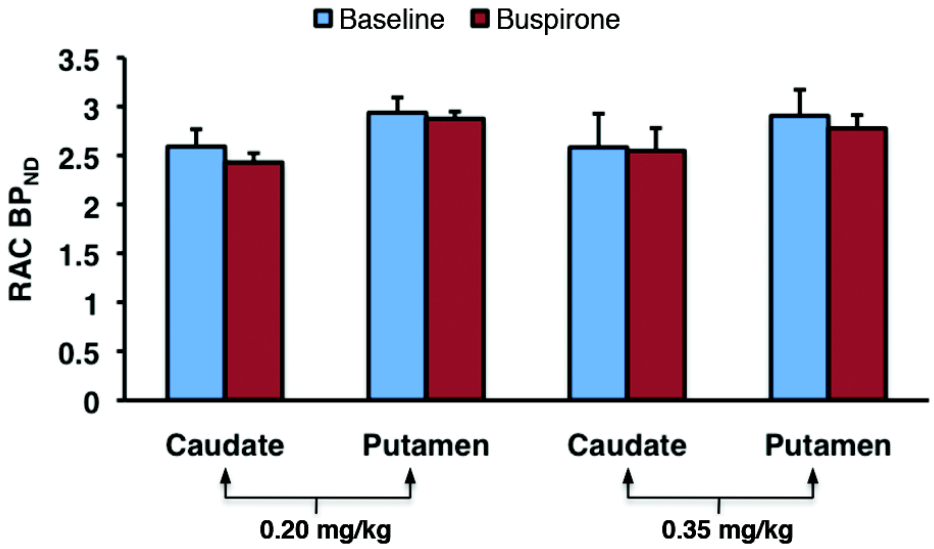
## Supplemental Materials for Politis *et al*
## "The role of serotonergic mechanisms in Parkinson's disease dyskinesias"

### SUPPLEMENTAL RESULTS

**Supplemental Table 1** Clinical characteristics of the two normal control subgroups that underwent the dose defining study with 0.20 mg/kg and 0.35 mg/kg bolus dose of the 5-$HT_{1A}$ agonist buspirone

| | **0.20 mg/kg buspirone** | **0.35 mg/kg buspirone** |
|---|---|---|
| Subjects | 6 | 6 |
| Sex | 5M / 1F | 5M / 1F |
| Age (years ± SD) | 62.85±6.06 | 63.77±8.40 |
| Weight (Kg ± SD) | 92.0 ± 14.47 | 92.9 ± 25.53 |
| BMI (units ± SD) | 28.78± 4.57 | 29.52± 6.64 |
| MMSE (mean ± SD) | 29.17 ± 0.75 | 29.67 ± 0.52 |
| BDI-II (mean ± SD) | 2.83 ± 2.14 | 3.33 ± 3.14 |
| HRSD (mean ± SD) | 2.5 ± 1.87 | 2.66 ± 3.50 |




**Supplemental Figure 1** $^{11}$C-raclopride (RAC) PET trial with buspirone in normal controls. No significant differences in caudate and putamen RAC non-displaceable binding potential ($BP_{ND}$) mean values in normal control subjects between baseline (blue bars) and following (red bars) low (0.20 mg/kg) and high bolus doses (0.35 mg/kg) of buspirone. Data represent mean + SD.

**Supplemental Table 2** Caudate and putamen $^{11}$C-raclopride $BP_{ND}$ mean values in a group of six normal controls at baseline and following 0.20 mg/kg dose of buspirone

| | **Caudate** | **Putamen** |
|---|---|---|
| OFF medication[a] | 2.59 ± 0.18 | 2.94 ± 0.16 |
| 0.20 mg/kg buspirone[a] | 2.43 ± 0.09 | 2.88 ± 0.07 |
| OFF medication vs 0.20 mg/kg buspirone[b] | NS[c] | NS |

[a]Data represent mean ± SD
[b]Paired t tests, two-tail *p* values
[c]NS = Not Significant

**Supplemental Table 3** Caudate and putamen $^{11}$C-raclopride $BP_{ND}$ mean values in a group of six normal controls at baseline and following 0.35 mg/kg dose of buspirone

| | Caudate | Putamen |
|---|---|---|
| OFF medication[a] | 2.58 ± 0.34 | 2.91 ± 0.27 |
| 0.35 mg/kg buspirone[a] | 2.55 ± 0.23 | 2.78 ± 0.13 |
| OFF medication vs 0.35 mg/kg buspirone[b] | NS[c] | NS |

