Decreased binding of the D₃ dopamine receptor-preferring ligand [¹¹C]-(+)-PHNO in drug-naïve Parkinson’s disease

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The D₃ dopamine (DA) receptor is a member of the D₂-like DA receptor family. While the D₂ receptor is abundant especially in motor-regions of the striatum, the D₃ receptor shows a relative abundance in limbic regions and globus pallidus. This receptor is of current interest in neurology because of its potential involvement in psychiatric and motor complications in Parkinson’s disease and the possibility that dopamine D₃-preferring agonist therapy might delay progression of the disorder. Preclinical data indicate that striatal levels of the D₃ (but not the D₂) DA receptor are decreased following lesion of nigrostriatal DA neurons; at present, there are no in vivo data on this receptor subtype in Parkinson’s disease. The objective of this positron emission tomography study was to compare [¹¹C]-(+)-PHNO (D₃ versus D₂ preferring) and [¹¹C]raclopride (D₃ = D₂) binding in brain of non-depressed, non-demented, dopaminergic drug-naïve patients with early-stage Parkinson’s disease (n = 10), relative to matched-controls (n = 9). Parkinson’s disease was associated with a trend for bilaterally decreased [¹¹C]-(+)-PHNO (but not [¹¹C]raclopride) binding in the D₃-rich ventral striatum (−11%, P = 0.07) and significantly decreased binding in globus pallidus (−42%, P = 0.02). In contrast, in the primarily D₂-populated putamen, both [¹¹C]-(+)-PHNO (25%, P = 0.02) and [¹¹C]raclopride (25%, P < 0.01) binding were similarly increased, especially on the side contra-lateral to the symptoms. In the midbrain, presumably containing D₃ receptors localized to the substantia nigra, [¹¹C]-(+)-PHNO binding was normal. Decreased [¹¹C]-(+)-PHNO to [¹¹C]raclopride ratio correlated with motor deficits and lowered-mood (P < 0.02). Our imaging data suggest that brain DA neuron loss in the human causes region-specific differential changes in DA D₂ and D₃ receptors with D₃ receptor ‘downregulation’ possibly related to some motor and mood problems in Parkinson disease. D₃ receptor levels might be a determinant vulnerability factor underlying side-effects associated with treatment; hence, these initial findings provide valuable baseline information to understand the role of D₃ receptors in response to Parkinson’s disease medication.

Keywords: [¹¹C]raclopride; [¹¹C]-(+)-PHNO; Dopamine D₂/D₃ receptors; Parkinson’s disease; positron emission tomography (PET)

Abbreviations: DA = dopamine; DC = dorsal caudate; DP = dorsal putamen; GP = globus pallidus; PET = positron emission tomography; ROI = region of interest; VS = ventral striatum
Introduction

As dopamine (DA) has an established (idiopathic Parkinson's disease) or suspected (schizophrenia, addiction) involvement in a variety of human brain disorders (Ehringer and Hornykiewicz, 1960; Snyder et al., 1974; Di Chiara and Imperato, 1988), there has been much interest in understanding the role of the different DA receptor subtypes (D1-like, including D1 and D5; and D2-like, including D2, D3 and D4) in these conditions. To date, D1 and especially D2 DA receptors have been the focus of most clinical interest. However, because of its preferential localization to the human limbic system (Landwehrmeyer et al., 1993; Murray et al., 1994), involved in motivation reward and emotion (for review see Ikemoto and Panksepp, 1999), the DA D3 receptor (Sokoloff et al., 1990) has been considered a potentially important drug target (Meltzer, 1991; Joyce, 2001) and possible pathophysiological factor (Joyce and Millan, 2007) in human conditions involving a brain DA abnormality.

The emerging D3 receptor literature in Parkinson's disease, based on experimental animal findings and human drug studies, implicates this receptor in the pathophysiology and treatment of the disorder. Loss of dopaminergic input to this receptor could provide the basis for some psychiatric problems associated with Parkinson's disease [e.g. depression or anhedonia; (Lemke et al., 2005)], whereas other disturbances [e.g. pathological gambling; Parkinson's disease [e.g. depression or anhedonia; (Lemke et al., 2005); Lader, 2008] consequent to DA agonist therapy, might be explained by excessive D3 receptor-linked activity (Bezard et al., 2003; Guillin et al., 2003; Richtand, 2006). Although this DA receptor subtype is not predominant in motor regions of the human striatum [e.g. putamen; (Murray et al., 1994)], some animal data suggest that the D3 receptor might be involved in normal motor function (Accili et al., 1996; Ekman et al., 1998; Gendreau et al., 1997; Xu et al., 1997; McNamara et al., 2006; Pritchard et al., 2007) and in development of abnormal motor responses caused by prolonged treatment with DA substitution therapy in Parkinson's disease. In this regard, increased D3 receptor levels, as inferred from [3H]7-OH-DPAT binding in MPTP-intoxicated monkeys have been associated with emergence of behavioural sensitization (levodopa-induced dyskinesias) which was attenuated by administration of a selective D3 partial-agonist (BP 897) (Bezard et al., 2003; Guigoni et al., 2005).

