

# Glutamate NMDA receptor dysregulation in Parkinson's disease with dyskinesias

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Levodopa-induced dyskinesias are a common complication of long-term therapy in Parkinson's disease. Although both pre- and post-synaptic mechanisms seem to be implicated in their development, the precise physiopathology of these disabling involuntary movements remains to be fully elucidated. Abnormalities in glutamate transmission (over expression and phosphorylation of N-methyl-D-aspartate receptors) have been associated with the development of levodopa-induced dyskinesias in animal models of Parkinsonism. The role of glutamate function in dyskinetic patients with Parkinson's disease, however, is unclear. We used <sup>11</sup>C-CNS 5161 [N-methyl-3(thiomethylphenyl)cyanamide] positron emission tomography, a marker of activated N-methyl-D-aspartate receptor ion channels, to compare *in vivo* glutamate function in parkinsonian patients with and without levodopa-induced dyskinesias. Each patient was assessed with positron emission tomography twice, after taking and withdrawal from levodopa. Striatal and cortical tracer uptake was calculated using a region of interest approach. In the 'OFF' state withdrawn from levodopa, dyskinetic and non-dyskinetic patients had similar levels of tracer uptake in basal ganglia and motor cortex. However, when positron emission tomography was performed in the 'ON' condition, dyskinetic patients had higher <sup>11</sup>C-CNS 5161 uptake in caudate, putamen and precentral gyrus compared to the patients without dyskinesias, suggesting that dyskinetic patients may have abnormal glutamatergic transmission in motor areas following levodopa administration. These findings are consistent with the results of animal model studies indicating that increased glutamatergic activity is implicated in the development and maintenance of levodopa-induced dyskinesias. They support the hypothesis that blockade of glutamate transmission may have a place in the management of disabling dyskinesias in Parkinson's disease.

**Keywords:** Parkinson; levodopa; dyskinesias; glutamate; PET; <sup>11</sup>C-CNS

**Abbreviations:** AMPA =  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; LID = levodopa-induced dyskinesias; NMDA = N-methyl-D-aspartate; UPDRS = Unified Parkinson's Disease Rating Scale;  $V_T$  = volumes of distribution

## Introduction

Dopamine replacement with oral levodopa remains the most effective treatment available for reversing the motor symptoms of Parkinson's disease. However, long-term use of levodopa in patients with Parkinson's disease is invariably associated with the development of abnormal involuntary movements known as dyskinesias, which may become as disabling as the parkinsonian symptoms themselves. A review of the cumulative literature suggests that levodopa-induced dyskinesias (LID) are experienced by 40% of patients with Parkinson's disease 4–6 years after starting levodopa, and up to 90% of patients by 9–15 years of treatment (Ahlskog and Muentner, 2001).

The pathogenic mechanisms underlying LID in Parkinson's disease are still being elucidated. The progressive loss of dopaminergic presynaptic terminal function associated with Parkinson's disease plays an important role in the development of LID, as the loss of striatal dopamine storage capacity results in non-physiological swings in striatal dopamine levels following levodopa administration. *In vivo* investigations of striatal dopamine fluxes with <sup>11</sup>C-raclopride PET have revealed that increases in synaptic dopamine concentration induced by levodopa administration in fluctuating dyskinetic patients are larger but shorter lived than those seen in sustained responders without dyskinesias (Tedroff *et al.*, 1996; de la Fuente-Fernandez *et al.*, 2004; Pavese *et al.*, 2006). Levels of synaptic dopamine after levodopa correlate with dyskinesia severity in Parkinson's disease (Pavese *et al.*, 2006). Pulsatile swings in brain dopamine may also result as a consequence of levodopa uptake by striatal serotonin terminals. These contain aromatic acid decarboxylase and can produce dopamine from exogenous levodopa though this is released in an unregulated manner (Carlsson *et al.*, 2007; Carta *et al.*, 2010).

Studies on animal models of Parkinson's disease indicate that pulsatile administration of high-dose oral levodopa results in abnormal expression of early genes (such as *c-jun* and *c-fos*) in the striatum (Svenningsson *et al.*, 2002), altered binding of non-dopaminergic neurotransmitter systems such as adenosine (Zeng *et al.*, 2000; Tomiyama *et al.*, 2004), raised the levels of peptide transmitters such as opiates dynorphin and enkephalin (Engber *et al.*, 1991; Henry *et al.*, 2003), and hyper-phosphorylation of glutamate *N*-methyl-D-aspartate (NMDA) receptors (Oh *et al.*, 1998; Calon *et al.*, 2002; Nash *et al.*, 2002).