[a]Data represent mean ± SD
[b]Paired t tests, two-tail *p* values
[c]NS = Not Significant

**Supplemental Table 4** Side-effect profile after administration of 0.20 mg/kg and 0.35 mg/kg of buspirone in groups of normal control subjects

| SIDE-EFFECT | No of Subjects | | |
|---|---|---|---|
| | 0.20 mg/kg buspirone | 0.35 mg/kg buspirone | Total |
| Drowsy | 3 | 1 | 4 |
| Dizziness | 1 | 0 | 1 |
| Nausea | 1 | 0 | 1 |
| Vomited | 1 | 0 | 1 |
| Lightheaded | 0 | 2 | 2 |
| None | 3 | 3 | 6 |

**Supplemental Table 5** Caudate and putamen $^{11}$C-DASB $BP_{ND}$ mean values in a group of 12 Parkinson's disease patients with stable response to levodopa (PD controls) and 24 with levodopa-induced dyskinesias (PD LIDs).

| | Caudate | Putamen |
|---|---|---|
| PD stable[a] | 0.95 ± 0.18 | 1.06 ± 0.14 |
| PD LIDs[a] | 0.86 ± 0.14 | 0.95 ± 0.19 |
| PD stable vs PD LIDs[b] | NS[c] | NS |

[a]Data represent mean ± SD
[b]Unpaired t tests, two-tail *p* values
[c]NS = Not Significant

**Supplemental Table 6** Caudate and putamen $^{11}$C-raclopride $BP_{ND}$ mean values in a group of 12 Parkinson's disease patients with stable response to levodopa (PD stable) OFF medication, following levodopa, and following levodopa preceded by buspirone.

| | Caudate | Putamen |
|---|---|---|
| OFF medication[a] | 2.32 ± 0.43 | 3.01 ± 0.41 |
| levodopa[a, b] | 2.16 ± 0.33 (6%) | 2.75 ± 0.31 (8%) |
| levodopa + buspirone[a, b] | 2.13 ± 0.34 (8%) | 2.69 ± 0.36 (10%) |
| $F_{(DFn, DFd)}$; *p* values[c] | $F_{(1.485,16.34)} = 19.89$; $p < 0.001$ | $F_{(1.713,18.84)} = 12.59$; $p < 0.001$ |
| OFF medication vs levodopa[d] | $p < 0.01$ | $p < 0.01$ |
| OFF medication vs levodopa + buspirone[d] | $p < 0.001$ | $p < 0.01$ |
| levodopa vs levodopa + buspirone[d] | NS[e] | NS |

[a]Data represent mean ± SD
[b]% change from baseline (OFF medication)
[c]Repeated-measures ANOVA, with the Greenhouse-Geisser correction (*F* and *p* values)
[d]*p* values following Bonferroni's multiple comparisons test
[e]NS = Not Significant

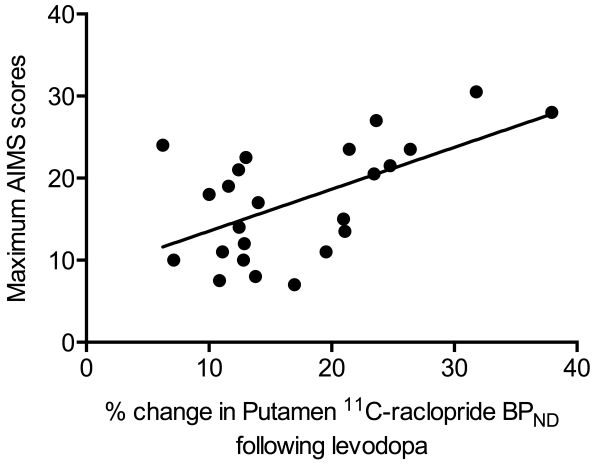
**Supplemental Table 7** Caudate and putamen $^{11}$C-raclopride $BP_{ND}$ mean values in a group of 24 Parkinson's disease patients with levodopa-induced dyskinesias (PD LIDs) OFF medication, following levodopa, and following levodopa and buspirone.