A separate line of investigation provides evidence, albeit controversial, that stimulation of the D3 receptor might actually protect nigrostriatal DA neurons from toxic damage. This was suggested in part by the ability of D3-prefering DA agonists (e.g. pramipexole) to protect against DA neuron damage caused by MPTP and 6-hydroxydopamine (for review see Joyce and Millan, 2007). Furthermore, in a single-photon emission computed tomography ([123I]I-CIT) imaging study, patients with Parkinson's disease who received pramipexole displayed a less severe loss of striatal DA transporter binding (at 46 months) relative to patients receiving levodopa (Parkinson Study Group, 2002). However, the incidence of dyskinesias was not significantly different between treatment groups (Constantinescu, 2007) and the issue of the D3 receptor and neuroprotection in Parkinson's disease is not yet established because of a variety of limitations to study design (for discussion see Clarke and Guttmann, 2002).

The present investigation focuses on understanding the response of the DA D3 receptor to loss of DA neurons in the human. Striatal D3 receptor levels [as measured by positron emission tomography (PET) imaging of the D2/D3 receptor probe [11C]raclopride] are typically elevated in Parkinson's disease patients who have yet to begin DA substitution therapy (Rinne et al., 1990; Brooks et al., 1992), as is the case in animals with induced-parkinsonism (Graham et al., 1990). This finding is generally considered to represent a form of denervation supersensitivity to compensate for diminished DA transmission (Lee et al., 1978). In the case of the D3 receptor, animal studies employing Parkinson's disease models generally show, paradoxically, the reverse, in which sustained interruption of DA transmission is associated with decreased striatal D3 receptor concentration as inferred from [3H]7-OH-DPAT binding and D3 receptor mRNA procedures (Levesque et al., 1995; Morissette et al., 1998). The biological significance of the D3 reduction, which appears to be related to decreased D1-stimulated brain-derived neurotrophic factor (BDNF) (Guillin et al., 2001), is as yet unknown (i.e. is reduction harmful or helpful in maintaining normal brain DA function?). To date, findings on D3 receptor expression in human Parkinson's disease are contradictory [decreased: (Piggott et al., 1999); and normal [3H]7-OH-DPAT binding: (Hurley et al., 1996b)]; and importantly, limited to post-mortem investigations (some employing a wide range of post-mortem times) involving patients who had received dopaminergic medications which might influence receptor expression (Antonini et al., 1994; Bordet et al., 1997). Although the function of the DA D3 receptor in the human is still unknown, the above considerations suggest that changes in striatal D3 receptor expression could lead to functional consequences related to behaviour, drug response and possibly neurodegenerative process in Parkinson's disease.

[11C]-(+)-propyl-hexahydro-naphtho-oxazin ([11C]-(+)-PHNO) is an agonist PET radioligand initially developed (Wilson et al., 2005) to distinguish between the DA receptor in the high (D2<sub>high</sub>): G-protein coupled state, responsible for the functional effects of DA) and low-affinity states (D2<sub>low</sub>: G-protein uncoupled, functionally inert) (Seeman et al., 1993). However, converging evidence suggests that [11C]-(+)-PHNO has a bio-distribution consistent with that of the D3 receptor in human brain (Willett et al., 2006; Graff-Guerrero et al., 2008) and preferential affinity for D3 over D2. The in vitro selectivity of [11C]-(+)-PHNO for the D3 receptor has been reported to be approximately 50-fold higher than for the D2 receptor (Freedman et al., 1994). In vivo, a study in the baboon using a D3 partial agonist (BP897) (Narendran et al., 2006) to block [11C]-(+)-PHNO signal, indicates a more modest D3 preference (4-fold). However, other studies in baboons using a selective antagonist (SB-277011) show as much as a 30-fold [11C]-(+)-PHNO preference for D3 over D2 (Rabiner et al., 2007, 2008a, b). A higher affinity of [11C]-(+)-PHNO for D3 over D2 has also been reported in a human PET study showing the selective displacement of [11C]-(+)-PHNO by a D3 antagonist (AB9225) (Abi-Saab et al., 2008).

