Frontal, midbrain and striatal dopaminergic function in early and advanced Parkinson’s disease
A 3D [18F]dopa-PET study


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Summary
We have studied focal changes in dopaminergic function throughout the brain volume in early and advanced Parkinson’s disease by applying statistical parametric mapping (SPM) to 3D [18F]dopa-PET. Data from seven early hemi-Parkinson’s disease and seven advanced bilateral Parkinson’s disease patients were compared with that from 12 normal controls. Parametric images of [18F]dopa influx rate constant ($K_{io}$) were generated for each subject from dynamic 3D [18F]dopa datasets and transformed into standard stereotactic space. Significant changes in mean voxel [18F]dopa $K_{io}$ values between the normal control group and each Parkinson’s disease group were localized with SPM. Conventional region of interest analysis was also applied to comparable regions on the untransformed image datasets. In early left hemi-Parkinson’s disease, significant extrastriatal increases in [18F]dopa $K_{io}$ were observed in the left anterior cingulate gyrus and the dorsal midbrain region ($P < 0.05$, corrected) along with decreases in striatal [18F]dopa $K_{io}$. In advanced Parkinson’s disease, significant extrastriatal decreases in [18F]dopa $K_{io}$ were observed in the ventral and dorsal midbrain regions ($P < 0.05$, corrected). No significant changes in [18F]dopa $K_{io}$ were observed in the anterior cingulate region. In a direct comparison between the early and late Parkinson’s disease groups, we observed relative [18F]dopa $K_{io}$ reductions in ventral and dorsal midbrain, and dorsal pontine regions along with striatal [18F]dopa $K_{io}$ reductions. Similar results were found with a region of interest approach, on non-transformed data, except for the focal midbrain [18F]dopa $K_{io}$ increase seen in early Parkinson’s disease. In conclusion, using SPM with [18F]dopa-PET, we have objectively localized changes in extrastriatal, pre-synaptic dopaminergic function in Parkinson’s disease. The significance of the increased dopaminergic activity of anterior cingulate in early Parkinson’s disease remains unclear, but may be compensatory. The [18F]dopa signal in dorsal midbrain and pontine regions suggests that [18F]dopa is taken up by serotonergic and noradrenergic neurons which also degenerate in advanced Parkinson’s disease. This suggests, therefore, that Parkinson’s disease is a monoaminergic neurodegenerative disorder.

Keywords: Parkinson’s disease; 3D [18F]dopa; focal changes; extrastriatal; progression

Abbreviations: AADC = aromatic amino acid decarboxylase activity; COMT = catechol-O-methyl transferase; $K_{io}$ = influx rate constant; MTGA = multiple time graphical analysis; rCBF = regional cerebral blood flow; SPM = statistical parametric mapping; UPDRS = Unified Parkinson’s Disease Rating Scale

Introduction
[18F]dopa-PET provides an in vivo method for assessing the functional integrity of pre-synaptic dopaminergic function in the basal ganglia in Parkinson’s disease (Garnett et al., 1983; Martin et al., 1989; Brooks et al., 1990a). It is known from post-mortem studies in Parkinson’s disease that there is degeneration of nigral and mesial frontal dopaminergic terminals as well as striatal loss (Bernheimer et al., 1973; Scatton et al., 1982; Kish et al., 1988; Fearnley and Lees, 1991). However, to date, it has been difficult to study these changes with [18F]dopa-PET because of the lower specific uptake of [18F]dopa in these regions, coupled with the limitations of resolution and sensitivity of 2D [18F]dopa-PET scanning.

We have implemented and validated 3D acquisition,
reconstruction and analysis of dynamic $^{18}$F]dopa-PET data (Rakshi et al., 1996), resulting in a fourfold increase in the sensitivity of brain signal to noise ratios in 3D compared with 2D acquired PET studies (Cherry et al., 1991; Bailey, 1992). In addition, the increased sensitivity enables a higher reconstructed resolution with a reduction in the partial volume effects leading to a further overall improvement in image quality. Therefore, 3D $^{18}$F]dopa-PET should be more sensitive and accurate at detecting focal changes within extrastriatal and striatal dopaminergic regions (see Fig. 1).

The conventional method of $^{18}$F]dopa analysis uses a region of interest approach with multiple time graphical analysis (MTGA) (Patlak and Blasberg, 1985) to sample putamen and caudate function. However, the size, shape and placement of these regions is arbitrary, producing inter- and intra-observer variation, and potential observer bias. Furthermore, in severe Parkinson’s disease it can be difficult to identify the striatal boundaries for placement because of the reduced $^{18}$F]dopa uptake.

We have applied statistical parametric mapping (SPM) to 3D $^{18}$F]dopa-PET studies, enabling us to objectively localize focal changes in dopaminergic function in Parkinson’s disease throughout the brain volume without having to make an a priori hypothesis as to their location. SPM was initially developed for PET activation studies and localizes significant differences in function in spatially normalized brain images on a voxel-by-voxel basis (Friston et al., 1991b). SPM has now been applied to a number of PET ligands including $^{11}$C]flumazenil to localize changes in GABA$_A$ receptor binding in focal epilepsy (Richardson et al., 1996) and to $^{11}$C]diprenorphine to study alterations in opioid receptor binding in Huntington’s and Parkinson’s disease (Weeks et al., 1995; Piccini et al., 1997).

