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Progressive striatal and cortical dopamine receptor dysfunction in Huntington's disease: a PET study

Nicola Pavese,[1] Thomasin C. Andrews,[1] David J. Brooks,[1] Aileen K. Ho,[2] Anne E. Rosser,[3] Roger A. Barker,[2] Trevor W. Robbins,[2] Barbara J. Sahakian,[2] Stephen B. Dunnett[3] and Paola Piccini[1]

[1]*MRC Clinical Sciences Centre and Division of Neuroscience, Faculty of Medicine, Imperial College, Hammersmith Hospital, London,* [2]*Centre for Brain Repair, University of Cambridge,* [3]*School of Biosciences, University of Cardiff, UK*

Correspondence to: Dr Paola Piccini, MRC Cyclotron Building, Faculty of Medicine, Imperial College School of Medicine, Hammersmith Campus, Du Cane Road, London W12 0NN, UK
E-mail: paola.piccini@csc.mrc.ac.uk

Summary

We have studied the progression of striatal and extrastriatal post-synaptic dopaminergic changes in a group of 12 patients with Huntington's disease using serial ^{11}C-raclopride PET, a specific marker of D2 dopamine receptor binding. All patients had two ^{11}C-raclopride PET scans 29.2 ± 12.8 months apart, and six of them had a third scan 13.2 ± 3.9 months later. We found a mean annual 4.8% loss of striatal ^{11}C-raclopride binding potential (BP) between the first and second scans, and a 5.2% loss between the second and third scans. Statistical Parametric Mapping (SPM) localized significant baseline reductions in ^{11}C-raclopride BP in both striatal and extrastriatal areas, including amygdala, temporal and frontal cortex in Huntington's disease compared with normal subjects matched for age and sex. When the ^{11}C-raclopride scans performed 29 months after the baseline scans were considered, SPM revealed further significant striatal, frontal and temporal reductions in ^{11}C-raclopride BP in Huntington's disease. Cross-sectional Unified Huntington's Disease Rating Scale (UHDRS) scores correlated with ^{11}C-raclopride binding, but there was no correlation between individual changes in UHDRS motor scores and changes in striatal binding. Performance on all neuropsychological measures deteriorated with time but only the accuracy score of the one-touch Tower of London test correlated significantly with striatal and putamen D2 binding. In summary, serial ^{11}C-raclopride PET demonstrates a linear progression of striatal loss of D2 receptors in early clinically affected Huntington's disease patients over 3 years. SPM also revealed a progressive loss of temporal and frontal D2 binding. Changes over time in clinical scores and in neuropsychological assessments, except for measures of planning, did not correlate with striatal D2 binding. This probably reflects both contributions from other affected brain structures and high variance in these measures.

Keywords: extrastriatal; Huntington's disease; PET; progression; raclopride

Abbreviations: BP = binding potential; CAPIT = Core Assessment Program for Intracerebral Transplantation; ROI = Region of Interest; SPM = Statistical Parametric Mapping; UHDRS = Unified Huntington's Disease Rating Scale

Introduction

Huntington's disease is an autosomal dominant neurodegenerative disorder with midlife onset characterized by motor, psychiatric and cognitive symptoms. The clinical symptoms primarily relate to the progressive loss of medium-spiny GABA-ergic neurons in the striatum, although pathological changes involving the cerebral cortex lead to further dysfunction in cortico-striatal-pallidal circuits (Albin *et al*., 1989).

To date, there is no effective treatment for preventing or slowing this neuronal degeneration. However, several neurotrophic factors have demonstrated the capacity to protect striatal neurons in experimental models of Huntington's disease (Anderson *et al*., 1996; Emerich *et al*., 1997; Kordower *et al*., 2000), and it has been proposed that implantation of foetal striatal cells into Huntington's disease striatum may provide another effective therapy for the disease by restoring inhibitory GABA-ergic control of the pallidal output neurons and by normalizing both cognitive and motor behaviour (Dunnett, 1995; Kendall *et al*., 1998; Bachoud-Levi *et al*., 2000).