The aim of our study was to measure binding of [11C]-(+)-PHNO in brain of drug-naive patients with Parkinson's disease...
and to compare the PHNO binding with that of the ‘classical’ DA D_{2/3} antagonist ligand [^{11}C]raclopride. Based on literature data, we hypothesized that [^{11}C]-(+)-PHNO binding would be decreased in D_{3}-rich (ventral striatum (VS) and globus pallidus, GP) whereas [^{11}C]raclopride binding would be elevated in D_{2}-rich (dorsal) striatum [as previously demonstrated in PET studies; for review see (Brooks, 2003; Hurley and Jenner, 2006)].

## Materials and Methods

### Subjects

Ten early-stage drug-naïve patients meeting UK Brain Bank Criteria for idiopathic Parkinson’s disease and nine healthy controls gave written informed consent to participate in a PET study approved by the Centre for Addiction and Mental Health research ethics board. None of the subjects, Parkinson’s disease or controls, had evidence of cognitive impairment [individual mini-mental state examination, MMSE scores greater than the cut-off score of 27 (O’Bryant et al., 2008)], significant medical conditions (except for Parkinson’s disease in the Parkinson’s disease group) or axis-I psychiatric disorders [as per structured clinical interview for DSM-IV disorders (First et al., 1994)]. All subjects completed mood and personality questionnaires. Table 1 summarizes characteristics of the subjects in the study.

### PET imaging

[^{11}C]-(+)-PHNO and [^{11}C]raclopride synthesis and image acquisition protocols have been described in detail elsewhere (Graff-Guerrero et al., 2008). PET images of [^{11}C]-(+)-PHNO (90-min) and [^{11}C]raclopride (60-min) scans were acquired using CPS-HRRT neuro-PET camera system (Siemens Medical Imaging, Knoxville, TN). Head movement minimization was achieved with a head immobilization system (Tru Scan Imaging, Annapolis USA). Transmission scans were obtained using a single-photon $^{137}$Cesium (Ey = 662 keV) point-source, and used to correct the emission scans for the attenuation of 511 keV photons through tissue and head support. The scans were initiated following a bolus injection of [^{11}C]-(+)-PHNO (303.4 ± 1.7 MBq; specific activity, 861.4 ± 240 mCi/μmol; mean mass, 2.31 ± 0.3 μg) or of [^{11}C]raclopride (355.2 ± 18 MBq; specific activity, 864.8 ± 268 mCi/μmol; mean mass, 4.52 ± 2.2 μg). Raw data were reconstructed by filtered-back projection (Defrise et al., 1997). Standard spin echo proton-density weight magnetic resonance images (MRI; TE = 17, TR = 6000, field of view = 22 cm 2D, 256 × 256, slice thickness = 2 mm, NEX = 2) were obtained (Sigma 1.5T MRI scanner, General Electric Medical Systems, Milwaukee, WI) for purpose of region of interest (ROI) delineation.

ROI delineation and analysis was performed by using in-house image analysis software for automated quantification of PET data (ROMI) (Rusjan et al., 2006). In brief, a standard brain template (International Consortium for Brain Mapping/Montreal Neurological Institute 152 MRI) containing a set of predefined cortical and subcortical ROIs [based on (Talairach et al., 1988) and (Kabani et al., 1998) atlases] was non-linearly transformed (SPM normalization and co-registration; Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm/) to fit individual high-resolution MRI. Each individual’s set of automatically created ROIs was then refined by iteratively including and deleting voxels based on the probability of each voxel belonging to grey matter (SPM2 segmentation, Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm). Each individual’s