In addition to applying SPM, we employed the standard region of interest and MTGA analytical approach (Brooks et al., 1990b) to sample striatal and midbrain regions in untransformed images and, subsequently, brain areas revealed by SPM to have altered dopaminergic function in Parkinson’s disease.
Subjects and method

Subjects

Seven patients with early hemi-Parkinson’s disease clinically affecting their left limbs [mean age 57 ± 5 years; mean motor Unified Parkinson’s Disease Rating Scale (UPDRS) score 9 ± 3 (range 4–12); Hoehn and Yahr stage 1; and mean symptom duration 19 ± 5 months] and seven patients with advanced bilateral Parkinson’s disease [five left dominant, two right dominant; mean age 54 ± 8 years; mean motor UPDRS score 41 ± 15 (range 22–66); Hoehn and Yahr stage 3–5; and mean symptom duration 12 ± 4 years] underwent [18F]dopa-PET scanning. Findings were compared with those of a group of 12 normal subjects, mean age 57 ± 11 years.

All patients fulfilled the UK Parkinson’s disease Brain Bank criteria for idiopathic Parkinson’s disease (Gibb and Lees, 1988). All Parkinson’s disease patients were on regular levodopa therapy except for two of the early Parkinson’s disease patients who were drug naive. In addition, three of the advanced Parkinson’s disease patients were taking a dopamine agonist, and two a monoamine oxidase-B inhibitor.

Each patient had their medication stopped at least 12 h before their PET scan and was clinically assessed by a single observer, using the UPDRS and Hoehn and Yahr rating scales (Hoehn and Yahr, 1967; Fahn and Elton, 1987) just before their scan. Parkinson’s disease patients with significant cognitive impairment, as assessed by the Folstein’s Mini-Mental Test (Folstein et al., 1975), were excluded from the study (<28 out of 30). All controls had a normal neurological examination and showed no evidence of rest tremor, rigidity or bradykinesia. No normal volunteer was taking medication.

All patients and normal volunteers gave written informed consent, after a full explanation of the procedure. Permission to perform these studies was granted by the Ethics Committee of the Royal Postgraduate Medical School, London, UK and the Administration of Radioactive Substances Advisory Committee (ARSAC), UK.

Scanning protocol

In addition to having their medication stopped 12 h before their PET scan, all subjects were fasted on the morning of the scan and received an oral bolus of 150 mg carbidopa and 400 mg entacapone, a peripheral catechol-O-methyl transferase inhibitor (COMT; Orion Farmos Pharmaceuticals Espoo, Finland) 1 h before scanning (Sawle et al., 1994; Ishikawa et al., 1996a).

The [18F]dopa-PET scans were performed with an ECAT 953B neuroscanner (CTI/Siemens, Knoxville, Tenn., USA), with an axial field of view of 10.8 cm, and in 3D acquisition mode with protocols that have been previously reported (Bailey, 1992), yielding 31 planes with a slice separation of 3.4 mm and an average in-plane resolution of 6 mm full-width half-maximum. A correction for tissue attenuation of 511 KeV gamma radiation was measured with a 10 min 2D transmission scan performed prior to tracer injection and acquired using retracted 60Ga/68Ge sources.

[18F]Dopa (140–180 Mbq in 10 ml of normal saline solution) was infused intravenously over 30 s. Scanning began at the start of the tracer injection with a protocol of 25 time frames over 94 min (4 × 1 min, 3 × 2 min, 3 × 3 min, 15 × 5 min). The subjects were positioned such that the orbitomeatal line was parallel to the transaxial plane of the tomograph and head position was carefully monitored throughout the scan.

Data analysis

Two methods of analysis were employed: (i) SPM and (ii) standard region of interest approach with MTGA. Analysis of data was performed on a SUN Sparc 10 workstation (Sun Microsystems, Silicon Valley, Calif., USA) using ANALYZE 7.0 (Mayo Foundation, Baltimore, Md., USA) (Robb and Hanson, 1991) and in-house software written in IDL image analysis software (Research Systems, Inc., Boulder, Col., USA). Parametric images of [18F]dopa influx rate constants (Ki) were generated with IDL image analysis software. Image transformation and SPM analysis was performed using SPM software (SPM95; Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab (Mathworks Inc., Sherborn, Mass., USA).

SPM analysis

Image transformation. From each subject’s dynamic 3D [18F]dopa-PET study, we generated [18F]dopa Ki on a voxel-by-voxel basis for all 31 image planes using the MTGA approach with an occipital tissue input function. Thus, we were able to produce parametric images of [18F]dopa Ki for each subject. Tissue count sampling was performed using time frames 14–25 (30–94 min) post-injection.

There is insufficient anatomical detail in parametric images of [18F]dopa Ki to enable accurate spatial normalization directly to the regional cerebral blood flow (rCBF) template within the SPM software (Friston et al., 1991a). We have, therefore, developed an indirect approach whereby we use the combined time frames 1–25 of the dynamic 3D [18F]dopa image to produce an integrated ‘add image’ (0–94 min). This ‘add image’ contains the earlier time frames which are blood flow dependent and so generates sufficient cortical as well as striatal detail to allow accurate spatial normalization to the SPM template (see Fig. 2). Applying this method we produced an ‘add image’ for each subject and spatially normalized it to the rCBF template. The resulting transformation parameters were then applied to the corresponding subject’s parametric image of [18F]dopa Ki, allowing all the parametric images to be transformed into the standard stereotaxic space of Talairach and Tournoux (Rakhi et al., 1998).

This then allowed comparisons to be made across scan...
datasets, in analogous voxel regions of the brain volume and to combine \([^{18}\text{F}]\text{dopa-PET datasets from different subjects to perform group analyses. Following spatial normalization of the parametric images (}\(K_i^{18}\)), a Gaussian kernel of \(8 \times 8 \times 6\, \text{mm}^3\) (full width half maximum in the \(x, y\) and \(z\) planes, respectively) was applied to remove high-frequency noise from the images.