The rate of progression of Huntington's disease is still unclear, and the ideal approach to measuring this remains

Table 1 *Clinical characteristics for each of the 12 patients with Huntington's disease*

	Sex	Age at onset (years)	CAG repeat	Age at first scan (years)	Disease duration at first scan (years)	UHDRS motor score at first scan
1	F	21	41	31	10	NA
2	F	43	45	47	4	NA
3	F	40	45	45	5	NA
4	M	52	43	68	16	NA
5*,**	M	54	NA	56	2	36
6*,**	M	37	48	42	5	49
7*,**	F	44	46	46	2	35
8*	M	47	NA	50	3	26
9*,**	M	56	42	63	7	30
10*,**	M	52	NA	56	4	14
11*	M	42	47	45	3	33
12*,**	M	37	40	39	2	24
Mean ± SD		43.7 ± 9.7		49 ± 10.3	5.2 ± 4.1	

Patients 5–12 were recruited to the UK CAPIT trial for Huntington's disease. *Patients with PET, and clinical and neuropsychological assessment performed three times. **Patients studied using SPM.
NA = not available; F = female; M = male.

controversial. In 1996, a Core Assessment Program for Intracerebral Transplantation (CAPIT) in Huntington's disease was published (Quinn *et al*., 1996). It combines a standardized set of neurological tests of motor, cognitive and neuropsychiatric function performed at standardized times before and after transplantation, and recommends ^{11}C-raclopride PET to visualize striatal grafts in terms of dopamine D2 receptor binding. The CAPIT Huntington's disease protocol has also been proposed for monitoring the outcome following implantation of genetically engineered cells and other experimental studies of potential disease-modifying treatments in Huntington's disease. However, because of the paucity of longitudinal studies some doubts have been expressed concerning several of the chosen clinical assessments, in particular the optimal ligand for PET imaging.

The aims of the present study were to verify: (i) whether ^{11}C-raclopride PET provides an objective and accurate method for the longitudinal assessment of loss of striatal dopaminergic D2 receptor binding in Huntington's disease patients; and (ii) whether serial changes in striatal D2 receptor binding potential (BP) correlate with changes in motor performances assessed with the Unified Huntington's Disease Rating Scale (UHDRS) (Huntington Study Group, 1996) and/or neuropsychological assessments recommended by CAPIT for Huntington's disease.

Methods

Subjects

Twelve clinically symptomatic Huntington's disease patients (eight males, four females; mean age 49 ± 10.3 years) were studied. Eight of these were recruited to the UK trial for studying intra-striatal transplantation of embryonic striatal tissue in Huntington's disease (CAPIT for Huntington's disease patients). Symptom duration in these patients ranged from 2 to 16 years, with a mean duration of 5.2 ± 4.1 years. All of them had an expanded CAG repeat in the Huntington's disease gene on chromosome 4. The clinical characteristics of the Huntington's disease patients are detailed in Table 1.

None of the patients were taking medication known to affect clinical status and/or to alter striatal binding of D2 receptor antagonist tracers used for PET scanning (tetramazine, neuroleptics or *N*-methyl-D-aspartate receptor blocking agents) at any time during the study. Smoking, and consumption of coffee and other caffeinated beverages were not allowed at least 6 h before scanning.

Data on repeated ^{11}C-raclopride scans in control subjects have been reported by our unit in a previous publication (Andrews *et al*., 1999). No further normal subjects underwent repeated scans in this study. Ten ^{11}C-raclopride studies of normal volunteers (seven males, three females; mean age 40.9 ± 6.8 years) from our database were used for Statistical Parametric Mapping (SPM) analysis.

The study received ethical approval from the Ethics Committee of Hammersmith, Queen Charlotte's & Chelsea and Acton Hospitals.

Permission to administer ^{11}C-raclopride was obtained from the Administration of Radioactive Substances Advisory Committee of the UK. All subjects gave informed written consent in accordance with the Declaration of Helsinki.

PET scanning

Each of the 12 Huntington's disease subjects had two serial ^{11}C-raclopride PET scans 29.2 ± 12.8 months apart. A third ^{11}C-raclopride PET scan was performed 13.2 ± 3.9 months later in six of the patients.

All patients had a volumetric MRI for coregistration purposes, performed at the time of each PET study.

Clinical and neuropsychological examination

The eight Huntington's disease patients who were candidates for the transplantation programme also had clinical and neuropsychological assessments according to the CAPIT for Huntington's disease criteria at the time of the first and second PET scans. Six had further assessments at the time of the third scan. The complete neuropsychological battery has been reported previously (Lawrence and Sahakian, 2001).

PET scanning procedure

Four Huntington's disease patients were scanned with a CTI 931/-08/12 scanner (CTI/Siemens, Knoxville, TN, USA). Once reconstructed, the spatial resolution for 15 planes of image data was 7.0 mm axially and 8.5 × 8.5 mm transaxially (full-width half maximum) (Spinks *et al*., 1988). For the eight patients taking part in the CAPIT for Huntington's disease, PET scans were all performed on a CTI/Siemens 953B PET camera (CTI/Siemens, Knoxville, TN, USA). The spatial resolution of this scanner for 31 planes of reconstructed image data in 2D mode was 8.5 × 8.5 mm transaxially, but 3.5 mm axially (full-width half maximum) (Spinks *et al*., 1992).