## Table 1 Characteristics of subjects

<table>
<thead>
<tr>
<th>Controls mean ± SD</th>
<th>Parkinson’s mean ± SD</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Age 54 ± 9 years</td>
<td>56 ± 11 years</td>
<td>0.70</td>
</tr>
<tr>
<td>Gender 5 Male/4 Female</td>
<td>5 Male/9 Female</td>
<td>0.12</td>
</tr>
<tr>
<td>Education 13.8 ± 1 Years</td>
<td>14.8 ± 1 Years</td>
<td>0.57</td>
</tr>
<tr>
<td>Premorbid IQ as per NART 117.3 ± 5.8</td>
<td>118.6 ± 2.1</td>
<td>0.67</td>
</tr>
<tr>
<td>MMSE 29.7 ± 0.7</td>
<td>29.8 ± 0.8</td>
<td>0.14</td>
</tr>
<tr>
<td>BDI 2.6 ± 2.1</td>
<td>6.5 ± 6.7</td>
<td>0.14</td>
</tr>
<tr>
<td>IDS 3.0 ± 3.2</td>
<td>7.9 ± 5.9</td>
<td>0.05</td>
</tr>
<tr>
<td>BPS 9.1 ± 2.3</td>
<td>10.0 ± 3.2</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Purdue Pegboard task</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPT - Dominant hand</td>
<td>14 ± 2</td>
<td>0.08</td>
</tr>
<tr>
<td>PPT - Non-dominant hand</td>
<td>14 ± 1</td>
<td>0.05</td>
</tr>
<tr>
<td>PPT - Both hands</td>
<td>11 ± 1</td>
<td>0.05</td>
</tr>
<tr>
<td>PPT - Symptomatic hand</td>
<td>11 ± 2</td>
<td>0.02</td>
</tr>
<tr>
<td>PPT - Asymptomatic hand</td>
<td>12 ± 3</td>
<td></td>
</tr>
<tr>
<td>Disease duration 3 ± 2 years</td>
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</tr>
<tr>
<td>Hoehn–Yahr stage 8 H&amp;Y I; 2 H&amp;Y II</td>
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<tr>
<td>UPDRS total 15 ± 3</td>
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<tr>
<td>UPDRS III motor score</td>
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<tr>
<td>UPDRS III asymptomatic score</td>
<td>1 ± 1</td>
<td></td>
</tr>
<tr>
<td>Symptomatic-side 3 L/7 R</td>
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</tbody>
</table>

Italic for P-value indicate significance at P < 0.05.

NART = National Adult Reading Task (Nelson, 1982); MMSE = Mini-mental state examination (Folstein et al., 1975); BDI = Beck Depression Inventory (Beck et al., 1961); IDS = Inventory of Depressive Symptomatology (Rush et al., 2000); BPS = Boredom Proneness Scale (Farmer and Sundberg, 1986); PPT = Purdue pegboard task (Lafayette-Instrument-Company, 1985); H&Y = Hoehn–Yahr stage (Hoehn and Yahr, 2001); UPDRS total = Unified Parkinson Disease Rating Scale (Fahn and Elton, 1987); UPDRS III = motor examination scale; UPDRS III symptomatic/asymptomatic-side rating = UPDRS III items including, leg agility, rapid alternating hand pronation and supination, open-close hand movement, finger taps, upper and lower limb rigidity, action or postural tremor and tremor at rest. a Significantly different from asymptomatic hand.
refined ROIs were aligned and resliced to match the dimension of the PET images [normalized mutual information algorithm implemented under SPM2; (Studholme et al., 1999)]. Three bilateral sub-compartment of the striatum [dorsal caudate (DC); dorsal putamen (DP); and VS] and GP were selected as ROIs and the cerebellar cortex (excluding vermis) was selected as the region of reference [details in (Rusjan et al., 2006)]. We also investigated $[^{11}C]$raclopride and $[^{11}C]$-(+)-PHNO time activity curves were obtained from the dynamic data and specific binding (BPND) was estimated in each ROI using the simplified reference tissue method (SRTM) (Lammertsma and Hume, 1996). Parameter estimation was performed with PMOD (Version 2.8.5; PMOD Technologies Ltd, Zurich, Switzerland). This method has been validated to reliably estimate BPND for both $[^{11}C]$raclopride and $[^{11}C]$-(+)-PHNO (Ginovart et al., 2006).

Comparisons between $[^{11}C]$-(+)-PHNO and $[^{11}C]$raclopride BPND in the different ROIs in the Parkinson's disease and control subjects were conducted by using standard repeated-measures ANOVAs (ROIs and ligands X group). When appropriate, Least Significant Difference tests, Bonferroni corrected, were applied to determine the significance of regional differences in BPND between groups. Based on the finding that relative to $[^{11}C]$raclopride, $[^{11}C]$-(+)-PHNO has a greater selectivity for D3 receptors in D3-rich areas, we estimated regional differences in the D3 binding fraction by calculating group differences in the ratio of $[^{11}C]$-(+)-PHNO to $[^{11}C]$raclopride BPND using the Mann–Whitney non-parametric U-test. We specifically investigated this question in two ROIs: one composed of the GP and VS, the 'D2-rich compartment' and the other composed of the DP and DC, the 'D3-rich compartment'. To adjust for potential differences in ROI size, BPND in the D2 and D3-rich compartments were assessed from the weighted (by ROI volume) time activity curves.

Parametric images of $[^{11}C]$-(+)-PHNO and $[^{11}C]$raclopride binding were generated by using DEPICT (Gunn et al., 2002). This method of parameter estimation requires no a priori decision about model structure (data-driven) and employs the basis-pursuit approach. The tissue time activity curve of the cerebellar cortex reference region served as input function. Each parametric map was spatially normalized to an anatomical template (MNI) using SPM2 normalization and co-registration tools. Once in the same space, BPND maps were statistically investigated to assess significant contrasts between groups in ROIs (explicit mask) using independent sample t-tests, Bonferroni corrected = 0.05.

