 2003). However, no order effects were observed in this study. Subjects may have not associated the pill as a rewarding stimulus since clinical coordinators primarily referred to it as a stimulant or by its generic name Dexedrine. Although prior conditioning was absent since no subjects had previous exposure to amphetamine, some conditioning may have been present if patients had received amphetamine first. However, since taking amphetamine the first day usually unblinded subjects, any conditioning may have been less pronounced. In line with other conditioning studies in which subjects believe they have received drug, rather than just evoke drug seeking sensations, no placebo effect was observed in the putamen or caudate (Boileau et al., 2006; Boileau et al., 2007). However, no placebo effect was observed in the ventral striatum either. The absence of the placebo effect could be due to the lack of conditioning or the excessive delay between placebo administration and the PET scan. Boileau et al. (2007) found that behavioral response to placebo was maximum 1 hr post-administration and rapidly declined afterwards, suggesting that the 1.5hr wait following amphetamine administration was too long in this study.

4.3 Subjective responses – Amphetamine Interview Rating Scale

Unfortunately, since no baseline AIRS scores were measured the ΔAIRS scores, a measure of the subjective difference between placebo and amphetamine conditions, were correlated with ΔBP_{drug}, which is a change of BP with amphetamine compared to placebo. Correlation analysis found a negative correlation between age and ΔAIRS. One study of presynaptic dopamine integrity has found that there is a 0.5% per year decline of type-2 vesicular monoamine transporter in both normal and PD subjects (Bohnen et al., 2006). Perhaps differences in presynaptic integrity could manifest as differences in subjective responses to amphetamine since age was not correlated with DA release in any regions of interest. Analysis revealed a positive

correlation between ΔBP_{drug} in the putamen and ventral striatum with depressive symptoms and dysphoria. These results could suggest that increased dopamine in these regions could induce depressive or dysphoria symptoms. Lastly, increased dopamine release in the putamen correlates with dopamine release in the caudate for both the amphetamine and placebo conditions. This correlation is consistent with the aforementioned feedforward striatal-nigralstriatal connection. The increased dopaminergic activity in the caudate results in disinhibition of the feedforward connections that control dopaminergic output to the putamen. Interestingly, no correlation was found between amphetamine release in the ventral striatum and euphoria (p=0.80) as previously reported (Drevets et al., 2001). Nor was there a correlation between depression severity, as measured by MADRS, and amphetamine induced release in the ventral striatum as hypothesized.

4.5 Study limitations

Interpreting these results is confounded by the fact that [^{11}C]raclopride has the capacity to quantify D2/D3 receptors but is also sensitive to changes of endogenous dopamine (Morris and Yoder, 2007). Therefore, [^{11}C]raclopride binding potential at baseline may be affected by both receptor density and endogenous dopamine levels.

OCCUPANCY MODEL

Numerous animal, primate and human studies have suggested that amphetamine induced changes in D2/3 radioligand binding reflect changes in endogenous dopamine (Laruelle, 2000). According to the occupancy model the observed decrease in [^{11}C]raclopride binding with amphetamine reflects increased endogenous dopamine release (Laruelle, 2000). Dopamine is released from vesicles of presynaptic neurons and subsequently binds to postsynaptic DA receptors (Figure 17). The function of [^{11}C]raclopride is to bind to postsynaptic DA receptors. If DA release is increased the neurotransmitter will "occupy" some of these radiotracer labeled