Glutamate is the principal excitatory neurotransmitter in the basal ganglia acting through ionotropic NMDA, kainate and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and G-protein-coupled metabotropic receptor subtypes. In the striatum, NMDA receptors primarily contain NR1, NR2A and NR2B subtypes (Küppenbender *et al.*, 2000). Increased glutamatergic neurotransmission associated with hyperphosphorylation of these subunits has been implicated in the development and maintenance of LID in both the 6-hydroxydopamine lesioned rodent model and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine lesioned primate models of Parkinson's disease (Oh *et al.*, 1998; Calon *et al.*, 2002; Nash *et al.*, 2002). However, evidence supporting an association between abnormal glutamate function and dyskinesias in patients with Parkinson's disease is circumstantial.

*N*-methyl-3(thiomethylphenyl)cyanamide (CNS 5161) is a non-competitive antagonist of NMDA receptors. It binds with high affinity to the MK801 site in the activated voltage-gated ion channels (Biegon *et al.*, 2007).

In this exploratory study, we have used <sup>11</sup>C-CNS 5161 PET to test the hypothesis that altered glutamatergic transmission is associated with LIDs in Parkinson's disease. The effect of levodopa on striatal and cortical NMDA receptor glutamate ion channel activity was assessed in patients with Parkinson's disease with and without dyskinesias in both 'OFF' (all anti-parkinsonian medication withdrawn 12 h prior to scanning) and 'ON' (levodopa administered 1 h before scanning) conditions.

## Materials and methods

### Subjects

Eighteen patients with Parkinson's disease [11 males; mean age  $\pm$  standard deviation (SD) = 66.6  $\pm$  8.2 years] with a clinical diagnosis of idiopathic Parkinson's disease (UK Parkinson's Disease Society Brain Bank diagnostic criteria for Parkinson's disease) and five healthy controls (two males; mean age  $\pm$  SD = 64  $\pm$  12.3 years) were recruited for this study. Patients with significant comorbidity, previous history of other neurological conditions (e.g. stroke, head injury, epilepsy), with dementia [Mini-Mental State Examination < 23 (Folstein *et al.*, 1975)] and depression [Hamilton Rating Scale for Depression (Hamilton, 1960) and Beck Depression Inventory > 9 (Beck *et al.*, 1961)] were not enrolled.

The patients were divided into two groups according to the presence (patients with Parkinson's disease-LID, *n* = 8) or absence (patients with Parkinson's disease-NLID, *n* = 10) of LID. Table 1 summarizes the demographics of all patients investigated. Two of the patients with Parkinson's disease-NLID had motor fluctuations; the remaining patients with Parkinson's disease-NLID had a stable response to their dopamine replacement therapy. None of the patients were taking amantadine or other medication targeting the glutamatergic system. Daily levodopa equivalent units were calculated as follows:

$$100 \text{ mg levodopa} = 2 \text{ mg cabergoline} = 1 \text{ mg pramipexole};$$

or

$$1 \text{ mg pergolide} = 5 \text{ mg ropinirole} = 4 \text{ mg rotigotine patch} = 0.5 \text{ mg rasagiline}.$$

The conversion factors below were used for controlled release levodopa and levodopa/carbidopa/entecapone combination:

$$125 \text{ mg controlled release levodopa} = 65 \text{ mg levodopa};$$

$$50 \text{ mg levodopa/carbidopa/entecapone combination} = 65 \text{ mg levodopa}$$

The study received approval from the Ethics Committee of Hammersmith, Queen Charlotte's and Chelsea and Acton Hospitals Trust. Permission to administer <sup>11</sup>C-CNS 5161 was obtained from the Administration of Radioactive Substances Advisory Committee (ARSAC), UK. Informed written consent was obtained from all participating subjects.

### Clinical assessment

Patients with Parkinson's disease were assessed with the Unified Parkinson's Disease Rating Scale (UPDRS) (Fahn *et al.*, 1987) subscale III (motor examination) and IV (complications of therapy) in both 'OFF' and 'ON' conditions.

**Table 1 Study population**

	Gender (M/F)	Age (years)	Disease duration (years)	Hoehn and Yahr stage	Levodopa equivalent units (mg/day)	UPDRS III (Motor score)		UPDRS IV (Dyskinesia score)
						OFF	ON	
Parkinson's disease-NLID patients ( <i>n</i> = 10, mean ± SD)	8/2	66.6 ± 8.4	7.1 ± 4.1	2.4 ± 0.2	774 ± 174	36.7 ± 1.6	29.4 ± 1.5	NA
Parkinson's disease-LID patients ( <i>n</i> = 8, mean ± SD)	3/5	66.8 ± 8.8	11.4 ± 4.3	2.6 ± 0.2	1307 ± 439**	41.8 ± 2.3**	33.9 ± 3.0**	6 ± 0.8
Healthy volunteers ( <i>n</i> = 5, mean ± SD)	2/3	64.7 ± 12.3	NA	NA	NA	NA	NA	NA

\*\**P* < 0.01, Mann–Whitney U-test.