A 10 min transmission scan was obtained using a retractable external ring source of ^{68}Ga/^{68}Ge to correct for attenuation of γ-radiation by the brain and skull. ^{11}C-raclopride (mean value 370 ± 8 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 22 serial time frames collected over 1 h. The patients were positioned such that the orbitomeatal line was parallel to the transaxial plane of the scanner and the head position was carefully monitored throughout the scan.

All follow-up scans were performed with the same camera as the baseline scan. The 10 normal volunteers used for SPM had PET performed with the same scanner and using the same procedure as the eight CAPIT for Huntington's disease patients.

Data analysis

ROI analysis

Region of Interest (ROI) image analysis was performed using Analyze software (version 7.5, BRU, Mayo Foundation, Rochester, MN, USA) on a Sun Sparc Ultra workstation.

Parametric images of ^{11}C-raclopride BPs were generated from the dynamic ^{11}C-raclopride scans, using a basis function implementation of the simplified reference region compartmental model, with the cerebellum as the reference tissue (Gunn *et al*., 1997). An integrated image of the data from the last four time-frames (20–60 min) was also created for coregistration purposes. Each individual MRI was coregistered to the corresponding PET using MPR software (Studholme *et al*., 1997). ROIs were traced around right and left head of caudate and dorsal putamen directly on the corresponding MRI coregistered to the PET images where these structures were clearly defined. Values of BP for caudate and putamen were then obtained by applying ROIs to corresponding parametric images.

For each patient, we calculated the averaged right and left putamen, caudate, and striatal BPs at each scan time. Striatal binding was calculated averaging the BP from the caudate and putamen regions.

Because four patients were serially studied with the 931 scanner and eight with the 953B scanner, we normalized patient data to control group means obtained with the respective scanners according to the formula (mean ^{11}C-raclopride BP for the group of healthy controls – subject BP)/mean ^{11}C-raclopride BP for the group of healthy controls. Normal BP values for the 931 scanner were 2.29 ± 0.16 for caudate and 2.35 ± 0.13 for putamen. For the 953 scanner these BP values were 2.32 ± 0.21 and 2.57 ± 0.30 for caudate and putamen, respectively.

We then calculated percentage change in striatal, caudate and putamen BPs between baseline and second PET scans, and between second and third PET scans for each subject. Finally, the mean percentage reductions in striatal, caudate and putamen BPs over time were calculated for the whole Huntington's disease cohort.

SPM analysis

SPM was applied to localize significant changes in D2 availability in parametric images of ^{11}C-raclopride BP at a voxel level. Stereotaxic image transformation and localization of peak significant changes were performed using SPM99 software (Wellcome Department of Cognitive Neuroscience, Institute of Neurology, London, UK) implemented in Matlab5.

Image transformation involved spatially normalizing the PET integrated image to a normal ^{11}C-raclopride template created with SPM software and then applying the transformation parameters to the BP image (Meyer *et al*., 1999). Parametric images were then spatially smoothed using a 6 × 6 × 6 mm (full-width at half maximum) isotropic Gaussian kernel. This spatial filter accommodates inter-individual anatomic variability and improves signal to noise for the statistical analysis.

SPM enables all the parametric images to be transformed into the standard stereotaxic space of Talairach and Tournoux (1988) and, consequently, allows comparisons to be made across scan datasets in analogous voxel regions of the brain volume and, combining datasets from different subjects, also allows between-group and within-group analyses.

A between-group comparison of the findings for the eight Huntington's disease patients taking part in the CAPIT for Huntington's disease at the time of the baseline scan, and those of 10 normal subjects was made.

Two within-group comparisons were also made: (i) findings for the eight Huntington's disease patients at the time of the first scan were compared with those for the same

subjects at the time of the second scan; and (ii) findings for six Huntington's disease patients at the time of the second scan were compared with the same subjects at the time of the third scan.

Between-group and within-group comparisons were performed using appropriately weighted contrasts to localize significant decreases in mean voxel BP values with SPM.

The contrasts were used to derive *Z* scores on a voxel basis using the general linear model (Friston *et al*., 1995). Regional brain differences were considered significant when maps of *Z* scores exceeded a threshold of 2.33 ($P < 0.01$) after correction for cluster size ($P < 0.05$).

No global BP normalization was applied.