**SPM data analysis.** Categorical comparisons of mean \([^{18}\text{F}]\text{dopa voxel } K_i^{18}\) values between the Parkinson’s disease and normal control groups were made applying SPM. Three between-group comparisons were made: (a) seven early left hemi-Parkinson’s disease patients versus 12 normal controls; (b) seven advanced bilateral Parkinson’s disease patients versus 12 normal controls; (c) seven early left hemi-Parkinson’s disease patients versus the seven advanced bilateral Parkinson’s disease patients.

Between-group comparisons were performed using appropriately weighted categorical contrasts to generate SPMs for both increases and decreases in mean voxel \([^{18}\text{F}]\text{dopa } K_i^{18}\) values on a voxel-by-voxel basis. No global normalization was applied since we used measured voxel \([^{18}\text{F}]\text{dopa } K_i^{18}\) values which were independent of rCBF. Significant differences in mean voxel \([^{18}\text{F}]\text{dopa } K_i^{18}\) values for the between-group comparisons (a), (b) and (c) were localized using SPM. The contrasts were used to derive between-group \(Z\) scores (unpaired \(t\) statistic transformed to the standard normal distribution) on a voxel-by-voxel basis using the general linear model (Friston et al., 1991b, 1995). The \(P\) values associated with these regional effects were corrected for multiple-dependent comparisons implicit in the SPM. Maps of \(Z\) scores surviving a threshold of \(>2.33\) \((P < 0.01)\) were further corrected for multiple comparisons. These surviving voxels were rendered on to a stereotactically normalized MRI (see Fig. 3).

**Region of interest analysis**

We applied standard region of interest and MTGA analysis to the original untransformed datasets to sample frontal, midbrain and striatal regions. The influx rate constants \([^{18}\text{F}]\text{dopa } K_i^{18}\)'s were calculated from the time frames 14–25 (30–94 min) post-injection for tissue count activity using occipital tissue counts as the input function. For the striatum, circular regions of 10 mm diameter were placed over each dorsal head of caudate and an elliptical region of 10 \(\times\) 24 mm over each dorsal putamen aligned to the long axis on three contiguous transverse planes.

With the increased resolution and sensitivity of our 3D \([^{18}\text{F}]\text{dopa-PET scanner, we were now able to identify the midbrain and frontal regions more clearly by visual image inspection and with reference to the stereotaxic atlas (Talairach and Tournoux, 1988). We sampled the midbrain region by placing a single circular region of 16 mm diameter, encompassing both ventral and dorsal midbrain regions, on two contiguous transverse planes. We retrospectively sampled...**
Cingulate, midbrain and pontine changes in Parkinson’s disease

Fig. 3 SPM[Z] transverse, sagittal and coronal maximum intensity projection maps rendered on to a stereotactically normalized MRI scan, showing areas of significant increases and decreases in [18F]dopa $K_i$ uptake in A and D, early left hemi-Parkinson’s disease (PD) compared with normal controls; in B and E, advanced bilateral Parkinson’s disease compared with normal controls; and in C, advanced Parkinson’s disease compared with early Parkinson’s disease. $Z$ score threshold for significance: $P < 0.05$, corrected.
the anterior cingulate region. This was achieved by initially identifying the region of interest on the stereotaxic atlas using the coordinates of the frontal focus determined from the SPM analysis. We then identified this region on the $[^{18}\text{F}]$dopa-PET scan by the position of the anterior cingulate cortex in relation to the striatum on the PET image. However, because this method is imprecise, we used regions encompassing a larger volume than the SPM focus so that the region of interest would be included. Two circular regions of interest of diameter 16 mm for right and left anterior cingulate regions corresponding to the foci where significant increases in $[^{18}\text{F}]$dopa $K_{io}$ values were observed with SPM analysis were applied.

For the input function, we sampled right and left occipital lobes with two circular regions of 32 mm diameter placed on the same three contiguous planes as those selected for the dorsal striatal regions. All regions of interest were placed manually using the IDL software.

The mean $[^{18}\text{F}]$dopa $K_{io}$ values for each frontal, midbrain, putamen and caudate region in the early and advanced Parkinson’s disease groups were compared with the $[^{18}\text{F}]$dopa $K_{io}$ values of normal subjects using Student’s unpaired two-tailed t test.

### Results

#### Frontal region

**SPM analysis**

In early left hemi-Parkinson’s disease we observed a significant increase in $[^{18}\text{F}]$dopa $K_{io}$ in the the left anterior cingulate gyrus (Brodmann area 24/32) compared with the normal control group (Table 1 and Fig. 3A). There was also a similar trend in the right cingulate but this did not reach statistical significance on correction ($Z = 3.8$, $P < 0.076$).

In advanced Parkinson’s disease, no significant change in mean cingulate $[^{18}\text{F}]$dopa $K_{io}$ was observed, either when compared with the normal controls or directly with the early hemi-Parkinson’s disease group.

#### Region of interest analysis

Following the unexpected frontal rise in $[^{18}\text{F}]$dopa $K_{io}$ in early Parkinson’s disease we sampled the corresponding regions on the original untransformed datasets, independent of SPM. In early left hemi-Parkinson’s disease, we found a 36% and 32% increase in mean right and left cingulate $[^{18}\text{F}]$dopa $K_{io}$s, respectively (Table 2). There was no significant change in $[^{18}\text{F}]$dopa $K_{io}$ in the advanced Parkinson’s disease group.