## <sup>11</sup>C-CNS 5161 positron emission tomography procedure

Patients with Parkinson's disease and healthy volunteers were scanned at the Cyclotron Building, Hammersmith Hospital.

Each patient had <sup>11</sup>C-CNS 5161 PET twice, once in a practically defined 'OFF' state following a 12-h withdrawal of medication and again, on a separate day, in an 'ON' state, 1 h after taking a standard oral 200 mg dose of levodopa. Healthy controls were only scanned once without levodopa. All subjects received ~444 Mbq of <sup>11</sup>C-CNS 5161 injected intravenously, followed by a 90 min dynamic emission scan. <sup>11</sup>C-CNS 5161 was manufactured by Hammersmith Imanet, GE Healthcare.

We used a Siemens ECAT EXACT HR+ scanner with an axial field of view of 15.5 cm. Sixty-three transaxial image planes were acquired as 2.46 mm slices with a reconstructed axial resolution of 5.4 mm and a transaxial resolution of 5.6 mm (Brix *et al.*, 1997). A 10 min transmission scan was acquired before every emission scan using a single rotating external photon point source of <sup>137</sup>Cs for subsequent attenuation and scatter corrections. Dynamic scans were acquired in 3D mode. PET data were corrected for attenuation, detector efficiency and scatter and reconstructed into tomographic images using filtered back projection.

All subjects had radial artery cannulation. Continuous sampling of blood activity was performed for 15 min with an online detector and then discrete blood samples for well counting were taken at baseline, 5, 10, 15, 20, 30, 40, 50, 60, 75 and 90 min. An aliquot of each discrete sample was rapidly centrifuged to obtain corresponding plasma and radioactivity concentrations that were measured in a NaI(Tl) well counter for blood and plasma separately. The continuous blood counts were corrected using the plasma/blood ratio to derive a plasma input function. The plasma was further analysed for radiolabelled metabolites using high-performance liquid chromatography and the plasma input function was corrected accordingly to produce a final parent radioligand input function to be used for quantification.

All subjects underwent volumetric T<sub>1</sub>-weighted MRI to allow PET coregistration and anatomical regional sampling, and T<sub>2</sub>-weighted MRI to exclude structural lesions.

## <sup>11</sup>C-CNS 5161 positron emission tomography analysis

### Frame-by-frame realignment procedure

A *post hoc* frame-by-frame realignment procedure was applied to compensate for head movement in the scanner. To reduce the

influence of redistribution of radiotracer producing erroneous realignments (Dagher *et al.*, 1998) non-attenuation corrected images were used. Non-attenuation corrected images are considered to be more useful for the realignment algorithm because these images include a significant scalp signal compared with attenuation corrected images. The non-attenuation corrected images were denoised using a level 2, order 64 Battle Lemarie wavelet filter (Turkheimer *et al.*, 1999). Frames were realigned to an early single, 'reference' frame that had a high signal-to-noise ratio, using a mutual information algorithm (Studholme *et al.*, 1997) and the transformation parameters were then applied to the corresponding attenuation corrected dynamic images.

### Regions of interest

The following regions of interest were investigated: caudate, putamen, precentral gyrus, occipital cortex, brainstem and cerebellum. Regions of interest were defined using a maximum probability atlas in Montreal Neurological Institute (MNI) space based on information from 30 subjects (Hammers *et al.*, 2003). Regions of interest were then transformed into the space of the individual PET images according to the following procedure. Individual MRI images were resliced (1 × 1 × 1 mm<sup>3</sup>) and coregistered to the corresponding subject's summed PET image using a normalized mutual information method which implements a rigid body (six parameters) transformation using SPM5 ([www.fil.ion.ucl.ac.uk/spm](http://www.fil.ion.ucl.ac.uk/spm)). The MNI T<sub>1</sub> template, available in SPM package, was then spatially normalized to each of the coregistered individual MRI images and the deformation parameters applied to the maximum probability atlas to delineate the regions of interest on the individual coregistered MRIs. This procedure has high reliability for the regions used in this study (Heckemann *et al.*, 2006). The normalized atlas, which is defined in MNI coordinates, was then resliced into the dimensions of the PET images and segmented to sample the radioactivity from grey matter alone.