Results

Both radioligands had high uptake and quantifiable signal in the striatum and pallidum. However, $[^{11}C]$-(+)-PHNO and $[^{11}C]$raclopride displayed different rank order of BPND in the ROIs for both the Parkinson's disease and control groups combined [ROI X ligand F(3,48) = 7.97, $P < 0.001$]. Specifically, $[^{11}C]$raclopride BPND was higher in DP and DC (D2-rich compartment) compared with VS and GP (D3-rich compartment) ($P < 0.01$), whereas $[^{11}C]$-(+)-PHNO BPND was higher in GP and VS compared with DP and DC ($P < 0.01$) (Fig. 1A and B). $[^{11}C]$-(+)-PHNO BPND (but not $[^{11}C]$raclopride) was also quantifiable in the SN; BPND ranged from 0.83 to 2.29 (mean ± SD 1.45 ± 0.15). As shown in Fig. 1A and B, Parkinson's disease was associated with a region-specific increase in $[^{11}C]$raclopride and $[^{11}C]$-(+)-PHNO BPND [ROI X group F(3,48) = 12.66, $P < 0.001$]. This effect, corresponding to a 25% greater $[^{11}C]$-(+)-PHNO (P corrected = 0.02) and $[^{11}C]$raclopride BPND (P corrected = 0.01) relative to control, was specific to the DP and more pronounced in the DP contra-lateral to Parkinson's disease symptoms (left DP: 7/10 patients having symptoms on the right) relative to the control side ($[^{11}C]$raclopride, $11\% \pm 2, P = 0.02$; $[^{11}C]$-(+)-PHNO, $13\% \pm 1, P = 0.0003$). In contrast, Parkinson's disease was associated with decreased $[^{11}C]$-(+)-PHNO BPND in the D3-rich GP (−42%, $P = 0.02$) and VS (−11%, $P = 0.07$). There was no difference between $[^{11}C]$-(+)-PHNO BPND in the SN of Parkinson's disease versus controls ($P = 0.99$). A significantly lower $[^{11}C]$-(+)-PHNO to $[^{11}C]$raclopride BPND ratio in Parkinson's disease relative to controls was observed in the D3 compartment (−23%) but not in the D2 (0%) compartment (U = 16, $P = 0.01$; Fig. 2), suggesting that decreased D3 binding might account for lower $[^{11}C]$-(+)-PHNO in Parkinson's disease. $[^{11}C]$-(+)-PHNO and $[^{11}C]$raclopride BPND in the DP, but not in any other regions, correlated with each other ($r = 0.78; P < 0.001$). Lower $[^{11}C]$-(+)-PHNO to $[^{11}C]$raclopride BPND ratio correlated with motor deficits [Purdue Pegboard Task symptomatic hand (Lafayette-Instrument-Company, 1985): $r = 0.74; P = 0.01$], and with lowered mood [Beck Depression Inventory (Beck et al., 1961): $r = -0.67; P = 0.03$] (Fig. 3A and B).

Voxel-wise statistical analyses (SPM5) were in line with ROI outcome, revealing bilateral clusters of reduced $[^{11}C]$-(+)-PHNO BPND in the internal and external portions of the prefrontal cortex suggesting increased D3 binding.
GP extending into a region that appears to correspond to the former ‘substantia inominata’ (Heimer, 2003) including the ventral pallidum, olfactory area, caudal ventral striatum and nucleus basalis (MNI coordinates, $20$, $-10$, $-4$; $t_{max} = 5.28$, $P$ corrected $= 0.04$) and increased $[^{11}C]$-(+)-PHNO $BP_{ND}$ (MNI coordinates, $-25$, $-4$, $-6$; $t_{max} = 3.98$, $P$ corrected $= 0.006$) in Parkinson’s disease relative to controls. Map thresholding $P < 0.005$ uncorrected; voxel extent $>6$ voxels; Coordinates are in the MNI brain space.