| | Caudate | Putamen |
|---|---|---|
| OFF medication[a] | 2.10 ± 0.30 | 2.98 ± 0.36 |
| levodopa[a, b] | 1.83 ± 0.27 (13%) | 2.45 ± 0.32 (17%) |
| levodopa + buspirone[a, b] | 1.91 ± 0.25 (9%) | 2.62 ± 0.22 (11%) |
| $F_{(DFn, DFd)}$; $p$ values[c] | $F_{(1.903, 43.77)} = 44.35$; $p < 0.001$ | $F_{(1.880, 43.24)} = 44.20$; $p < 0.001$ |
| OFF medication vs levodopa[d] | $p < 0.001$ | $p < 0.001$ |
| OFF medication vs levodopa + buspirone[d] | $p < 0.001$ | $p < 0.001$ |
| levodopa vs levodopa + buspirone[d] | $p < 0.05$ | $p < 0.05$ |

[a]Data represent mean ± SD
[b]% change from baseline (OFF medication)
[c]Repeated-measures ANOVA, with the Greenhouse-Geisser correction ($F$ and $p$ values)
[d]$p$ values following Bonferroni's multiple comparisons test

**Supplemental Table 8** Side-effect profile after administration of 0.35 mg/kg of buspirone in Parkinson's disease patients with mild-moderate (MM) and moderate-severe (MS) levodopa-induced dyskinesias (PD LIDs)

| SIDE-EFFECT | No of Subjects | | | |
|---|---|---|---|---|
| | PD stable | PD MM LIDs | PD MS LIDs | TOTAL |
| Drowsy | 1 | 5 | 3 | 10 |
| Dizziness | 1 | 2 | 1 | 4 |
| Nausea | 1 | 0 | 0 | 1 |
| Vomited | 1 | 0 | 0 | 1 |
| Lightheaded | 1 | 0 | 0 | 1 |
| Excessive Sweating | 1 | 0 | 0 | 1 |
| Stomach Upset | 1 | 0 | 0 | 1 |
| Confusion | 0 | 1 | 1 | 2 |
| None | 8 | 6 | 8 | 22 |




**Supplemental Figure 2** Higher percentage (%) reductions in putamen $^{11}$C-raclopride $BP_{ND}$ correlate with higher maximum abnormal involuntary movement scale (aims) scores ($r = 0.58$; $p < 0.01$) following administration of levodopa in the group of parkinson's disease patients with levodopa-induced dyskinesias.

**Supplemental Table 9** Caudate and putamen $^{11}$C-DASB $BP_{ND}$ mean values in a group of 12 normal controls, 12 Parkinson's disease patients with stable response to levodopa (PD controls), 12 with mild-moderate levodopa-induced dyskinesias (PD MM LIDs), and 12 with moderate-severe levodopa-induced dyskinesias (PD MS LIDs).

| | **Caudate** | **Putamen** |
|---|---|---|
| Normal Controls[a] | 1.36 ± 0.13 | 1.36 ± 0.11 |
| PD stable[a] | 0.95 ± 0.18 | 1.06 ± 0.14 |
| PD MM LIDs[a] | 0.92 ± 0.09 | 1.02 ± 0.12 |
| PD MS LIDs[a] | 0.79 ± 0.15 | 0.89 ± 0.22 |
| $F_{(DFn, DFd)}$; $p$ values[b] | $F_{(3, 44)} = 35.60$; $p < 0.001$ | $F_{(3, 44)} = 19.92$; $p < 0.001$ |
| Normal Controls vs PD stable[c] | $p < 0.001$ | $p < 0.001$ |
| Normal Controls vs PD MM LIDs[c] | $p < 0.001$ | $p < 0.001$ |
| Normal Controls vs PD MS LIDs[c] | $p < 0.001$ | $p < 0.001$ |
| PD stable vs PD MM LIDs[c] | NS[d] | NS |
| PD stable vs PD MS LIDs[c] | NS | NS |
| PD MM LIDs vs PD MS LIDs[c] | NS | NS |

[a]Data represent mean ± SD
[b]One-way ANOVA, with Brown-Forsythe and Bartlett's tests ($F$ and $p$ values)
[c]$p$ values following Bonferroni's multiple comparisons test
[d]NS = Not Significant

**Supplemental Table 10** Caudate and putamen $^{11}$C-raclopride $BP_{ND}$ mean values in a group of 12 Parkinson's disease patients with mild-moderate levodopa-induced dyskinesias (PD MM LIDs) OFF medication, following levodopa, and following levodopa and buspirone.