### Data quantification

Rank-shaping regularization of exponential spectral analysis (Turkheimer *et al.*, 2003) together with the metabolite-corrected plasma input function was used to quantify <sup>11</sup>C-CNS 5161 binding in brain tissue relative to the radioligand concentration in arterial plasma by producing regional estimates of the total tracer volume of distribution (V<sub>T</sub>). Rank-Shaping regularization of exponential spectral analysis was the method of quantification of choice because of the slow wash-out of <sup>11</sup>C-CNS 5161 that, even 90 min from injection, renders the quantification through traditional compartmental models too susceptible to measurement of noise and therefore unreliable. Rank-Shaping regularization of exponential spectral analysis is a

development of the original exponential spectral analysis approach (Cunningham *et al.*, 1993; Turkheimer *et al.*, 1994) that does not require a predefined compartmental structure for the target region and produces reliable estimates of volumes of distribution for both plasma and reference modelling with significant levels of measurement noise (Turkheimer *et al.*, 1994, 2007).

## Statistical analysis

The left–right averaged  $^{11}\text{C}$ -CNS 5161  $V_T$  values in caudate, putamen, precentral gyrus, occipital cortex and cerebellum were used for statistical analysis. Statistical analyses of clinical data were performed with InStat3 for Macintosh (University of Medicine and Dentistry, NJ, USA).

We used non-parametric ANOVA (Kruskal–Wallis test) to assess the differences in PET measurements among groups. The non-parametric unpaired Mann–Whitney U-test was used to compare PET measurements between patients with Parkinson's disease-NLID and Parkinson's disease-LID in both 'ON' and 'OFF' conditions. Finally, the paired Wilcoxon test was used to assess significant changes in PET measurements following levodopa administration within each group.

## Results

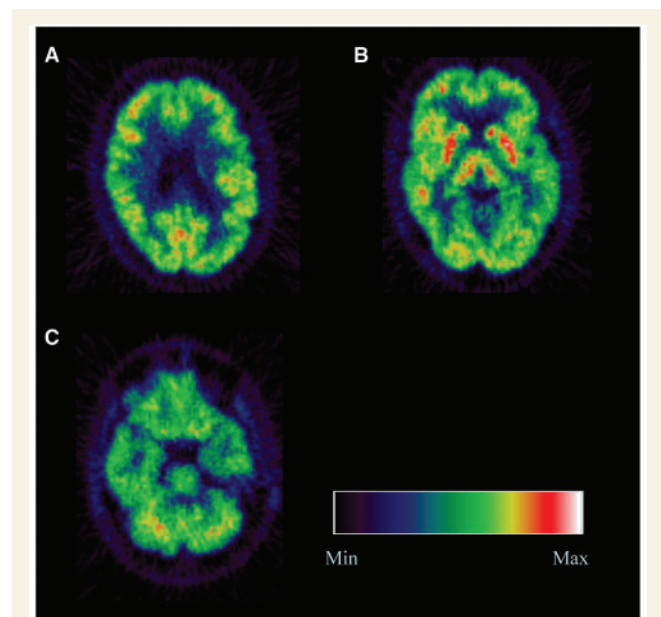
Figure 1 shows PET images of  $^{11}\text{C}$ -CNS 5161 uptake from a healthy volunteer. Seven patients with Parkinson's disease-NLID had  $^{11}\text{C}$ -CNS 5161 PET twice—once after and once withdrawn from levodopa. The remaining three patients with Parkinson's disease-NLID only had the 'OFF' medication scan, as they withdrew their consent after the first PET session. The eight patients with Parkinson's disease-LID were scanned twice, once with and once without levodopa, and they all developed involuntary movements following levodopa administration on the day of the 'ON' condition PET scan. In six of these patients the dyskinesia severity was mild and did not interfere with the scanning procedure. The remaining two patients had moderate dyskinesias with significant head movement. The same two patients also had significant head movement during the 'OFF' condition scan. None of the images of these two patients could be analysed.

Regional mean  $^{11}\text{C}$ -CNS 5161  $V_T$  values in controls, patients with Parkinson's disease-NLID and patients with Parkinson's disease-LID are shown in Table 2 and Fig. 2. Table 3 shows

regional mean  $^{11}\text{C}$ -CNS 5161  $V_T$  values in the seven patients with Parkinson's disease-NLID and six patients with Parkinson's disease-LID who were scanned in both 'ON' and 'OFF' states.

In the 'OFF' medication state, patients with Parkinson's disease-NLID and Parkinson's disease-LID had similar  $^{11}\text{C}$ -CNS 5161  $V_T$  values in all the examined regions. Kruskal–Wallis one-way analysis of variance showed no statistically significant effect of group (controls, Parkinson's disease-NLID and patients with Parkinson's disease-LID in the 'OFF' medication state) on  $^{11}\text{C}$ -CNS 5161  $V_T$  values for caudate, putamen, precentral gyrus, occipital cortex, brainstem and cerebellum.