**Discussion**

Our major finding is a reduction in $[^{11}C]$-(+)-PHNO binding in $D_3$-rich brain areas of ‘drug-naïve’ patients with Parkinson’s disease. This finding, which is strengthened by the contrasting change in $[^{11}C]$-raclopride binding, suggests that the human brain, like that of the experimental animal, might ‘downregulate’
Differential regional distribution of $[^{11}C]$-(+)-PHNO and $[^{11}C]$raclopride

Consistent with previous reports in humans (Graff-Guerrero et al., 2008), baboons (Narendran et al., 2006) and cats (Ginovart et al., 2006), we found that in vivo $[^{11}C]$-(+)-PHNO binding co-existed spatially with $[^{11}C]$raclopride distribution, but differed in that the $[^{11}C]$raclopride signal was more intense in dorsolateral aspects of the striatum (DP, DC) whereas $[^{11}C]$-(+)-PHNO uptake was predominant in ventromedial regions including the VS and GP. Furthermore, BP$_{ND}$ (with relatively low variance) could be measured in the midbrain SN (1.45 ± 0.15) with $[^{11}C]$-(+)-PHNO, but not with $[^{11}C]$raclopride.

The regional distribution of $[^{11}C]$raclopride and $[^{11}C]$-(+)-PHNO binding in the present study is generally consistent with that of D$_2$ and D$_3$ binding and mRNA measured in post-mortem human brain (Meador-Woodruff et al., 1996; Suzuki et al., 1998; Gurevich and Joyce, 1999). It is also in line with in vivo (Narendran et al., 2006; Rabiner et al., 2007, 2008a, b) and in vitro (Freedman et al., 1994) data suggesting that $[^{11}C]$-(+)-PHNO is a D$_3$-preferring agonist, whereas raclopride has equal affinity for both D$_2$-like DA receptor subtypes (Malmberg et al., 1994). Indeed, $[^{11}C]$-(+)-PHNO is estimated to have a 14- to 19-fold higher affinity for the D$_3$ receptor binding site (in vitro) relative to $[^{11}C]$raclopride (Freedman et al., 1994; van Vliet et al., 2000) as well as a higher affinity for the D$_3$ receptor relative to DA (Freedman et al., 1994; Seeman et al., 2006). The higher affinity of this ligand for the D$_3$ receptor and its potency at displacing endogenous DA from this receptor [unlike raclopride (Seeman et al., 2006)] can explain its higher binding in D$_3$-rich areas compared with that of $[^{11}C]$raclopride. However, our observation that $[^{11}C]$-(+)-PHNO binding was greater in GP than in VS is in variance with the results of D$_3$ binding studies in post-mortem brain (Landwehrmeyer et al., 1993; Murray et al., 1994). One possible explanation is that the very low level of DA in the GP relative to striatum (Ehringer and Hornykiewicz, 1960) leads to greater receptor concentration in response to loss of nigrostriatal DA neurons.

Opposing changes in $[^{11}C]$raclopride and $[^{11}C]$-(+)-PHNO BP$_{ND}$ in Parkinson’s disease

Our present study replicates earlier PET studies in drug-naı̂ve Parkinson’s disease showing elevated striatal $[^{11}C]$raclopride BP$_{ND}$, interpreted as an adaptive increase in postsynaptic D$_2$ receptor level in response to decreased DA availability (for review see Nandhagopal et al., 2008). We now extend this finding to $[^{11}C]$-(+)-PHNO; further supporting the notion that this ligand primarily binds to D$_2$ receptors in the DP. Although the general assumption, based on preclinical findings, is that increased tracer binding reflects increased receptor density (B$_{max}$) (for review see Wichmann and DeLong, 2003), it is possible that very low DA levels in the putamen also contribute to increased BP$_{ND}$ (decreased K$_D$). Although Parkinson’s disease-related increases in BP$_{ND}$, observed with both ligands, were of equal magnitude and inter-correlated in the DP, $[^{11}C]$-(+)-PHNO might have been more sensitive to lower DA levels when compared with $[^{11}C]$raclopride, thus accounting for a portion of the increased BP$_{ND}$. In this regard, greater sensitivity of the agonist ligand $[^{11}C]$-(+)-PHNO to fluctuations in endogenous DA levels has been demonstrated in a pharmacological challenge study in humans (Willeit et al., 2008).

Amongst the different explanations for reduced $[^{11}C]$-(+)-PHNO binding in GP and VS is the generic possibility that binding is decreased because of above-normal levels of endogenous DA in these two brain areas (decreased tracer affinity; 1/K$_D$). Although the possibility of increased DA in any brain area of patients with Parkinson’s disease would appear to be unlikely, increased $[^{18}F]$dopa uptake in the internal segment of the GP has been reported in early-stage Parkinson’s disease (Whone et al., 2003). However, in a non-human primate model (MPTP) of very early Parkinson’s disease, clinically asymptomatic animals showed decreased DA levels in GP (Pifli et al., 1992).