Statistical analysis

Statistical analyses of clinical data were performed with InStat3 for MacIntosh (University of Medicine and Dentistry, NJ, USA). Comparisons among groups were made using ANOVA (analysis of variance) followed by a Bonferroni multiple comparison *post hoc* test. Linear regressions were used for correlations between ^{11}C-raclopride BP and variables of interest.

Results

PET data

ROI analysis

The mean annual change in striatal D2 dopamine binding was 4.8% (range 2.3–7.3%) between the baseline and second scans for the entire cohort of 12 Huntington's disease patients. The mean annual rate of D2 binding reduction was higher in caudate (5.4%, range 2.9–9.5%) than in putamen (4.15%, range 0.7–6.9%). Comparable results were found when we assessed the annual loss between the second and the third scan in the subgroup of six patients: 5.2% (range 3.3–6.8%) for striatum, 5.2% (range 2.2–7.7%) for caudate, and 4.8% (range 3.2–6.3%) for putamen. Figure 1 shows the mean values of striatal loss of ^{11}C-raclopride BP in the subgroup of six patients with three scans.

Striatal D2 binding was negatively correlated with disease duration at each time-point evaluated (first scan $r = -0.56$, $P = 0.01$; second scan $r = -0.60$, $P = 0.03$; third scan $r = -0.78$, $P = 0.05$).

Negative correlations were also found between caudate and putamen D2 binding and disease duration (for caudate: first scan $r = -0.58$, $P = 0.04$; second scan $r = -0.60$, $P = 0.05$; third scan $r = -0.80$, $P = 0.04$; and for putamen: first scan $r = -0.60$, $P = 0.05$; second scan $r = -0.59$, $P = 0.04$; third scan $r = -0.78$, $P = 0.05$).

No correlation was observed between individual annual

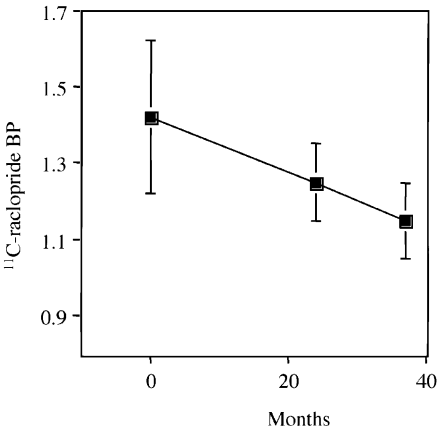

Fig. 1 Mean absolute values of striatal loss of ^{11}C-raclopride BP over the study's time-frame in the subgroup of six patients who had a PET scan repeated three times.

Table 2 *Between-group SPM findings showing the locations of significant decreases in ^{11}C-raclopride BP in Huntington's disease compared with normal patients at baseline*

Area	Talairach coordinates			*Z* score	*P* value
	x	*y*	*z*		
Right putamen	24	–15	4	5.23	$P < 0.001$*
Left putamen	–30	–10	4	5.30	$P < 0.001$*
Right caudate	10	16	4	4.89	$P < 0.001$*
Left caudate	–8	16	4	4.71	$P < 0.001$*
Right amygdala	20	–6	–12	3.41	$P < 0.001$**
Left amygdala	–24	–2	–16	3.25	$P < 0.001$**
Right Brodmann area 38	52	12	–20	3.44	$P < 0.001$**
Left Brodmann area 38	–51	11	–20	3.1	$P < 0.001$**
Right Brodmann area 21	62	–35	–8	3.3	$P < 0.001$**
Left Brodmann area 21	–62	–35	–8	3.1	$P < 0.001$**
Right Brodmann area 10	34	48	8	3.08	$P < 0.001$**
Left Brodmann area 10	–20	48	8	2.9	$P < 0.001$**
Right Brodmann area 9	42	30	28	2.93	$P < 0.001$**
Left Brodmann area 9	–42	30	28	2.91	$P < 0.001$**

*Corrected for whole brain volume; **corrected for cluster.

rates of striatal D2 binding reduction and either age at onset or disease duration (data not shown).

SPM analysis

The categorical comparison of the baseline scans of Huntington's disease patients with those of the normal control group localized significant reductions in ^{11}C-raclopride BP throughout the right and left caudate, and the right and left putamen in the former. We also observed a significant bilateral decrease in the amygdala, temporal cortex and frontal cortex of Huntington's disease patients (Table 2).

The within-group comparison of the baseline ^{11}C-raclopride scans and the second scans for the Huntington's disease group showed further decreases in mean ^{11}C-raclopride BP throughout the above-mentioned areas in the latter (Table 3). Similar, but only marginally significant, further reductions ($P < 0.05$, uncorrected) were found when comparing the third scans with the second scans, reflecting the reduced power due to the lower number of subjects and the shorter time interval between the final two sets of scans (data not shown).