#### Midbrain and pons regions

**SPM analysis**

In the early Parkinson’s disease group we observed a significant increase in dorsal midbrain $[^{18}\text{F}]$dopa $K_{io}$ compared with the normal control group (Table 1 and Fig. 3A).

In advanced bilateral Parkinson’s disease there was a significant reduction in both ventral and dorsal midbrain $[^{18}\text{F}]$dopa $K_{io}$ compared with the normal control group involving the substantia nigra, midbrain tegmentum and dorsal raphe nuclei (Table 1 and Fig. 3B).

In the direct comparison of advanced bilateral Parkinson’s disease with early hemi-Parkinson’s disease significant reductions in mean $[^{18}\text{F}]$dopa $K_{io}$ were observed in the dorsal pons as well as the dorsal and ventral midbrain regions and striata (Table 1 and Fig. 3C).

**Region of interest analysis**

No significant changes in midbrain $[^{18}\text{F}]$dopa $K_{io}$ were found in early hemi-Parkinson’s disease (Table 2).

In the advanced Parkinson’s disease group there was a 28% reduction in mean midbrain $[^{18}\text{F}]$dopa $K_{io}$ at the level of the nigra compared with normal controls.

**Striatal regions**

**SPM analysis**

A comparison of the early left hemi-Parkinson’s disease with the normal control group showed significant reductions in mean $[^{18}\text{F}]$dopa $K_{io}$ throughout the right and left putamen; these were more widespread and significant in the right putamen (Table 1 and Fig. 3D). In addition to the main putamen focus, there was also a second focus located in the right anterior putamen which extended into right head of caudate and a further separate focus in the dorsal body of the right caudate (Table 1 and Fig. 3D).

In advanced Parkinson’s disease compared with the normal control group, more extensive and highly significant reductions in $[^{18}\text{F}]$dopa $K_{io}$ values were found throughout both the dorsal and ventral striata (Table 1 and Fig. 3E).

In the direct comparison of advanced bilateral Parkinson’s disease (left-dominant) with early left hemi-Parkinson’s disease, interestingly, only the relative further reduction in left putamen reached statistical significance at $P < 0.05$, corrected (Table 1). There was a trend, however, towards relative reductions throughout the striata ($P < 0.05$, uncorrected).

**Region of interest analysis**

In the early left hemi-Parkinson’s disease group there was a 64% and 44% reduction in mean right and left putamen $[^{18}\text{F}]$dopa $K_{io}$, respectively (Table 2). There were corresponding 36% and 27% reductions in mean right and left caudate $[^{18}\text{F}]$dopa $K_{io}$s (Table 2).

In the advanced bilateral Parkinson’s disease group there were mean reductions of 79% and 70% in right and left putamen $[^{18}\text{F}]$dopa $K_{io}$ values (Table 2). There were...
Table 1 SPM findings for in between-group comparisons showing the locations of significant increases and decreases in [18F]dopa uptake in Parkinson’s disease

<table>
<thead>
<tr>
<th>Comparison of early left hemi-Parkinson’s disease with normal control group</th>
<th>Z score</th>
<th>Talairach co-ordinates</th>
<th>P value (corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left anterior cingulate gyrus</td>
<td>4.98</td>
<td>–2</td>
<td>32</td>
</tr>
<tr>
<td>Dorsal midbrain</td>
<td>4.05</td>
<td>–4</td>
<td>–38</td>
</tr>
<tr>
<td>Decreases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right putamen</td>
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<td>26</td>
<td>–4</td>
</tr>
<tr>
<td>Left putamen</td>
<td>5.03</td>
<td>–26</td>
<td>–8</td>
</tr>
<tr>
<td>Right anterior putamen</td>
<td>4.12</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>Dorsal body right caudate</td>
<td>4.00</td>
<td>22</td>
<td>–16</td>
</tr>
</tbody>
</table>

Comparison of advanced bilateral Parkinson’s disease with normal control group

<table>
<thead>
<tr>
<th>Decreases</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal and ventral midbrain</td>
<td>4.56</td>
<td>4</td>
<td>–32</td>
</tr>
<tr>
<td>Left anterior putamen</td>
<td>6.84</td>
<td>–18</td>
<td>14</td>
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<tr>
<td>Right putamen</td>
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<tr>
<td>Dorsal body right caudate</td>
<td>6.78</td>
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<td>Right anterior putamen</td>
<td>6.63</td>
<td>20</td>
<td>10</td>
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Comparison of advanced bilateral Parkinson’s disease with early left hemi-Parkinson’s disease

<table>
<thead>
<tr>
<th>Decreases</th>
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<tbody>
<tr>
<td>Dorsal pons</td>
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<tr>
<td>Dorsal and ventral midbrain</td>
<td>4.45</td>
<td>–14</td>
<td>–32</td>
</tr>
<tr>
<td>Left putamen</td>
<td>4.42</td>
<td>–24</td>
<td>–10</td>
</tr>
</tbody>
</table>

Early Parkinson’s disease group, n = 7; advanced Parkinson’s disease group, n = 7; normal control group, n = 12.

Discussion

We have applied SPM to spatially normalized parametric images of [18]dopa $K_o$, and localized similar reductions in striatal [18]dopa $K_o$ in early and advanced Parkinson’s disease to those detected using the standard region of interest approach. SPM, however, enabled us to localize objectively focal [18]dopa $K_o$ changes in anterior cingulate, midbrain and pontine regions which could not have been predicted by visual inspection of [18]dopa-PET images. This is because of their lower specific uptake of [18]dopa.