Given the selectivity of $[^{11}C]$-(+)-PHNO for the D$_3^{high}$ receptor (i.e.: unlike $[^{11}C]$raclopride which binds to both the DA receptor in high- and low-affinity states) (Seeman et al., 1993), an alternative explanation is that decreased $[^{11}C]$-(+)-PHNO binding in Parkinson’s disease is due to a reduction in G protein-coupled receptors (D$_3^{high}$). This possibility however is not supported by in vitro (Rubinstein et al., 1990; Cai et al., 2002;
Chefer et al., 2008) or in vivo (Cumming et al., 2003) studies in animal models of Parkinson’s disease. Furthermore, although the existence of D3

Although the findings are not entirely consistent, animal data generally demonstrate that D3 receptor binding in one or more dopaminomeric field is decreased following treatment with toxins (6-OHDA; MPTP) that damage brain DA neurons (Levesque et al., 1995; Hurley et al., 1996a; Morissette et al., 1998; Wade et al., 2001). It is less certain from preclinical studies however, whether decreased DA transmission per se is sufficient to cause reduction in receptor concentration: both no change (Levesque et al., 1995) and decreased (Stanwood et al., 2000) D3 receptor expression have been reported in resepinerized and antipsychotic treated animals. Given that D3 binding reductions reported in (post-mortem) animal studies are not likely to be confounded by possible competition between DA and D2 radioligand at the receptor site (as the tissue is washed and binding conducted in some studies at saturation), the simplest explanation for our finding of low [11C]-(+)-PHNO binding in VS and GP is that decreased binding of the PET tracer represents, at least in part, decreased concentration of the D3 receptor in this context. In contrast, low D3 binding could be explained by loss of either cellular elements containing the receptor or the receptor itself. In principle, decreased [11C]-(+)-PHNO binding could be due to loss of D3-containing DA neurons (e.g. autoreceptors) or postsynaptic cells [e.g. GABA-dynorphinergic substance P neurons (Surmeier et al., 1996)]. Data from 6-OHDA lesioned-animals argue against a mere disappearance of autoreceptors on DA axon terminals since parallel changes in D3 receptor binding and mRNA expression in post-synaptic cells have been shown to occur (Levesque et al., 1995). This conclusion is also consistent with the limited destruction, in early stage Parkinson’s disease, of ventral tegmental innervation to limbic striatum (Javoy-Agid et al., 1981) and with the finding of minimal expression of D3 receptor mRNA in ventral tegmental DA cells (Gurevich and Joyce, 1999). Thus, the most probable explanation is that part of the [11C]-(+)-PHNO binding decrease is consequent to a downregulation of the D3 receptor in intact postsynaptic cells. Animal findings suggest that expression of the D3 receptor is regulated by a D1/5 receptor stimulation-dependent elevation of brain-derived neurotrophic factor [BDNF, a neurotrophin anterogradely released upon DA neuron activation (Guillin et al., 2001)], which is reported to be reduced in Parkinson’s disease (Parain et al., 1999).

Investigations of D3 mRNA distribution in the human midbrain show D3 expression ranging from low in SN reticulata (presumably on GABA neurons) to very low in SN compata, to virtually absent in the ventral tegmental area. However, D3 receptor mRNA is present in tyrosine-hydroxylase positive neurons, suggesting that D3 receptors in midbrain are in part localized to DA neurons (Gurevich and Joyce, 1999). Our failure to find decreased [11C]-(+)-PHNO binding in midbrain is consistent with some data in the MPTP-treated monkey showing no loss of D3 receptor binding in the SN despite a massive loss of the DA transporter, a DA neuron marker (Quik et al., 2000). In our study, normal midbrain [11C]-(+)-PHNO binding [presumably exclusively to D3 (Rabiner et al., 2008b)] could be explained by an only modest loss of DA neuron cell bodies in early-stage Parkinson’s disease, upregulation of D3 receptor number in remaining cell bodies, or simply by preponderance of D3 receptors localized to non-nigral DA neurons in the examined midbrain area.