Table 2 and Fig. 2 provide numerical and graphical representations, respectively, of the regional differences in ^{11}C-raclopride BP obtained by comparing the group of patients at the time of the first scan with healthy controls. Table 3 and Fig. 3 provide numerical and graphical representations, respectively, of the regional differences in ^{11}C-raclopride BP obtained by comparing the group of patients at the time of the first and second scans.

Correlation of the clinical assessment with ^{11}C-raclopride BP

We observed a mean annual increase in UHDRS score of 6.3 (20%) between the baseline and the second scans, and of 5.3 (12.3%) between the second and the third scans. Cross-sectional UHDRS total motor scores correlated negatively with striatal ^{11}C-raclopride binding ($r = –0.47$, $P = 0.03$), but we did not find any significant correlations between striatal ^{11}C-raclopride binding and scores on single items of the UHDRS, nor with changes in UHDRS motor scores and changes in striatal binding ($r = 0.12$, $P = 0.69$).

Correlation of the neuropsychological assessment with ^{11}C-raclopride BP

Performance on all neuropsychological measures deteriorated consistently over time (Table 4).

The accuracy score of the one-touch Tower of London test correlated significantly with ^{11}C-raclopride BP in the striatum (evaluations at the time of second and third scans: $r = 0.74$, $P = 0.04$ and $r = 0.82$, $P = 0.05$, respectively) and putamen ($r = 0.79$, $P = 0.02$ and $r = 0.85$, $P = 0.04$, respectively) such that the deterioration in performance on the test was related to the extent of receptor reduction in those structures.

There were no significant correlations between individual changes in other neuropsychological tests scores and changes in dopamine D2 binding in the striatum, putamen or caudate (data not shown).

Discussion

In this study, we found a linear rate of reduction in striatal D2 receptor binding in early Huntington's disease, as measured using serial ^{11}C-raclopride PET over a 3-year time-frame.

Deterioration in UHDRS scores and in neuropsychological assessments also occurred, but did not correlate well with deterioration of striatal function and so provided complementary information. Additionally, we observed loss of D2 receptor binding in extra-striatal structures, including amygdala, and frontal and temporal cortex.

Several approaches, including clinical, neuropsychological and PET assessments, have been used previously to determine rates of disease progression in Huntington's disease, but in none of these studies was the time course of the disease studied using all these different means of evaluation together.

^{11}C-raclopride PET is a sensitive indicator of striatal degeneration and restoration, showing good correlations with both histological and behavioural measures of reconstruction after intra-striatal grafting in lesioned rats (Torres *et al*.,

Table 3 *Within-group SPM findings showing the locations of further significant decreases in ^{11}C-raclopride BP in Huntington's disease by the time of the second scan*

Area	Talairach coordinates			*Z* score	*P* value
	x	*y*	*z*		
Right striatum	24	–15	4	4.00	$P < 0.001$*
Left striatum	–30	–10	4	3.7	$P < 0.001$*
Right Brodmann area 21	62	–35	–8	3.26	$P < 0.001$**
Left Brodmann area 21	–62	–35	–8	3.4	$P < 0.001$**
Right Brodmann area 10	34	48	8	3.9	$P < 0.001$**
Left Brodmann area 10	–20	48	8	3.8	$P < 0.001$**

*Corrected for whole brain volume; **corrected for cluster.

1995). It has been proposed as the most appropriate modality for monitoring disease progression in Huntington's disease patients (Quinn *et al*., 1996; Besret *et al*., 2000), and has been used to detect progressive striatal changes in both pre-symptomatic (Antonini *et al*., 1996; Hussey *et al*., 1998; Andrews *et al*., 1999) and symptomatic gene carriers (Hussey *et al*., 1998; Andrews *et al*., 1999). However, the rate of progression in more advanced patients has not been studied extensively. Two studies have examined disease progression in clinically affected subjects between two time points: Hussey and colleagues reported a 7% annual loss of striatal ^{11}C-raclopride binding in six patients (Hussey *et al*., 1998), while Andrews and colleagues found an annual 3% loss in a cohort of four patients (Andrews *et al*., 1999).