sites, which is observed as a decline in binding potential. In our experiment amphetamine was used to increase endogenous dopamine release. Amphetamine acts by reversing the action of the dopamine transporter, which functions to reuptake DA present in the synapse. At high doses, amphetamine not only extrudes DA from the cytoplasmic pool but also releases DA already stored in vesicles (Sulzer et al., 1995). DA release is necessary for radiotracer to be displaced. Studies using α-methyl-para-tyrosineor reserpine, which block DA production or storage respectively, have shown blunted AMP induced decreases in radiotracer binding (Laruelle et al., 1997).
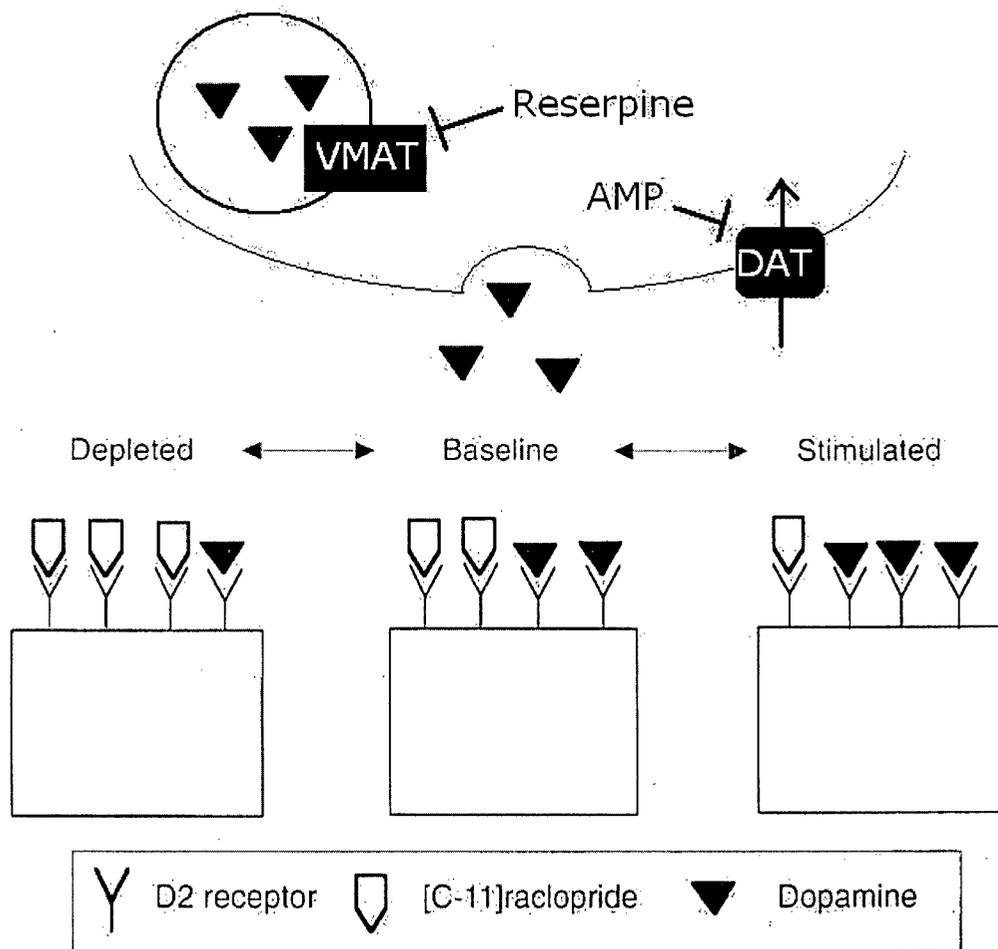
Figure 17 Classical occupancy model. Drugs that deplete dopamine levels like reserpine by blocking vesicular monoamine transporters (VMAT) permit more [^{11}C]raclopride to bind to D2 receptors. Conversely, drugs like amphetamine (AMP) that reverse the dopamine transporter (DAT) increase levels of synaptic dopamine and effectively displace [^{11}C]raclopride, consequently decreasing its observed binding potential. (Adapted by permission from Macmillan Publishers Ltd: Laruelle 2000; © 2000).