Our main findings should be considered in the light of the following limitations. In particular, although emerging data support the use of [11C]-(+)-PHNO to investigate the D3 system by PET, validation of this hypothesis has not been fully achieved in humans (e.g. with occupancy studies) and interpretation of our binding data is confounded to some extent by the binding of the radioligand to the D2 receptor. Indeed, quantification of the D3 receptor has historically been problematic. Given the lack of total selectivity of [11C]-(+)-PHNO and the higher D2 signal in striatum, decreases in D3 BPND can only be imprecisely measured and are likely to be underestimated. In this regard, we estimated a relative ‘D3 to D2 fraction’ by calculating the ratio of [11C]-(+)-PHNO to [11C]raclopride BPND. Although we recognize that this calculation can at best provide only a rough index of the D3 signal, the results do suggest yet greater decreases in [11C]-(+)-PHNO BPND in Parkinson’s disease. Despite the likely possibility of underestimating the difference between groups, the present study still found rather robust reductions in binding in Parkinson’s disease relative to controls. Other limitations relate to imaging measurement in the GP. Investigations (including our own) have reported that GP (the ROI having the most marked [11C]-(+)-PHNO change) time-activity curves typically have lower peak-uptake and slower washout when compared with other striatal regions, consequently leading to more variable BPND measurements (Ginovart et al., 2006). It has been suggested that the unusual GP kinetics could result from the D3 binding fraction in this brain area. However, despite our finding of different D3 receptor fraction in Parkinson’s disease and control subjects, we found that GP BPND was estimated with equal precision across groups (covariance of the fit controls: 9%; Parkinson’s disease: 12.5%) and that the coefficients of variation were similar (controls: 12.3%; Parkinson’s disease: 9.1%). Another factor which could potentially influence BPND measurement is brain morphological differences between groups. However, the possibility of brain atrophy in Parkinson’s disease explaining decreased [11C]-(+)-PHNO BPND is minimized because of the absence of grey-matter density differences in ROIs across groups (data not shown). Furthermore, adjustment for the effect of partial-volume (which minimizes the impact of morphological differences on outcome measure) resulted in a similar reductions in BPND in Parkinson’s disease (GP: –30%; VS: –8%).

What is the functional significance of decreased [11C]-(+)-PHNO binding?

Assuming that decreased [11C]-(+)-PHNO binding is in fact explained by some loss of the DA D3 receptor, the significance of our findings depends upon the biological role of the D3 receptor in human brain, which has yet to be established. In general, animal data suggest that the D3 receptor might exert an inhibitory role.
effect on motor function. This is supported by some animal findings of increased locomotor activity in transgenic mice lacking the D3 receptor (Accili et al., 1996; Xu et al., 1997; McNamara et al., 2006) and, in normal mice exposed to D3 antisense treatment (Ekmén et al., 1998) or to low doses of D3-prefering DA antagonists (Gendreau et al., 1997; Pritchard et al., 2007). Similarly, in the DA neuron-lesioned non-human primate, D3 agonist exposure enhances the anti-parkinsonian effects of levodopa (Silverdale et al., 2004). Taken together, these findings suggest that downregulation of the D3 receptor might represent an adaptation to compensate for the loss of motor function associated with DA neuron damage in Parkinson’s disease. However, seemingly at odds with the animal data, we found a positive correlation between decreased performance on the Purdue Pegboard motor task and D3 binding suggesting that D3 receptor stimulation could be involved in normal control over motor function. The literature is also unclear as to the role of DA in the GP. Interestingly, some imaging data suggest that decreased DA activity in the internal segment of the GP (which appeared to be affected in our study; see Results section) is associated with the loss of the ‘honeymoon phase’ to levodopa therapy and the onset of motor fluctuations (Brooks, 2003).

Given the preferential distribution of the D3 receptor in limbic brain it could be anticipated that loss of the receptor could impair mood, emotional and motivational functions. In this regard, our finding of a positive relationship between lowered-mood and decreased [11C]-(+)-PHNO binding suggests that reduced D3 stimulation could be a plausible candidate for the aetiology of certain symptoms of depression common in Parkinson’s disease (e.g. affective flattening, anhedonia and feelings of emptiness). Consistent with this possibility is the clinical observation (in an open study) that the D3 preferring anti-parkinsonian medication pramipexole might have anti-depressive and anti-anhedonic properties (Lemke et al., 2005). It must be emphasized, that the relationship we observed between [11C]-(+)-PHNO binding in D3-rich regions and several clinical outcome measures was correlational and not necessarily causal in nature. However, our data do raise the possibility that some behavioural consequences of DA neuron loss in Parkinson’s disease could be explained not only by loss of DA stimulation of the D3 receptors but, in addition, by some loss of the receptor itself.

The possibility of estimating D3 receptor levels in vivo through [11C]-(+)-PHNO imaging, although complicated by the limitations described above, has potentially important clinical implications. On one hand, it has been suggested that D3 receptor is implicated in treatment-induced complications in Parkinson’s disease, particularly psychiatric side-effects including pathological gambling and other compulsive behaviours. On the other hand, D3 receptor activation has a speculated (though still unconfirmed in the human) neuroprotective action on DA neurons. Future investigations of the association between brain D3 receptor levels and treatment-induced psychiatric problems and disease status in Parkinson’s disease as well as more fundamental studies of the factors responsible for controlling receptor expression [e.g. the dopamine D1 receptor; (Guillin et al., 2001)] are warranted.

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