Even less is known about the kinetics of disease progression. A cross-sectional study by Antonini and colleagues indicated that striatal degeneration in Huntington's disease patients might proceed in a non-linear fashion (Antonini *et al*., 1998). They found a correlation between CAG repeat length and the estimated percentage loss of striatal D2 binding after

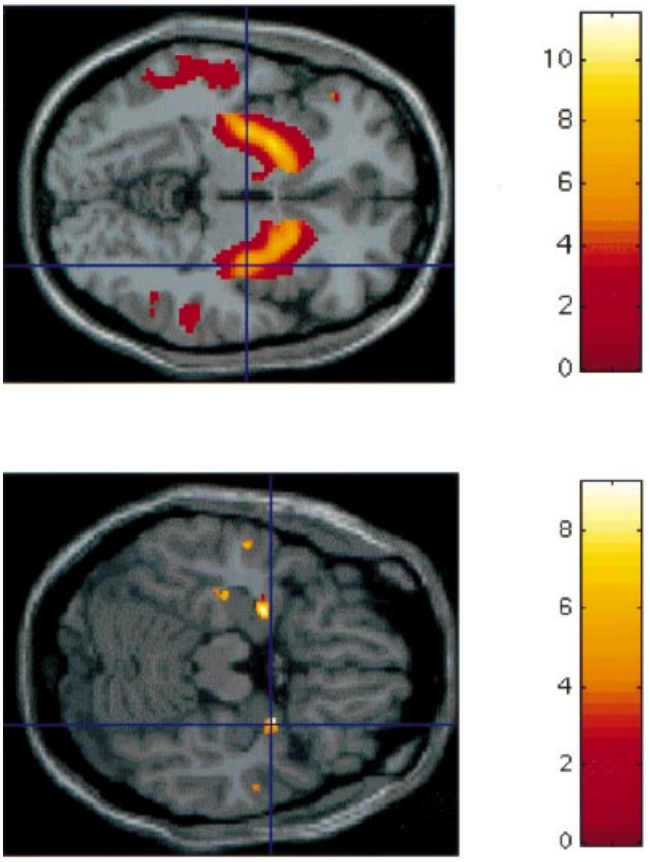

Fig. 2 Transaxial projection of SPMs. Between-group comparison: areas of significant between-group differences in ^{11}C-raclopride BP for the group of normal subjects and patients with Huntington's disease. The areas are superimposed on a standard MRI template. The lower image shows the decreases in BP in amygdala.

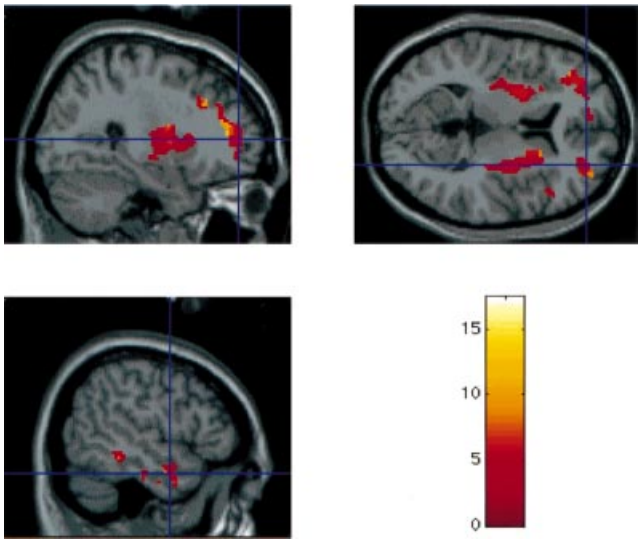

Fig. 3 Transaxial and sagittal projections of SPMs. Within-group comparison: areas of significant differences in ^{11}C-raclopride BP at the first scan compared with the second scan in eight Huntington's disease patients. The areas are superimposed on a standard MRI template.

Table 4 *Neuropsychological assessment at each scan time-point*

	Baseline (±SD)	First follow-up (±SD)	Second follow-up (±SD)
Verbal fluency	26.7 ± 4.2	27.4 ± 4.3	23.4 ± 6.2
Digit symbol	26.4 ± 1.8	24.4 ± 2	22 ± 3.1
Mini Mental State Examination	28.3 ± 0.6	26.7 ± 0.6	27.6 ± 0.8
Trails A	73.6 ± 9.2	77 ± 8.6	83.3 ± 12
Trails B	163 ± 21.7	235.7 ± 40.2	259.4 ± 50
Digit span (maximum forward)	6.7 ± 0.2	6.7 ± 0.3	6.2 ± 0.4
Block span	4.57 ± 0.3	4.86 ± 0.5	4.1 ± 0.6
CANTAB ID/ED stages passed	8.14 ± 0.6	8.14 ± 0.6	8.3 ± 0.9
Spatial Working Memory strategy	35 ± 2.65	33.3 ± 2.78	36.8 ± 2.8
One-touch Tower of London accuracy	22.4 ± 2.1	26.7 ± 3.45	28.9 ± 3.4
Stroop colour naming (correct responses)	71.1 ± 5.8	65.7 ± 7.4	57.2 ± 13.6
Stroop word reading (correct responses)	46.4 ± 2.2	40.6 ± 3.21	41.6 ± 4.5
Stroop interference (correct responses)	27 ± 3.1	26.9 ± 2.6	24 ± 4.2