The physiological effects of amphetamine such as regional cerebral blood flow, peripheral rate of clearance of radiotracer and radiotracer plasma protein binding are also accounted for within this model. Studies using a bolus plus constant infusion paradigm, in which radiotracer levels reach equilibrium before the intervention drug is administered have shown robust AMP-induced decreases (Laruelle et al., 1995; Carson et al., 1997; Laruelle et al., 1997). Note that a ceiling effect occurs with [^{11}C]raclopride displacement because the antagonist radiotracer binds ~80% of all the receptors whereas DA can only bind to ~50% of DA receptors considered to be in a high-affinity configuration. Furthermore, tonic release of endogenous DA occupies ~20% of these high-affinity receptors. This results in ~37.5% of the entire receptor population available to be displaced (Figure 18).
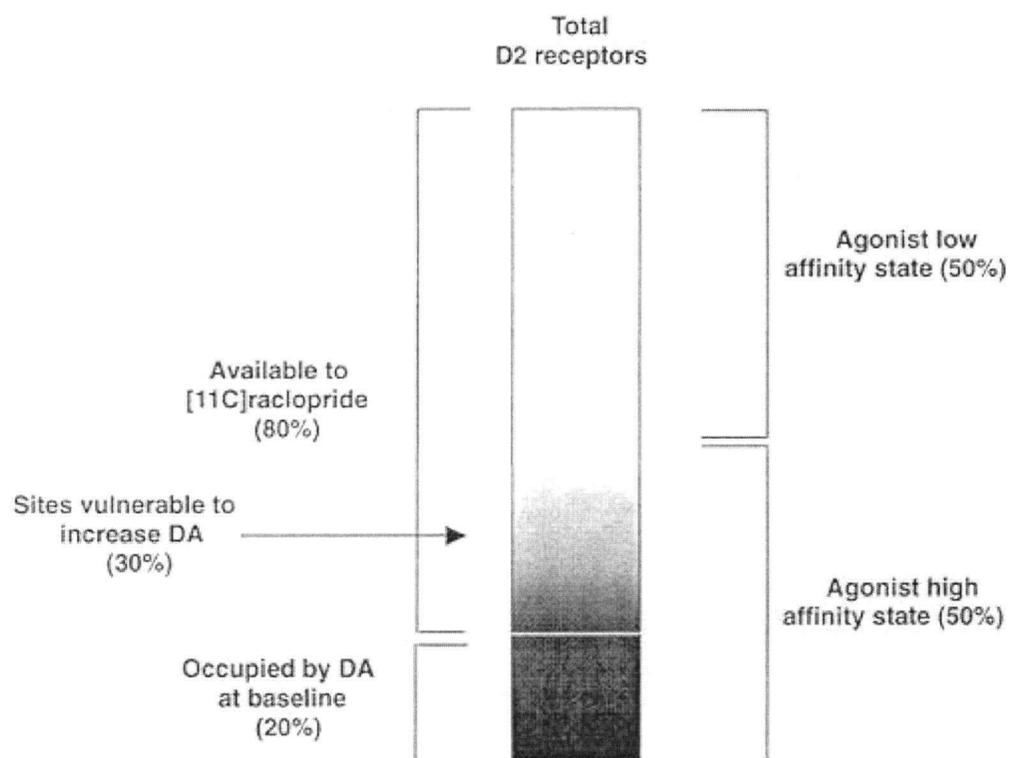
Figure 18 Schematic representation of D2 receptor states. Studies have suggested that 50% of

D2 receptors are in high and low affinity states and that 20% of receptors are occupied by

endogenous DA at baseline leaving only 30% available to be bound following drug intervention.

With [^{11}C]raclopride capable of binding 80% of D2 receptors this leaves approximately 37.5% of

receptors able to be displaced (Reprinted by permission from Macmillan Publishers Ltd: Laruelle

2000; © 2000).