Following levodopa, the patients with Parkinson's disease-NLID showed a significant decrease in  $^{11}\text{C}$ -CNS 5161  $V_T$  values in caudate ( $P = 0.02$ ), putamen ( $P = 0.02$ ) and precentral gyrus ( $P = 0.03$ ), whereas patients with Parkinson's disease-LID showed a significant increase in  $V_T$  values compared to baseline in putamen ( $P = 0.03$ )



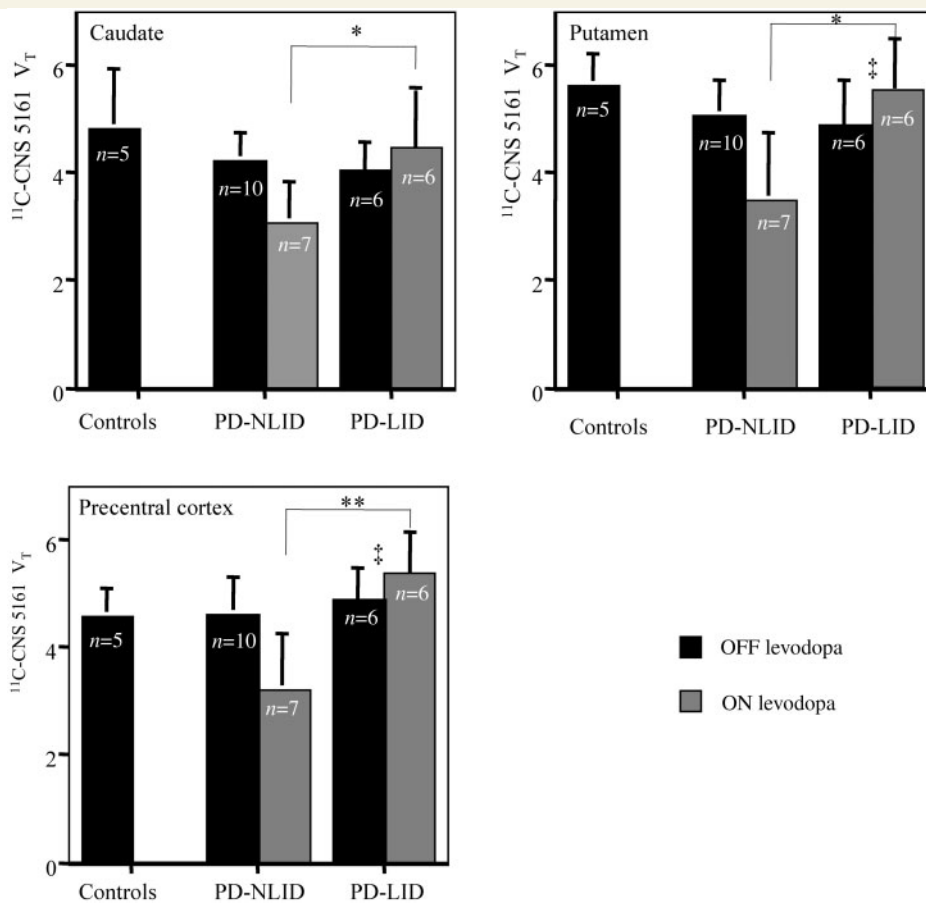
**Figure 1** PET images of  $^{11}\text{C}$ -CNS 5161 uptake from a healthy volunteer, showing cortical (A), basal ganglia (B), and cerebellar (C) uptake.

**Table 2**  $^{11}\text{C}$ -CNS 5161 volume of distribution ( $V_T$ )

Region	Healthy volunteers	PD-NLID patients		PD-LID patients		Mann–Whitney U-test	
		OFF L-dopa (n = 10)	ON L-dopa (n = 7)	OFF L-dopa (n = 6)	ON L-dopa (n = 6)	OFF L-dopa PD-NLID versus PD-LID	ON L-dopa PD-NLID versus PD-LID
Caudate (mean $\pm$ SD)	4.82 $\pm$ 1.10	4.21 $\pm$ 0.55	3.06 $\pm$ 0.77	4.1 $\pm$ 0.94	4.44 $\pm$ 1.13	NS	$P = 0.022$
Putamen (mean $\pm$ SD)	5.62 $\pm$ 0.60	5.05 $\pm$ 0.67	3.49 $\pm$ 1.26	4.98 $\pm$ 1.1	5.49 $\pm$ 1.0	NS	$P = 0.022$
Precentral cortex (mean $\pm$ SD)	4.57 $\pm$ 0.51	4.60 $\pm$ 0.71	3.19 $\pm$ 1.05	4.80 $\pm$ 0.8	5.37 $\pm$ 0.84	NS	$P = 0.005$
Occipital cortex (mean $\pm$ SD)	5.22 $\pm$ 0.74	4.27 $\pm$ 1.0	3.32 $\pm$ 1.56	4.98 $\pm$ 1.02	4.96 $\pm$ 0.82	NS	NS
Brainstem (mean $\pm$ SD)	3.32 $\pm$ 1.29	3.24 $\pm$ 0.71	3.37 $\pm$ 1.63	3.87 $\pm$ 0.73	3.91 $\pm$ 0.64	NS	NS
Cerebellum (mean $\pm$ SD)	4.85 $\pm$ 0.67	4.37 $\pm$ 1.89	3.62 $\pm$ 1.89	5.80 $\pm$ 1.60	5.20 $\pm$ 1.03	NS	NS