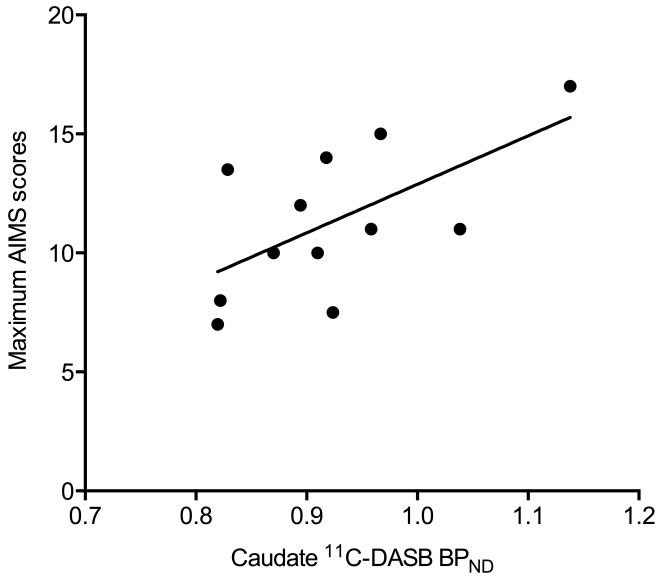
| | **Caudate** | **Putamen** |
|---|---|---|
| OFF medication[a] | 2.01 ± 0.16 | 2.80 ± 0.23 |
| levodopa[a, b] | 1.77 ± 0.15 (12%) | 2.36 ± 0.20 (15%) |
| levodopa + buspirone[a, b] | 1.89 ± 0.11 (6%) | 2.60 ± 0.20 (7%) |
| $F_{(DFn, DFd)}$; $p$ values[c] | $F_{(1.592, 17.51)} = 22.50$; $p < 0.001$ | $F_{(1.752, 19.27)} = 56.02$; $p < 0.001$ |
| OFF medication vs levodopa[d] | $p < 0.001$ | $p < 0.001$ |
| OFF medication vs levodopa + buspirone[d] | $p < 0.01$ | $p < 0.001$ |
| levodopa vs levodopa + buspirone[d] | $p < 0.01$ | $p < 0.001$ |

[a]Data represent mean ± SD
[b]% change from baseline (OFF medication)
[c]Repeated-measures ANOVA, with the Greenhouse-Geisser correction ($F$ and $p$ values)
[d]$p$ values following Bonferroni's multiple comparisons test

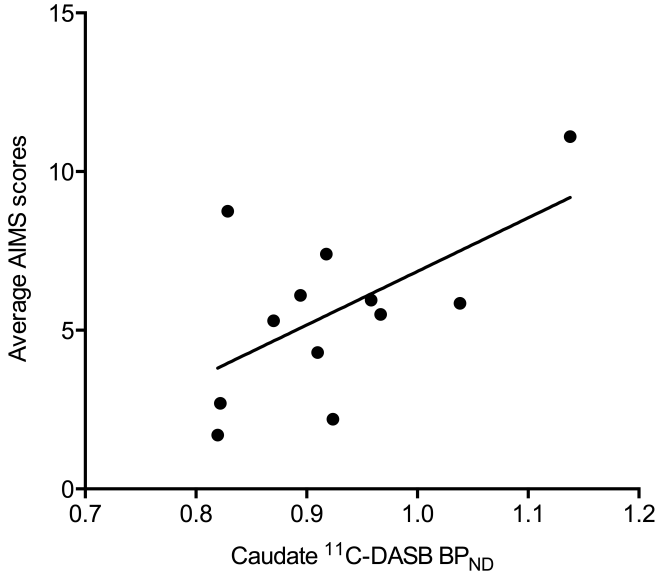
**Supplemental Table 11** Caudate and putamen $^{11}$C-raclopride $BP_{ND}$ mean values in a group of 12 Parkinson's disease patients with moderate-severe levodopa-induced dyskinesias (PD MS LIDs) OFF medication, following levodopa, and following levodopa and buspirone.