CANTAB ID/ED = Cambridge Neuropsychological Test Automated Battery, Intradimensional/Extradimensional shift tests.

age correction in asymptomatic Huntington's disease mutation carriers and symptomatic patients. While CAG repeat length influenced the rate of disease progression, the slopes of the correlation for asymptomatic mutation carriers and patients were significantly different, implying that the rate of disease progression is faster during the earlier asymptomatic stages of the disease.

In the present study, for the first time, the course of the disease was studied using ^{11}C-raclopride PET over three time-points. We found a mean annual loss of striatal D2 binding of 4.8% between the baseline and second scans, 29.17 $\pm$ 12 months apart, and a similar rate (5.2%) between the second and third scans 1 year later. Striatal D2 binding at each time-point was significantly correlated with disease duration, but not with age at onset. No correlation was observed between the annual rate of striatal D2 binding reduction and either age at onset or disease duration. These results suggest that in early clinically affected Huntington's disease patients, striatal loss of dopamine binding proceeds in a linear fashion, at least over an interval of 3 years. Consequently, ^{11}C-raclopride PET should provide a valuable approach for monitoring the efficacy of putative neuroprotective therapies in Huntington's disease, and a linear modelling design for longitudinal clinical trials might be used.

A methodological issue concerns the combination of PET data acquired with two different scanners. To address this, we normalized patient data to control groups scanned with the respective cameras to avoid systematic bias in the various correlations between BP values obtained with two different cameras and clinical variables.

SPM interrogation of ^{11}C-raclopride binding potentials across the whole brain volume, comparing normal subjects and Huntington's disease patients at the time of the baseline scan, localized significant reductions in caudate and putamen D2 receptor binding in the Huntington's disease cohort. It also revealed extra-striatal D2 receptor availability reduction in the Huntington's disease group. In particular, bilateral decreases of D2 binding were seen in the amygdala, and temporal (Brodmann areas 21 and 38) and frontal areas (Brodmann areas 9 and 10). In addition, we also found progression of extrastriatal loss of D2 binding in Huntington's disease over 3 years.

^{11}C-raclopride is known to have the potential to bind at extrastriatal D2 receptors, as binding of specific D2 agonists, such as ^{125}I-epidepride and ^{11}C-epidepride, to striatal and extrastriatal regions is inhibited by cold raclopride (Kessler *et al*., 1993; Langer *et al*., 1999). However, ^{11}C-raclopride has been reported to be an unsuitable tracer for measuring extrastriatal D2 receptor function using a ROI approach because of the low signal/noise ratio (Farde *et al*., 1988). SPM compares mean tracer binding between groups on a voxel-by-voxel basis. By smoothing the images, noise is reduced at the expense of spatial resolution, and this gives SPM additional power compared with ROI-based analysis to detect subtle local binding changes.

The locations of the extrastriatal reductions of ^{11}C-raclopride BP that we detected with SPM are, in fact, consistent with the distribution of D2 receptors reported in post-mortem studies of human brain (Joyce *et al*., 1991; Kessler *et al*., 1993; Hall *et al*., 1996). Dopamine D2 receptors were found in amygdala, thalamic nuclei, anterior cingulate and anterior hippocampus, and neocortex. In particular, the frontal cortex had low levels of D2 receptors, while the inferior and medial temporal cortex had relatively higher levels.

We acknowledge that our sample size was relatively small and our results require confirmation with a larger study; however, our SPM findings draw attention to the potential significance of the extrastriatal dopamine system in the pathophysiology of cognitive disturbances in early Huntington's disease. Decreases of D2 binding were found in areas such as: (i) amygdala, which is involved in emotional memory as well as spatial and motor learning (Rasia-Filho *et al*., 2000; Davis and Whalen, 2001); (ii) Brodmann area 38, corresponding to the anterior pole of the temporal lobe, which is involved in complex memory and imaging processes; and (iii) Brodmann area 21, corresponding to the middle temporal gyrus and concerned with auditory memory. Loss of D2 receptor binding in the amygdala and temporal cortex has also been reported in patients with Alzheimer's disease (Joyce *et al*., 1993, 1998). Bäckman and colleagues reported that cortical volumetric measurements and D1 binding correlated with cognitive measures in Huntington's disease, suggesting that a reduction in the number of D1 receptors in these areas may contribute to disturbances in cognitive processes (Bäckman *et al*., 1997).