PD = Parkinson's disease; NS = not significant.





**Figure 2** Regional <sup>11</sup>C-CNS 5161 volume of distribution (V<sub>T</sub>) values (mean ± SD) 'OFF' and 'ON' levodopa in controls, patients with Parkinson's disease without dyskinesias (PD-NLID) and patients with Parkinson's disease with dyskinesias (PD-LID). *n* = the number of subjects in each group. \*\**P* < 0.01; \**P* < 0.05; Mann–Whitney U-test; ‡*P* < 0.05 paired Wilcoxon test.

**Table 3** <sup>11</sup>C-CNS 5161 volume of distribution (V<sub>T</sub>) results of within group comparison between OFF and ON conditions, Wilcoxon matched-pairs signed-ranks test

Region	Parkinson's disease-NLID patients		Parkinson's disease-LID patients	
	OFF L-dopa (n = 7)	ON L-dopa (n = 7)	OFF L-dopa (n = 6)	ON L-dopa (n = 6)
Caudate (mean ± SD)	4.07 ± 0.50	3.06 ± 0.77*	4.1 ± 0.94	4.44 ± 1.13
Putamen (mean ± SD)	5.0 ± 0.73	3.49 ± 1.26*	4.98 ± 1.1	5.49 ± 1.0*
Precentral cortex (mean ± SD)	4.3 ± 0.71	3.19 ± 1.05*	4.80 ± 0.8	5.37 ± 0.84*
Occipital cortex (mean ± SD)	4.22 ± 1.0	3.32 ± 1.56	4.98 ± 1.02	4.96 ± 0.82
Brainstem (mean ± SD)	3.17 ± 0.77	3.37 ± 1.63	3.87 ± 0.73	3.91 ± 0.64
Cerebellum (mean ± SD)	4.09 ± 0.7	3.62 ± 1.89	5.80 ± 1.60	5.20 ± 1.03

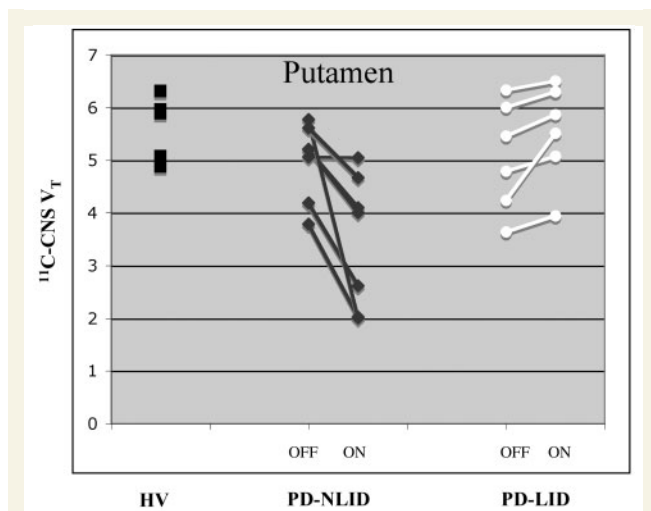
\**P* < 0.05.

and precentral gyrus (*P* = 0.03). In both groups of patients, levodopa administration did not result in any significant changes in <sup>11</sup>C-CNS 5161 V<sub>T</sub> in occipital cortex, brainstem and cerebellum (Table 3).

When comparing the 'ON' state between groups (Mann–Whitney U-test) (Table 2 and Fig. 2), patients with Parkinson's disease-LID had significantly higher mean <sup>11</sup>C-CNS 5161 V<sub>T</sub> for caudate, putamen and precentral gyrus relative to patients with Parkinson's disease-NLID. <sup>11</sup>C-CNS 5161 V<sub>T</sub> values for occipital

cortex, brainstem and cerebellum in the 'ON' state were not different between patients with Parkinson's disease-NLID and Parkinson's disease-LID. Figure 3 shows individual plots of putamen <sup>11</sup>C-CNS 5161 uptake in controls and in the two groups of patients with Parkinson's disease in both 'OFF' and 'ON' conditions.