| | **Caudate** | **Putamen** |
|---|---|---|
| OFF medication[a] | 2.19 ± 0.38 | 3.15 ± 0.39 |
| levodopa[a, b] | 1.88 ± 0.36 (14%) | 2.54 ± 0.40 (19%) |
| levodopa + buspirone[a, b] | 1.93 ± 0.34 (12%) | 2.63 ± 0.24 (16%) |
| $F_{(DFn, DFd)}$; $p$ values[c] | $F_{(1.921, 21.13)} = 28.25$; $p < 0.001$ | $F_{(1.947, 21.42)} = 23.03$; $p < 0.001$ |
| OFF medication vs levodopa[d] | $p < 0.001$ | $p < 0.001$ |
| OFF medication vs levodopa + buspirone[d] | $p < 0.001$ | $p < 0.01$ |
| levodopa vs levodopa + buspirone[d] | NS[e] | NS |

[a]Data represent mean ± SD; [b]% change from baseline (OFF medication); [c]Repeated-measures ANOVA, with the Greenhouse-Geisser correction (*F* and *p* values) [d]*p* values following Bonferroni's multiple comparisons test; [e]NS = Not Significant




**Supplemental Figure 3** Higher caudate $^{11}$C-DASB non-displaceable binding potential ($BP_{ND}$) values correlated with higher maximum abnormal involuntary movement scale (aims) scores ($r = 0.61$, $p < 0.05$) during an 150 min period after levodopa administration.




**Supplemental Figure 4** Higher caudate $^{11}$C-DASB non-displaceable binding potential ($BP_{ND}$) values correlated with higher average abnormal involuntary movement scale (aims) scores ($r = 0.58$, $p < 0.05$) during an 150 min period after levodopa administration.

## SUPPLEMENTAL METHODS

### *Levodopa Equivalent Dose calculation formulas*

**Supplemental Table 12** Calculation of daily and lifetime dopaminergic medication equivalent dose based on theoretical equivalence to levodopa

| |
|---|
| **D or L – $LED_{TOTAL}$ (mg or g) =** (1 X levodopa) + (0.77 X levodopa CR) + (1.43 X levodopa + Entacapone) + (1.11 X levodopa CR + Entacapone) + (20 X Ropinirole) + (20 X Ropinirole ER) + (100 X Pramipexole) + (30 X Rotigotine) + (10 X Bromocriptine) + (8 X Apomorphine) + (100 X Pergolide) + (67 X Cabergoline) |

[a]Levodopa with carbidopa or benserazide hydrochloride; [b]In levodopa / carbidopa or benserazide hydrochloride, only levodopa is calculated

**Supplemental Table 13** Calculation of daily and lifetime levodopa equivalent dose

| |
|---|
| **D or L - $LED_{LEVODOPA}$ (mg or g) =** (1 X levodopa) + (0.77 X levodopa CR) + (1.43 X levodopa + Entacapone) + (1.11 X levodopa CR + Entacapone) |

[a]Levodopa with carbidopa or benserazide hydrochloride; [b]In levodopa / carbidopa or benserazide hydrochloride, only levodopa is calculated

**Supplemental Table 14** Calculation of daily and lifetime dopamine agonists equivalent dose

| |
|---|
| **D or L - $LED_{DAg}$ (mg or g) =** (20 X Ropinirole) + (20 X Ropinirole ER) + (100 X Pramipexole) + (30 X Rotigotine) + (10 X Bromocriptine) + (8 X Apomorphine) + (100 X Pergolide) + (67 X Cabergoline) |