Methodological issues

In practice, as with all methods and measurements, there are sources of error in the technique, and their understanding is required for the proper interpretation of the results. The main methodological issues are: 3D PET data acquisition, quantification and interpretation of [18]dopa uptake, and the application of SPM to [18]dopa datasets.

3D scanning has a great sensitivity advantage over 2D acquisition. The technique, however, is complex and some of the problems involved have yet to be completely resolved (Badawi, 1997). In 3D mode or volume imaging, the interplane septa of the PET scanner are removed and coincidence events may be detected by pairs of detectors located in any ring. This greatly increases the camera sensitivity to true coincident events, but also increases the sensitivity to scattered coincidences. One therefore has to employ a scatter correction method for 3D datasets (Cherry et al., 1993; Bailey and Meikle, 1994). Another difficulty relates to the requirement that all pairs of detectors in coincidence should have the same sensitivity, i.e. detector normalization. It has been shown that there is no unique normalization for both scatter and true coincidences or for all counts rates. Detector normalization is not perfect and is still being improved. Finally, because of the high count rates that detectors have to process in 3D acquisition, there is an increase in dead-time and random coincidence events detected. Ingenious methods to overcome these limiting factors in 3D mode are currently being developed.

Nevertheless, accepting the problems and limitations above, we have implemented 3D [18]dopa-PET and successfully performed a validation experiment. We have also demonstrated improved image quality and reproducibility compared with our previous 2D protocol (Rakshi et al., 1996; Trebossen et al., 1996) and were able to completely discriminate both contralateral and ipsilateral putamen [18]dopa $K_o$ values in early hemi-Parkinson’s disease from normal controls.

The objective of a parameter estimation using [18]dopa-
Table 2 Mean regional right and left putamen, caudate, mesial frontal and midbrain $K_i$ values in normal controls, early left hemi-Parkinson’s disease and advanced bilateral Parkinson’s disease

<table>
<thead>
<tr>
<th></th>
<th>Right putamen</th>
<th>Left putamen</th>
<th>Right caudate</th>
<th>Left caudate</th>
<th>Right frontal</th>
<th>Left frontal</th>
<th>Midbrain</th>
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<tbody>
<tr>
<td>Normal ($n = 12$)</td>
<td>0.0171</td>
<td>0.0171</td>
<td>0.0168</td>
<td>0.0168</td>
<td>0.0016</td>
<td>0.0017</td>
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<td>Mean</td>
<td>0.0171</td>
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<td>0.0168</td>
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</tr>
<tr>
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<td>0.0018</td>
<td>0.0028</td>
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<td>0.0096</td>
<td>0.0108</td>
<td>0.0123</td>
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<td>Mean</td>
<td>0.0061</td>
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<td>0.0016–0.0037</td>
<td>0.0018–0.0035</td>
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<td>Bilateral Parkinson’s disease ($n = 7$)</td>
<td>0.0036</td>
<td>0.0051</td>
<td>0.0081</td>
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<td>Mean</td>
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<td>0.0011</td>
<td>0.0018</td>
<td>0.0016</td>
<td>0.0006</td>
<td>0.0004</td>
<td>0.0007</td>
</tr>
<tr>
<td>Range</td>
<td>0.0012–0.0052</td>
<td>0.0033–0.0062</td>
<td>0.0046–0.0102</td>
<td>0.0012–0.0109</td>
<td>0.0009–0.0027</td>
<td>0.0014–0.0025</td>
<td>0.0050–0.0067</td>
</tr>
<tr>
<td>$P$ values</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.006</td>
</tr>
</tbody>
</table>

n.s. = not significant.
PET is to measure a biological variable of interest to the pathophysiology of Parkinson’s disease. With \([^{18}F]dopa-PET\), we would ideally like to measure \(k_3\), the rate constant which directly reflects aromatic amino acid decarboxylase activity (AADC) and thus pre-synaptic dopaminergic function (Dhawan et al., 1996). However, \([^{18}F]dopa\) is mainly peripherally metabolized in the presence of a peripheral AADC inhibitor (carbidopa), to 3-0-methyl-\([^{18}F]dopa\) by COMT (Boyes et al., 1986), which then crosses the blood–brain barrier and contributes to non-specific and striatal \([^{18}F]\) activity. This, along with the formation of other brain dopamine metabolites, makes direct kinetic modelling of \(k_3\) very complex (Gjedde et al., 1991; Kuwabara et al., 1995) and to date has not provided additional biological or clinical information on Parkinson’s disease over simpler methods of deriving an \([^{18}F]dopa\) \(K_i^o\) reflecting AADC activity (Dhawan et al., 1996; Ishikawa et al., 1996b; Cumming et al., 1997; Ichise and Ballinger, 1997).

We have therefore employed the simpler but less specific MTGA approach to measure the net transfer of \([^{18}F]dopa\) across the blood–brain barrier as the \([^{18}F]dopa\) \(K_i^o\), where \(K_i^o\) is the slope of the Patlak plot and reflects the rate of unidirectional striatal \([^{18}F]dopa\) accumulation into dopaminergic pre-synaptic terminals, its subsequent decarboxylation and vesicular storage. \([^{18}F]dopa\) \(K_i^o\), however, is also influenced by factors other than those primarily related to \([^{18}F]dopa\) accumulation and decarboxylation, such as plasma and amino acid concentrations, peripheral and central COMT activities and cerebral blood flow. We have attempted to minimize these effects by fasting all subjects prior to scanning and by using a peripheral COMT inhibitor (Sawle et al., 1994). We have also used occipital counts for the input function to minimize errors due to attenuation correction, scan detector sensitivity or metabolite correction in 3D. Finally, it should be noted that brain \([^{18}F]dopa\) uptake is a measure of its capacity to decarboxylate exogenous dopa and, as such, measures pre-synaptic dopaminergic function. It does not provide a measure of the endogenous rate of dopamine synthesis.