Cognitive and psychiatric features in Huntington's disease, which may be present some time before the onset of the clinical movement disorder, are thought to be mainly due to a disruption at the level of the basal ganglia of re-entrant loop systems from the cortex. In groups of patients with Huntington's disease of various levels of severity, a recent cross-sectional study showed significant correlations between striatal dopamine binding and performances using the symbol digit test, the Stroop reading condition, the trail making test and, to a lesser extent, the Mini Mental State Examination (Sanchez-Pernaute *et al*., 2000).

In the present study, only the accuracy score of the one-touch Tower of London test correlated significantly with D2 binding potential in the striatum and putamen, such that deterioration in test performance was related to the extent of receptor reduction in those structures.

The accuracy score is an index of sequencing and planning ability, and is indicative of higher-order executive function known to be mediated by the prefrontal cortex. Our results show that deterioration in planning ability over time is accompanied by a reduction in D2 receptors in patients' basal ganglia structures, which project to the frontal lobe. This finding builds upon previous reports, where caudate and putamen binding correlated with performance on the Tower of London task in a cross-sectional paradigm involving both

presymptomatic and symptomatic Huntington's disease patients (Lawrence *et al*., 1998*a*, *b*). Other neuropsychological tests did not show such a correlation. This may be attributed to the demonstrated practice effect common in tests of executive function (Bachoud-Levi *et al*., 2001) and/or a lack of sensitivity of these tests given the relatively short period of time between the two assessment time-points. The relatively small number of patients in our cohort, and the narrow ranges of ^{11}C-raclopride BP values and/or clinical scores, should also be taken into account as possible factors for the lack of correlation between performances in neuropsychological tests and reduction in D2 receptors in the basal ganglia. Dysfunction in cortical post-synaptic receptors other than in basal ganglia may also be responsible for the impairment in neuropsychological performance in our Huntington's disease patients. Further investigations are therefore required to clarify this issue.

We observed a mean annual increase in UHDRS score of 6.3 (20%) by the time of the second scan in our Huntington's disease patients and of 5.3 (12.3%) by the time of the third scan. These results are in agreement with Siesling and colleagues who reported a significant decline in the motor score of 78 Huntington's disease patients over 2 years (Siesling *et al*., 1998), showing that the UHDRS is appropriate for repeated administration and allows comparisons of inter- and intra-individual clinical signs.

In a recent study, both UHDRS total motor score and bradykinesia showed a good correlation with putaminal ^{11}C-raclopride binding (Sanchez-Pernaute *et al*., 2000). We did not find any correlation between striatal ^{11}C-raclopride binding and single items of UHDRS, although we observed that cross-sectional UHDRS motor scores correlated with striatal ^{11}C-raclopride binding. In addition, in our patients there was no correlation between individual changes in UHDRS motor scores and changes in striatal binding. Our results are similar to those reported in an earlier study by Turjanski and colleagues (Turjanski *et al*., 1995). The heterogeneous population studied by Sanchez-Pernaute and colleagues, and the broad definition of bradykinesia they have used may account for the difference in findings (Sanchez-Pernaute *et al*., 2000).

The lack of correlation between individual changes in ^{11}C-raclopride binding and UHDRS motor scores suggests that this clinical scale does not reflect progression of striatal reduction of dopaminergic receptors alone. Sequential ^{11}C-raclopride PET and UHDRS might, therefore, reflect and monitor different aspects of the underlying neurodegenerative process in Huntington's disease.

Conclusion

In conclusion, our study indicates that striatal loss of D2 dopamine receptors in early clinically affected Huntington's disease patients progresses in a linear fashion over 3 years and that ^{11}C-raclopride PET is suitable for detecting restoration of dopamine receptor binding due to the effects of putative neuroprotective and/or cell implantation therapy in this disease.

Changes over time in clinical scores and in neuropsychological assessments, except for measures of planning, did not correlate with striatal D2 binding, probably reflecting different aspects of the underlying neurodegenerative process in Huntington's disease rather then striatal dopaminergic changes alone. These different approaches could, therefore, be used in combination in clinical trials to evaluate the different aspects of Huntington's disease.

In addition, by investigating changes in ^{11}C-raclopride binding potential across the whole brain, we have detected a decrease of D2 receptors in the cortical areas involved in cognition and memory in Huntington's disease patients. The extent of this impairment and its progression over time, however, need to be investigated further using larger cohorts.

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