### *Blood sample analysis*

*Genotyping*

Samples of 10ml EDTA-anticoagulated whole blood were taken venously and stored at -80°C until DNA extraction. Samples were only identified by a coded subject number and DNA isolation and genotyping was performed by investigators unaware of any subject information using standard methods (Autopure LS system, Gentra Systems). DNA yield was measured with UV at 260 nm and with the 260/280 ratio as a quality check. An MJ Research PTC-200 Pertier thrmocycler (Watertown) was used for all polymerase chain reaction (PCR) amplifications. The PCR reactions included 30 to 80 ng of genomic DNA, 1 U Taq polymerase (Qiagen), 0.3X corresponding Qiagen PCR buffer, 3.75 µg of sense and antisense primers, and 2.5mM dinucleoside triphosphate (dNTP) in a final volume of 50 µL. All PCR products were electrophoresed in a 3% MetaPhor agarose gel (Cambrex) in Tris-borate/ethylene-diaminetetraacetic acid buffer (TBE) and stained with ethidium bromide. Three variants of SLC6A4 gene were genotyped: 5-HTTLPR and rs25531in the promoter region, and 5-HTTVNTR in intron 2.

*5-HTTLPR and rs25531 polymorphism*

The following primer sequences designed for PCR amplification were: Forward: 5'-GGCGTTGCCGCTCTGAATGC-3' and Reverse. 5'-GAGGGACTGAGCTGGACAACCAC-3'. Samples were amplified on a PCR thermocycler with an initial denaturation step of 10 min at 94°C followed by 32 cycles consisting of denaturation for 30 s at 95°C, annealing for 30 s at 57°C and elongation for 30 s at 72°C and one final elongation step for 5 min at 72°C. This yields a "short" 486 bp and a "long" 529 bp fragment. The PCR products were digested for 12 h at 37°C with 0.1 µl MSP1 (New England Biolabs) and 1 µl buffer per sample. MSP1 recognizes and cuts a 5'-C/CGG-3' sequence resulting in the following fragments: 340 bp, 127 bp and 62 bp for the LA allele, 297 bp, 127 bp and 62 bp for the SA allele, 174, 166, 127 and 62 bp for the LG allele and 166, 131, 127 and 62 bp for the SG allele. Fragments were run on a polyacrylamide gel. All biallelic 5-HTTLPR genotypes were thus determined using two different protocols that yielded identical results.

*5-HTTVNTR polymorphism*

The 5-HTTVNTR has 3 alleles that are 250 bp, 267 bp, and 300 bp corresponding to 9, 10, and 12 repeats, respectively. The following primer pairs were modified from previous (1): 5'-GTCAGTATCACAGGCTGCGAG-3' and 5'-TGTTCCTAGTCTTACGCCAGTG-3'. The cycling conditions were 94°C for 5 minutes, 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 60 seconds followed by a final extension at 72°C for 10 minutes.

***PET methods: Movement correction***

Head movement was corrected using a frame-by-frame realignment procedure (2). Non–attenuated corrected (non-AC) images were used for realignment, to provide additional information by reducing the influence of redistribution of radiotracer producing erroneous realignments (3). The non-AC image was denoised using a level 2, order 64 Battle Lemarie wavelet filter (4). The denoised frames were then realigned using a mutual information algorithm (5), excluding the first three frames for $^{11}$C-raclopride and the first seven for $^{11}$C-DASB containing little information. Frames 10 for $^{11}$C-raclopride and 13 for $^{11}$C-DASB were chosen as the reference frames because they offer good signal-to-noise ratio. Frames 4-20 for $^{11}$C-raclopride and 8-28 for $^{11}$C-DASB of the original time series were then resliced and reassembled into a movement-corrected dynamic scan. Decay-corrected time–activity curves were derived and compared to those without movement correction. Amount and timing of any movement were assessed graphically and compared with intrascan records.

***PET methods: $^{11}$C-raclopride***

Region-of-interest (ROI) analysis was performed using ANALYZE medical imaging software (Mayo Foundation, Rochester, MN, USA; Version 8.0b). Coregistration and reslicing was performed using the Mutual Information Registration algorithm in the statistical parametric mapping (SPM; Version 5) software package (Wellcome Department of Cognitive Neuroscience, Institute of Neurology, London, UK) implemented in Matlab (The Mathworks Inc., Natick, MA, USA; Version 7.5).