Another important consideration is the effect of levodopa administration on \([^{18}F]dopa\) uptake in Parkinson’s disease. We have found no significant difference in \([^{18}F]dopa\) uptake in early or advanced Parkinson’s disease subjects scanned twice within 1 month, on one occasion taking their levodopa medication and on the other occasion off levodopa medication, stopped 12 h prior to their PET scan (R. Ceravalo, unpublished observations). The long-term effects of exposure to levodopa therapy on AADC activity in Parkinson’s disease are, however, unknown.

Finally, it has been suggested that to improve region of interest placement, one can co-register each patient’s individual MRI to their \([^{18}F]dopa-PET\) scan and place the regions on the co-registered image. We have not found this approach to significantly change \([^{18}F]dopa\) \(K_i^o\) values or improve reproducibility when compared with the standard region of interest method (unpublished data). This may be because the various steps involved in image co-registration also introduce sources of error. With advancing PET technology, this method may ultimately improve the accuracy of region of interest placement.

With respect to SPM, there are three main issues: (i) accurate spatial normalization; (ii) applying Gaussian smoothing; and (iii) the assumption that the voxel \([^{18}F]dopa\) \(K_i^o\) values across the parametric images are normally distributed for all subject groups.

It is possible that the relative differences in contrast and intensity between \([^{18}F]dopa\) ‘add images’ and the rCBF template might affect spatial normalization. The normalization process is partly dependent on these factors, but the degree to which it might be affected will also depend on the particular constraints of the normalization programme. In practice, however, the \([^{18}F]dopa\) ‘add images’ closely resemble the rCBF template (Fig. 2) because the cortex and striatum have relatively high blood flow and the ‘add image’ incorporates the earlier time frames which are highly blood flow dependent. Furthermore, if the spatial normalization was imprecise this would represent a substantial source of error variance and we would not have obtained high Z scores for \([^{18}F]dopa\) \(K_i^o\) changes in Parkinson’s disease. Accurate spatial normalization is further supported by the SPM maps being accurately rendered on to the putamen and caudate regions of the standard normalized MRI, with the maximal scores identified in Talairach space corresponding to putamen and caudate structures (see Fig. 3).

In future, however, there is a strong case for developing an \([^{18}F]dopa\) template, rather than using an rCBF template for direct spatial normalization. SPM has now been successfully applied to a number of other PET radioligands (Weeks et al., 1995; Richardson et al., 1996; Piccini et al., 1997). We arbitrarily applied minimal Gaussian smoothing (8 mm) in order to increase the signal to noise ratio and therefore the sensitivity. This step did not alter our results and could have been omitted. In general, irrespective of the size of the structure giving a signal, smoothing will increase signal to noise and therefore sensitivity, but will degrade resolution (Friston et al., 1995).

Finally, addressing the issue of the normal distribution of voxel \([^{18}F]dopa\) \(K_i^o\) values. This assumption is supported by no outliers being observed at the maximal Z score plots in the three Parkinson’s disease studies and can be inferred from a normal distribution of region of interest \([^{18}F]dopa\) \(K_i^o\) values in normal and Parkinson’s disease populations, both from this and previous PET studies. The unpaired \(t\) test is generally considered to be a robust statistical test, capable of handling suboptimal normally distributed data. At \(Z\) scores greater than 4.0, the permutation test is thought to be unnecessary (Holmes et al., 1996).

**Extrastralial changes in early Parkinson’s disease**

An unexpected finding revealed by SPM was increased \([^{18}F]dopa\) uptake in the anterior cingulate and dorsal midbrain...
regions in early hemi-Parkinson’s disease (see Fig. 3A). In addition to the reasons already discussed, these extrastriatal $[^{18}F]_{dopa} K_b$ changes are unlikely to be artefactual false positives because the anterior cingulate result was confirmed retrospectively, independent of stereotaxic transformation and SPM, by applying conventional region of interest analysis to the corresponding cingulate regions on the original untransformed images.

With region of interest analysis, we observed similar mean right and left anterior cingulate $[^{18}F]_{dopa} K_b$ increases but only a significant increase in the left anterior cingulate with SPM. (SPM showed a trend in right cingulate $Z = 3.8$, $P < 0.076$.) However, we applied a larger region of interest than the SPM focus which would have reduced the value of the higher left cingulate $[^{18}F]_{dopa} K_b$.

The significance of increased $[^{18}F]_{dopa} K_b$ is unclear but suggests increased AADC activity. There are a number of possible explanations for this finding which are now discussed. Our knowledge of brain AADC activity in health and disease is limited because there are no direct in vivo human studies. Furthermore, there are no post-mortem studies in early hemi-Parkinson’s disease to determine directly early striatal and extrastriatal dopaminergic changes. Therefore, the exact pathophysiological changes occurring in dopaminergic neurons in the very early stages of Parkinson’s disease are unknown.