No correlation was found between striatal <sup>11</sup>C-CNS 5161 V<sub>T</sub> values and disease severity both 'OFF' and 'ON' medication, as measured by the UPDRS ('OFF' medication, caudate: *P* = 0.2,



**Figure 3** Individual plots of putamenal <sup>11</sup>C-CNS 5161 volume of distribution ( $V_T$ ) values in healthy volunteers (HV) and in the two groups of Parkinson's disease patients in both 'OFF' and 'ON' conditions. PD-NLID = patients with Parkinson's disease without dyskinesias; PD-LID = patients with Parkinson's disease with dyskinesias.

$r = 0.31$  and putamen:  $P = 0.6$ ,  $r = 0.14$ ; 'ON' medication, caudate:  $P = 0.1$ ,  $r = 0.53$  and putamen:  $P = 0.1$ ,  $r = 0.52$ ). Similarly, we found no correlation between striatal <sup>11</sup>C-CNS 5161  $V_T$  values both 'OFF' and 'ON' medication and levodopa equivalent units ('OFF' medication, caudate:  $P = 0.2$ ,  $r = 0.31$  and putamen:  $P = 0.2$ ,  $r = 0.31$ ; 'ON' medication, caudate:  $P = 0.2$ ,  $r = 0.39$  and putamen:  $P = 0.3$ ,  $r = 0.33$ ).

## Discussion

This is the first *in vivo* study to report glutamatergic activity in patients with Parkinson's disease with and without LIDs. We found that levodopa administration acted to depress <sup>11</sup>C-CNS 5161 uptake, a use-dependent marker of NMDA ion channel activation, in the caudate, putamen and motor cortex (precentral gyrus) of non-dyskinetic patients with Parkinson's disease. This was not true, however, of dyskinetic patients with Parkinson's disease suggesting that they had relatively enhanced glutamate receptor activity in motor areas. Levodopa administration did not result in any significant changes in <sup>11</sup>C-CNS 5161 uptake in non-motor occipital cortex, brainstem and cerebellum of Parkinson's disease cases.

The NMDA receptor is a voltage-gated ion channel. When glutamate molecules bind to their binding sites on the extracellular part of the receptor, they trigger opening of the ion channel with subsequent influx of calcium into the cell. Non-competitive glutamate antagonists such as MK801, Phencyclidine and Thienylphencyclidine bind selectively to specific intrachannel sites in the open NMDA channel (Foster and Wong, 1987). CNS 5161 binding sites are also located inside the ion channel. In fact, binding of tritium labelled CNS 5161 in rat brain is increased by more than 2-fold in the presence of glutamate and glycine, a required

cofactor of NMDA receptor activation (Biegon *et al.*, 2007). It is, therefore, likely that <sup>11</sup>C-CNS 5161, the PET ligand used in our study, binds in a use-dependent fashion to the activated (open) NMDA receptor channel and that its distribution reflects channel function.

If <sup>11</sup>C-CNS 5161 uptake reflects levels of NMDA receptor activation, our PET findings would suggest that dyskinetic and non-dyskinetic patients in an 'OFF' medication state have similar levels of NMDA receptor activity, which are comparable to those observed in healthy volunteers. However, acute administration of levodopa resulted in differential glutamatergic transmission in the two groups; whilst NLID-patients with Parkinson's disease showed a reduction of <sup>11</sup>C-CNS 5161 uptake in both striatal and cortical areas, LID-patients with Parkinson's disease showed a relative increase from baseline of tracer uptake in the same areas, suggesting that NMDA receptor function following levodopa administration is inhibited in the former but not in the latter.

The reduced striatal NMDA receptor activity after levodopa in NLID-patients with Parkinson's disease might result from dopamine D2 receptor stimulation, as these sites are expressed on the terminals of corticostriatal projections and act to reduce glutamate release and dampen striatal excitation (Bamford *et al.*, 2004; Surmeier *et al.* 2007).

Dopamine D2 receptor stimulation could also be responsible for the reduced NMDA activity seen in precentral cortex. Indeed, D2 receptors depress glutamate responses in hippocampal neurons (Kotecha *et al.*, 2002), and D2 inhibitory action on prefrontal cortical pyramidal cell excitability has been observed in immature animals (Gulledge and Jaffe, 1998).

In contrast, an NMDA activity increase from baseline, as seen in the dyskinetic patients, may reflect the uncoupling of hyperphosphorylated NMDA receptors from D2 receptor influence; these are reported to be present in parkinsonian animals with dyskinesias (Chase *et al.*, 2000).