Percentage (%) decreases of regional $^{11}$C-raclopride $BP_{ND}$ reflecting increases of DA release following a challenge with levodopa or a challenge with buspirone preceding the administration of levodopa compared to the practically defined OFF medication condition were calculated according to the following formulas:

*Percentage changes from the practically defined OFF medication phase of ROIs $^{11}$C-raclopride $BP_{ND}$ following administration of levodopa*

$$\%\Delta_{\text{11C-raclopride}} = \frac{\text{11C-raclopride BP}_{\text{ND}}\ (\text{OFF-levodopa})}{\text{11C-raclopride BP}_{\text{ND}}\ \text{OFF}} \times 100$$

*Percentage changes from the practically defined OFF medication phase of ROIs $^{11}$C-raclopride $BP_{ND}$ following administration of levodopa and buspirone*

$$\%\Delta_{\text{11C-raclopride}} = \frac{\text{11C-raclopride BP}_{\text{ND}}\ (\text{OFF-levodopa+buspirone})}{\text{11C-raclopride BP}_{\text{ND}}\ \text{OFF}} \times 100$$

***PET methods: $^{11}$C-DASB***

ROI analysis was performed using ANALYZE medical imaging software. Coregistration and reslicing was performed using the Mutual Information Registration algorithm in SPM5 software package implemented in Matlab7.5.

The $BP_{ND}$ was calculated as the ratio at equilibrium of specifically bound (striatal area) radioligand to that of nonspecifically bound (cerebellum; 6). Both cerebellar and striatal regions were sampled from frames 8-28 of the dynamic scan after scans were corrected for head movement.

***PET methods: ROIs***

ROIs were traced guided by the Talairach and Tournoux (7) stereotaxic atlas in combination with the Duvernoy (8) 3D sectional atlas on the individual coregistered MRIs and these were then used to sample the parametric PET images. For each patient, we calculated the averaged right and left ROIs. Anatomical borders were defined manually for: caudate, putamen and cerebellum which were defined in both hemispheres and were standardized for volume throughout subjects.

Anatomical borders were defined manually for:

(a) Cerebellum: On the axial section. The anterior border is defined by the inferior semilunar lobule, the posterior border and the lateral border is defined by the transverse sinus, and the medial border is defined by the cerebellar falx.

(b) Caudate nucleus: On the axial section. The anterior border is defined by the lateral ventricle, the posterior border is defined by the internal capsule, the medial border is defined by the lateral ventricle and fornix, and the lateral border is defined by the external capsule.

(c) Putamen: On the axial section. The anterior border is defined by the anterior limb of the internal capsule, the posterior border is defined by the posterior limb of the internal capsule, the lateral border is defined by the external capsule/claustrum, and the medial border is defined by the lamina medullaris lateralis.

ROIs were standardized for volume throughout subjects and were manually defined on both hemispheres for cerebellum (8100mm$^3$), caudate nucleus (1400mm$^3$), putamen (2100mm$^3$).

**SUPPLEMENTAL REFERENCES**


1. Li T, Holmes C, Sham PC, Vallada H, Birkett J, Kirov G, et al. Allelic functional variation of serotonin trans- porter expression is a susceptibility factor for late onset Alzheimer's disease. Neuroreport 1997; 8: 683–6.
2. Montgomery AJ, Thielemans K, Mehta MA, Turkheimer F, Mustafovic S, Grasby PM. Correction of head movement on PET studies: comparison of methods. J Nucl Med 2006; 47: 1936–44.
3. Dagher A, Gunn RN, Lockwood G, Cunningham VJ, Grasby PM, Brooks DJ. Measuring neurotransmitter release with PET: Methodological Issues. In: Carson RE, Daube-Witherspoon ME, Herscovitch P, editors. Quantitative Functional Brain Imaging with Positron Emission Tomography. London: Academic Press; 1998.
4. Turkheimer FE, Brett M, Visvikis D, Cunningham VJ. Multiresolution analysis of emission tomography images in the wavelet domain. J Cereb Blood Flow Metab 1999; 19: 1189–1208.
5. Studholme C, Hill DL, Hawkes DJ. Automated three-dimensional registration of magnetic resonance and positron emission tomography brain images by multiresolution optimization of voxel similarity measures. Med Phys 1997; 24: 25–35.
6. Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, et al. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J Cereb Blood Flow Metab 2007; 27: 1533–39.
7. Talairach J, Tournoux P. Co-planar stereotaxic atlas of the human brain. New York: Thieme;1988.
8. Duvernoy HM. The human brain: surface, blood supply, and three-dimensional sectional anatomy. New York: Springer-Verlag Wien; 1999.