The rate limiting step of endogenous dopamine formation within pre-synaptic dopaminergic nerve terminals is tyrosine hydroxylase which converts L-tyrosine to L-dopa. Under normal physiological conditions AADC which converts L-dopa to dopamine is not thought to be regulated. However, a number of studies have suggested that AADC can be regulated under pathophysiological conditions. Human post-mortem brain studies have suggested that AADC may be up-regulated in dopamine neurons that are spared during ageing (Agid et al., 1987; Kish et al., 1995), and increased AADC activity has been demonstrated in patients with psychosis (Reith et al., 1994). It is possible, therefore, that AADC may undergo regional regulatory changes in Parkinson’s disease.

The anterior cingulate observation (Brodmann area 24/32) corresponds neuroanatomically to the mesocortical region which receives dopaminergic projections from the ventral tegmental area A10 (Lindvall and Bjorklund, 1974; Simon and Le Moal, 1984). This projection forms part of the mesocorticolumbic dopaminergic system which is involved in motor, cognitive and behavioural functions (Glowinski et al., 1984; Le Moal and Simon, 1991). Dopaminergic projections to the cerebral cortex, however, also arise from dopamine neurons in the substantia nigra (Bjorklund and Lindvall, 1984). The mesocortical system, which includes the pre-frontal region, is distinct from other ascending dopaminergic projections, i.e. nigrostriatal and mesolimbic, and has unique intrinsic properties. For example, they completely lack or have greatly reduced numbers of autoreceptors on their cell bodies, dendrites and nerve terminals. They also have a higher neuronal firing rate, different activity pattern, higher dopamine turnover and a diminished response to dopamine agonists/antagonists (Bannon and Roth, 1983).

Another interesting feature of the mesocortical system, which could possibly explain our PET finding, is the inverse relationship demonstrated between the mesocortical and nigrostriatal dopaminergic systems (Pycock et al., 1980). This was first demonstrated in animal studies where dopaminergic neurons projecting to the mesocortex were specifically lesioned in rats. This led to an increase in dopamine levels, its metabolites and D2 receptor binding and sensitivity in the striatum. Similar findings have now been demonstrated in non-human primates (Roberts et al., 1994). It has therefore been proposed that the mesocortical dopaminergic system has an inhibitory regulatory effect on nigrostriatal dopaminergic systems. Conversely, nigrostriatal lesions could produce an increase in dopamine turnover associated with increased AADC activity in the mesocortex. Surprisingly, there are no studies investigating the reverse relationship.

A further unique feature of the mesocortical dopaminergic neurons is their preferential sensitivity to physical and psychological stress (Roth and Elsworth, 1995). Therefore our finding could instead represent a stress related response in early Parkinson’s disease.

On a general dopaminergic perspective, it has been proposed that there are compensatory or adaptive processes in Parkinson’s disease to counterbalance the initial nigral dopaminergic cell loss and that symptoms appear when dopamine depletion reaches a critical threshold and these adaptive processes fail (Zigmond et al., 1990). One such possible compensatory mechanism could involve increased synthesis and release of dopamine from residual midbrain dopaminergic neurons. Animal studies have shown that partial or unilateral midbrain dopaminergic lesions result in increased dopamine content and turnover in the remaining striatal dopaminergic terminals (Agid et al., 1973; Andersson et al., 1980; Melamed et al., 1982) perhaps related to the sprouting of residual dopaminergic nerve terminals (Blanchard et al., 1996).

In our $[^{18}F]_{dopa}$-PET study we have demonstrated increased $[^{18}F]_{dopa} K_b$ suggestive of increased AADC activity. The question is whether this results from, or leads to, increased dopamine synthesis or turnover in early Parkinson’s disease. If so, another alternative interpretation of our finding would be that up-regulation of AADC activity in the A10 projection is a compensatory or secondary effect, reflecting early dysfunction in that projection. One would then speculate that similar up-regulation of AADC occurs in the nigrostriatal projection in pre-clinical Parkinson’s disease, but by clinical presentation; although there is increased AADC in individual dopaminergic neurons, the total striatal AADC is reduced because of the continuing neuronal loss. $[^{18}F]_{dopa}$-PET would then underestimate dopaminergic neuronal loss in early disease. In summary, there is no clear explanation of our finding; however, there are a number of interesting possibilities which we have proposed.
The SPM finding of increased dorsal midbrain $^{[18]}$F]-dopa $K_i^o$ in early hemi-Parkinson’s disease was not observed on region of interest analysis. SPM detected a specific midbrain area of increased $^{[18]}$F]-dopa $K_i^o$, whereas region of interest sampled whole midbrain activity being unable to distinguish clearly between ventral and dorsal midbrain regions on an integrated ‘add image’. Although the focus occurred at the level of the superior colliculus, it extended forward and may represent the rostral component of the raphe nuclei.

We now know that a single AADC enzyme catalyses the decarboxylation of both L-dopa in catecholaminergic neurons and 5-hydroxytryptophan in serotonergic neurons (Tison et al., 1991). $^{[18]}$F]-dopa will therefore be taken up by both serotonergic and noradrenergic neurons and reflect their function within the brainstem, i.e. $^{[18]}$F]-dopa-PET is not specific for dopaminergic neurons. With improved $^{[18]}$F]-dopa-PET images, we have demonstrated high signal uptake in the dorsal brainstem corresponding to the regions of the serotonergic neurons of the median raphe nuclei, and the noradrenergic neurons of the locus coeruleus (Figs 1 and 3). Therefore, our dorsal midbrain finding may represent increased AADC activity in serotonergic neurons in this region.