According to the classical model of the basal ganglia function, increased glutamatergic transmission generates LIDs by inducing increased activity in the direct striatopallidal pathway, with resultant loss of normal inhibitory output to thalamocortical pathways (Calabresi *et al.*, 2000).

Results of animal model studies also indicate that increased striatal glutamatergic activity is implicated in the development and maintenance of LIDs (Oh *et al.*, 1998; Chase *et al.*, 2000; Calon *et al.*, 2002; Nash *et al.*, 2002; Robelet *et al.*, 2004). Raised levels of extracellular glutamate and increased expression of the glial glutamate transporter GLT1 have been observed in the striatum and substantia nigra of dyskinetic 6-hydroxydopamine lesioned rats after chronic levodopa treatment (Robelet *et al.*, 2004). Dyskinesias have also been associated with increased phosphorylation of striatal NMDA receptor subunits in lesioned rats (Chase *et al.*, 2000) and with NMDA and AMPA receptor upregulation in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine lesioned monkeys (Calon *et al.*, 2002).

In this study, we did not find any significant increase in <sup>11</sup>C-CNS 5161 uptake in our patients with Parkinson's disease compared to the control subjects. However, in the 'ON' state, the patients with Parkinson's disease-LID had significantly higher mean <sup>11</sup>C-CNS

5161  $V_T$  in the caudate, putamen and precentral gyrus than patients with Parkinson's disease-NLID. Our findings therefore provide further supportive evidence for a role of relatively raised glutamatergic transmission in dyskinetic compared to non-dyskinetic Parkinson's disease.

Since our LID-patients with Parkinson's disease had more severe UPDRS scores than NLID-patients with Parkinson's disease, it could be argued that our findings could simply reflect differences in Parkinson's disease severity. However, the two groups were at a similar Hoehn and Yahr stage. In the 'OFF' medication state, they had similar  $^{11}\text{C}$ -CNS 5161  $V_T$  values in all the examined regions and they were not different from healthy volunteers. Additionally, no correlation was found between UPDRS scores and striatal  $^{11}\text{C}$ -CNS 5161 uptake. It is therefore, unlikely that the difference in non-medicated UPDRS scores is the sole explanation for the differences in  $^{11}\text{C}$ -CNS 5161  $V_T$  values seen after administration of levodopa to the dyskinetic and non-dyskinetic Parkinson's disease groups.

Our findings rationalize the use of blockers of glutamate transmission to improve LIDs in Parkinson's disease. The potential anti-dyskinetic effect of glutamate antagonists is well recognized. Clinical studies have demonstrated the efficacy of low affinity NMDA antagonists such as amantadine and dextromethorphan for alleviating motor fluctuations and peak dose dyskinesias in Parkinsonian patients (Verhagen-Metman *et al.*, 1998a, b; Del Dotto *et al.*, 2001). In these studies, the introduction of adjunct amantadine or dextromethorphan resulted in significant 40–60% reductions in LIDs without exacerbating the underlying parkinsonian symptoms.

Other non-selective glutamate antagonists, including memantine, remacemide and the glutamate release inhibitor riluzole, have failed to show clear efficacy in reducing LID when used in Parkinson's disease (Merello *et al.*, 1999; Parkinson Study Group, 2001; Braz *et al.*, 2004). The reason for lack of efficacy of these compounds remains to be clarified. A possible explanation, however, is differences in subtype specificity. Changes in expression of both AMPA and metabotropic subtypes of glutamate receptors have been reported in patients with Parkinson's disease (Calon *et al.*, 2003; Samadi *et al.*, 2009). It is possible that combined blockade of specific subtypes of the glutamate receptor is required to achieve a significant anti-dyskinetic effect. This view is supported by the observation that simultaneous AMPA and NMDA blockade in rodent and primate models of Parkinson's disease provides a greater reduction of LID than inhibition of either of these receptors alone (Bibbiani *et al.*, 2005).

Since  $^{11}\text{C}$ -CNS 5161 is a selective antagonist of the NMDA receptor, our study does not provide any direct information about the functional role of other glutamate receptor subtypes. It is likely, however, that other glutamate receptor subtypes become sensitized to levodopa. Novel PET ligands are now available as markers of metabotropic glutamate receptor type 5 function and ligands for AMPA receptor are being developed. Further *in vivo* investigation into the role of different subtypes of glutamate receptor is crucial and it will be essential in the development of new strategies for the pharmacological treatment of dyskinesias in patients with Parkinson's disease.

## Acknowledgements

The authors wish to thank Hope McDevitt, Andreanna Williams, James Anscombe and Andrew Blyth for their help with scanning and the patients who kindly agreed to take part into the study.

## Funding

The study was supported by the Parkinson's Disease Society, UK.

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