However, some results are discrepant with the occupancy model. First, amphetamine induced

decreases in benzamides such as [^{11}C]raclopride are sustained for at least six hours after

administration in both double bolus (Cardenas et al., 2004) and bolus plus infusion paradigms

(Laruelle et al., 1997). This binding potential decrease lasts longer than the change in

extracellular DA as measured by microdialysis. This observation may be reconciled with the fact

that amphetamine may sustain elevated synaptic levels of DA by inhibiting monoamine oxidase

(Green and el Hait, 1978) and stimulating DA synthesis (Uretsky and Snodgrass, 1977). In

addition, microdialysis does not reflect intrasynaptic DA because pharmaceuticals that

equivalently increase intrasynaptic DA, as measured by radiotracer displacement, will have a

50-fold difference of increase in extracellular DA levels. Agonist-mediated receptor

internalization has been postulated to account for the discrepancy between the time course of DA

release measure by microdialysis and the observed decreases in binding potential. In brief, when

agonists stimulate receptors, the agonist-receptor complexes are translocated from the membrane

to acidified endosomes where they are degraded or held for return to the membrane. Since

raclopride has low lipophilicity and the low sodium, high proton environment of the internalized

receptors decreases its affinity, raclopride only binds to externalized receptors. However, this

latter point remains equivocal.

POSTSYNAPTIC& PRESYNAPTIC FACTORS

The prominent effects of amphetamine in the ventral striatum may be accounted for by several

post-synaptic factors. First, endogenous dopamine has a higher affinity for D3 receptors

(Sokoloff et al., 1990). Since the proportion of D3 receptors relative to D2 receptors is higher in

the ventral striatum (32%) than the caudate (18%) and putamen (12%), it would suggest that the

ventral striatum would likely bind more released dopamine (Gurevich and Joyce, 1999). As well,

microdialysis experiments have revealed that the ventral striatum has lower baseline levels of

dopamine, thus increasing the availability of displaceable postsynaptic receptors (Bradberry et al., 2000). Additionally, regional differences may exist in the number of autoreceptors versus postsynaptic, synaptic versus extrasynaptic receptors, high versus low affinity receptors and neuromachinery regulating internalization. Differences in all these factors may also explain the amphetamine induced profile exhibited in these PD subjects.

Regional and disease state differences in presynaptic factors should also be taken into consideration. For instance, the midbrain dopamine neurons that project to the ventral striatum express fewer D2 autoreceptors and dopamine transporters (Hurd et al., 1994; Haber et al., 1995). Decreased D2 autoreceptor expression would elevate amphetamine induced DA release because they serve to inhibit DAT function (Han et al., 1999). On the other hand, this effect may be counterbalanced by the expression of fewer DAT, which amphetamine uses to release DA into the synapse.

4.6 Future Directions

The first future objective of this study is to complete recruitment and scanning of 10 PD depressed (n=10) and non-depressed subjects (n=10). The next phase of this study is to compare the PD population with non-PD depressed (n=10) and controls (n=10). Beyond these crucial objectives, other questions may be investigated including the distribution of extrastriatal dopamine receptors, connections between the prefrontal cortex and the basal ganglia, and presynaptic dopaminergic terminals of the basal ganglia.

EXTRASTRIATAL RADIOTRACERS

Many metabolic imaging studies have identified extrastriatal sites such as the amygdala, anterior cingulate cortex, and anterior insula to be abnormal in depression (Mayberg, 1997). Future work

will continue to investigate differences in thalamic and prefrontal cortex dopamine profiles of depressed and non-depressed subjects. Since the affinity for D2/D3 receptors by [^{11}C]raclopride is limited outside of the striatum the use of newly developed extrastriatal radiotracers such as [^{11}C] FLB-457 and [^{18}F] fallypride may be considered. The use of these tracers will be well suited as a comparison to the metabolic studies in depression. In non-human primates studies using these tracers, large decreases of binding potential by amphetamine in the dorsal striatum, thalamus, temporal cortex and hippocampus have been reported (Chou et al., 2000; Slifstein et al., 2004; Mukherjee et al., 2005). A [^{18}F]fallypride study in humans also found significant decreases in the ventral striatum, substantia nigra and anterior cingulated (Riccardi et al., 2006). However, the use of these extrastriatal radiotracers does come with some caveats. With [^{11}C]FLB-457, only a reference tissue model can adequately measure striatal and extrastriatal binding potential simultaneously, whereas peak and transient equilibrium underestimate binding potential where the D2 receptor density is above 7nM such as with the striatum (Olsson and Farde, 2001). Second, the high affinity of these radiotracers has enabled specific activity to be detected in the cerebellum, which is commonly used as the reference tissue (Asselin et al., 2006). This poses the problem of underestimating binding potential, particularly at extrastriatal sites, when the cerebellum is used as the reference region.

REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION (RTMS)

Another future direction of investigating dopamine release in depressed versus non-depressed subject would involve repetitive transcranial magnetic stimulation (rTMS). Studies have found that stimulating the motor cortex or dosolateral prefrontal cortex can induce decreases in [^{11}C]raclopride binding in the caudate, putamen and ventral striatum via cortical glutamatergic projections (Strafella et al., 2001; Strafella et al., 2003; Ohnishi et al., 2004). There is evidence suggesting that glutamate may play a role mood disorders. Thus rTMS studies stimulating medial

and orbitofrontal cortical areas may link frontal dysfunctions with impaired dopamine release. Of interest, rTMS is currently considered one of the new approaches to treating depression, however, further refinement of methodology is required before becoming a standard practice (Loo and Mitchell, 2005).

PRESYNAPTIC FUNCTION

To better understand what it occurring at the synapse during amphetamine administration further in vitro and in vivo examination will be required. At an in vitro level the contribution of autoreceptors during DA release should be elucidated and to determine the distribution of raclopride among the presynaptic, postsynaptic and extrasynaptic sites. As this study is limited to observing effects on postsynaptic measures of dopamine nerve terminals, it would be useful to explore the presynaptic function and integrity of dopamine terminals in vivo. The density of dopaminergic terminals can be evaluated using the radiotracer [^{11}C]dihydrotetrabenazine, which binds to vesicular monoamine transporter 2. The turnover of dopamine can be examined using [^{18}F]-dopa and dopamine transporter can be specifically visualized using [^{11}C]methylphenidate rather than the [^{11}C]RTI-32 or [^{123}I]B-CIT, which bind to multiple monoamine transporters.

4.7 Conclusion

The amphetamine induced DA release in the ventral striatum measured in this study has been well replicated in normal subjects but is demonstrated for the first time in Parkinson disease patients. As well, these PD patients exhibit a different amphetamine induced profile in which putamen DA release is reduced. The lack of a placebo effect in this study may be due to the absence of conditioning, appropriate timing or expectation. Although no difference was observed between depressed and non-depressed subjects there continues to be a large of body of literature that supports changes in presynaptic and postsynaptic dopaminergic function of depressed

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individuals. As well, the results of this study may be contaminated by the use of antidepressants and that most patients were mildly depressed. In interpreting these results it is important to take into account the properties of $[^{11}C]$raclopride, evidence supporting the occupancy model and contributing synaptic factors. Lastly, future directions will expand the analysis into extrastriatal regions, directly stimulate mesostriatal and mesolimbocortical sites with rTMS and further explore synaptic function in vitro and in vivo.

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APPENDIX

UBC

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

PRINCIPAL INVESTIGATOR:	DEPARTMENT:	UBC CREB NUMBER:
A. Jon Stoessl	UBC/Medicine, Faculty of/Medicine, Department of/Neurology - Med	H06-70035

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
Elliott Bogusz
Thomas J. Ruth
Lakshmi N. Yatham

SPONSORING AGENCIES:
Canadian Institutes of Health Research - "Role of Dopamine in Depression"
Michael Smith Foundation for Health Research

PROJECT TITLE:
The Role of Dopamine in Depression

The current UBC CREB approval for this study expires: February 7, 2008

AMENDMENT(S):			AMENDMENT APPROVAL DATE:
Document Name	Version	Date	February 7, 2007
Protocol:			
Study Protocol	3	January 16, 2007	
Consent Forms:			
Normal Consent Form	5.1	January 29, 2007	
PD Consent Form	6.1	January 29, 2007	
Depressed Subject Consent	5.1	January 29, 2007	
Letter of Initial Contact:			
Information Letter PD	5	January 16,	