In a previous PET study of early Parkinson’s disease with $^{[11]}$C]-WIN 35,428, a cocaine analogue which specifically labels the dopamine transporter, an 84% reduction in midbrain binding was reported (Frost et al., 1993). We did not observe a reduction in midbrain $^{[18]}$F]-dopa uptake in our early hemi-Parkinson’s disease cases. This may reflect either a milder degree of involvement at this stage (their five patients had more advanced and bilateral Parkinson’s disease), and/or relative preservation of dopa decarboxylase activity compared with dopamine re-uptake site binding. Alternatively, it has been suggested that dopamine re-uptake sites may be down-regulated (Bannon et al., 1992).

**Extrastralial changes in advanced Parkinson’s disease**

No significant changes in anterior cingulate $^{[18]}$F]-dopa $K_i^o$ were observed with SPM or conventional region of interest analysis. This result is perhaps surprising given that post-mortem data have shown a reduction in frontal dopamine and its metabolites in Parkinson’s disease (Scatton et al., 1982). One possible explanation could be that our advanced Parkinson’s disease group did not have significant cognitive impairment. Alternatively, AADC activity may be maintained in mesocortical areas in advanced Parkinson’s disease despite a fall in tyrosine hydroxylase activity and dopamine levels.

In the midbrain region significant decreases in $^{[18]}$F]-dopa $K_i^o$ were demonstrated by region of interest analysis and were shown by SPM to involve both ventral and dorsal regions, in particular, the substantia nigra, ventral tegmentum and the median raphe nuclei.

In the SPM comparison of advanced Parkinson’s disease with early hemi-Parkinson’s disease (reflecting Parkinson’s disease progression) the most significant further $^{[18]}$F]-dopa $K_i^o$ reductions were in the lower dorsal midbrain to the upper dorsal pons regions, corresponding to dorsal raphe nuclei and locus coeruleus, respectively (see Fig. 3C). Surprisingly, these reductions were much more significant than the further striatal losses and suggest marked monoaminergic rather than simply dopaminergic neuronal degeneration in Parkinson’s disease. Their exact role in the clinical development and late complications of Parkinson’s disease, e.g. motor fluctuations and freezing, and problems of long-term levodopa therapy, e.g. dyskinaesia, is unknown.

**Striatal changes in Parkinson’s disease**

Employing 3D $^{[18]}$F]-dopa-PET, we have demonstrated bilateral putamen dopaminergic dysfunction in early hemi-Parkinson’s disease (Hoehn and Yahr stage 1). Furthermore, all individual putamen $^{[18]}$F]-dopa $K_i^o$ values contralateral to the clinically unaffected side fell outside the normal range. This contrasts with our previous 2D $^{[18]}$F]-dopa-PET study of early hemi-Parkinson’s disease where most corresponding putamen $^{[18]}$F]-dopa $K_i^o$ values were within the normal range (Morrish et al., 1995). In this study, we used a higher resolution 3D PET scanner with greater sensitivity and improved image quality. This resulted in an ~40% increase in normal striatal $^{[18]}$F]-dopa $K_i^o$ values and a wider separation from the Parkinson’s disease range suggesting normal $^{[18]}$F]-dopa $K_i^o$ values were previously underestimated (Rakshi et al., 1996). Therefore, the conclusion, based on the results of the earlier 2D study, that the ipsilateral putamen in early hemi-Parkinson’s disease is not significantly affected, is no longer supported. With further advances in PET technology, we may demonstrate even greater differences.

In Parkinson’s disease, involvement but relative sparing of caudate dopaminergic function is well recognized; however, it is still unclear when and how it develops. With SPM, we detected a significant region of reduced $^{[18]}$F]-dopa uptake in the dorsal body of the right caudate in early left hemi-Parkinson’s disease. This suggests preferential targeting of the body as well as head of caudate in early Parkinson’s disease. In support of our finding, a post-mortem study has demonstrated severe dopamine depletion in dorsorostral caudate regions comparable with dorsocaudal putamen losses in established Parkinson’s disease (Kish et al., 1988).

In advanced bilateral Parkinson’s disease, both SPM and region of interest analyses demonstrated greater and more extensive loss of $^{[18]}$F]-dopa uptake throughout the putamen and caudate, compared with early hemi-Parkinson’s disease. The differences in mean symptom duration and mean motor UPDRS scores between the early and severe Parkinson’s disease patients were ~10 years and ~30 points, respectively. One might then have expected a greater difference in mean putamen and caudate $^{[18]}$F]-dopa $K_i^o$s between the two Parkinson’s disease groups from region of interest analysis. However, the contralateral putamen in early hemi-Parkinson’s...
disease has already lost 65% of the normal level of $[^{18}\text{F}]$dopa uptake with our 3D measure and, therefore, further smaller reductions in $[^{18}\text{F}]$dopa $K_i$ are likely to be associated with large increases in motor disability. The relationship between motor UPDRS and $[^{18}\text{F}]$dopa $K_i$ is, therefore, likely to be non-linear, possibly exponential.

**Conclusion**

With recent advances in PET technology, $[^{18}\text{F}]$dopa-PET has become more sensitive at detecting extrastriatal and preclinical changes in Parkinson’s disease. Applying SPM and region of interest analysis to 3D $[^{18}\text{F}]$dopa-PET data we have identified patterns of subregional dopaminergic dysfunction in cingulate, brainstem and striatal regions in Parkinson’s disease. Our study suggests increased AADC activity in anterior cingulate and dorsal midbrain regions in early Parkinson’s disease and significant serotonergic and noradrenergic as well as dopaminergic neuronal degeneration within the brainstem in advanced Parkinson’s disease, in addition to the known striatal dopaminergic changes. Parkinson’s disease, therefore, is most likely a monomeric neurodegenerative disorder. This may have important implications for future treatment strategies.

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