

Hypothalamic involvement in Huntington's disease: an *in vivo* PET study

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Recent studies have shown alterations in metabolism, sleep and circadian rhythms as well as in several neuropeptides derived from the hypothalamic–pituitary axis in Huntington's disease patients; however, the pathology underlying these abnormalities is not known. Our aim was to assess *in vivo* D₂ receptor's loss/dysfunction and increases in microglial activation in the hypothalamus of symptomatic Huntington's disease patients and premanifest Huntington's disease gene carriers using PET with ¹¹C-raclopride (RAC), a specific D₂ receptor ligand and ¹¹C-(R)-PK11195 (PK), a marker of microglial activation. We have studied 9 symptomatic Huntington's disease patients (age = 46.8 ± 4.7 years; mean ± SD) and 10 premanifest Huntington's disease gene carriers (age = 41.9 ± 8.2 years; mean ± SD). RAC and PK findings for these subjects were compared with those of a group of normal controls (RAC, n = 9; PK, n = 10). In the symptomatic Huntington's disease group, we found a significant decrease (P = 0.0012) in mean hypothalamic RAC binding potential (BP) and a significant increase in mean hypothalamic PK BP (P = 0.0008). Similarly, a significant decrease (P = 0.0143) in mean hypothalamic RAC BP and a significant increase in mean hypothalamic PK BP (P = 0.0057) were observed in the premanifest Huntington's disease group. Hypothalamic RAC and PK BP values correlated with each other in combined Huntington's disease groups (r = -0.6180, P = 0.0048) but not with striatal RAC and PK BP values. Our data demonstrate, for the first time, significant D₂ receptor loss and microglia activation in the hypothalamus of Huntington's disease. These pathological changes occur very early in the course of the disease and may partly explain the development of commonly reported symptoms in Huntington's disease including progressive weight loss, alterations in sexual behaviour and disturbances in the wake–sleep cycle.

Keywords: hypothalamus; Huntington's disease; positron emission tomography (PET); raclopride; PK11195

Abbreviations: BP = binding potential; PET = positron emission tomography; PK = ¹¹C-(R)-PK11195; RAC = ¹¹C-raclopride; ROI = region of interest; SD = standard deviation; SPM = statistical parametric mapping; VOI = volume of interest

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Introduction

Several studies have now shown alterations of neuropeptides derived from the hypothalamic–pituitary axis in Huntington's disease, as well as a range of other hypothalamically mediated behaviours such as sleep, circadian rhythms and metabolism (Kirkwood *et al.*, 2001; Morton *et al.*, 2005; Goodman *et al.*, 2008; and for reviews see Petersén and Björkqvist, 2006; Aziz *et al.*, 2007). In respect of the former, in Huntington's disease patients, the regulation of cortisol and adrenocorticotrophic hormone (Heuser *et al.*, 1991; Leblhuber *et al.*, 1995; Björkqvist *et al.*, 2006), growth hormone and prolactin (Caraceni *et al.*, 1977; Chalmers *et al.*, 1977; Hayden *et al.*,

1977; Levy *et al.*, 1979), testosterone (indirect hypothalamic effect through the influence of follicle-stimulating hormone–luteinizing hormone) and luteinizing hormone (Markianos *et al.*, 2005) and vasopressin (Wood *et al.*, 2008) has been reported to be altered. In Huntington's disease transgenic mice, the regulation of oxytocin, vasopressin, cocaine-amphetamine regulated transcript, neuropeptide Y, prepro-thyroid-stimulating hormone-releasing hormone and prepro-somatostatin (Kotliarova *et al.*, 2005), gonadotropin-releasing hormone (Papalexi *et al.*, 2005) and adrenocorticotrophic hormone (Björkqvist *et al.*, 2006) have also been reported to be altered.

Additionally, Petersen *et al.* (2005) found remarkable atrophy and loss of orexin immunoreactive neurons in the lateral hypothalamus of R6/2 transgenic mice and significant atrophy and loss of orexin immunoreactive neurons in Huntington's disease patients.

Moreover, structural MRI imaging studies have shown grey matter density loss in the hypothalamus of Huntington's disease patients, which is already present in the early stages of the disease (Kassubek *et al.*, 2004; Douaud *et al.*, 2006).

Finally, abnormalities in metabolism (Goodman *et al.*, 2008; Van der Burg *et al.*, 2008), circadian rhythms and sleep (Morton *et al.*, 2005) have also now been reported in the R6/2 transgenic mice and Huntington's disease patients, pointing to significant hypothalamic pathology.

According to a post-mortem study of human brain tissue (Gurevich and Joyce, 1999), D₂ receptors are observed without substantial difference in binding densities in all major hypothalamic sites. D₃ receptor sites have also been observed across all hypothalamic sites but the densities are lower compared with those of the D₂ receptors. Hypothalamic D₂ mRNA expression levels are ~25% of those of caudate nucleus (Hurd *et al.*, 2001), which is one of the richest brain sites in expressing dopamine D₂ and D₃ receptors.

¹¹C-raclopride (RAC) with PET is a marker of the dopamine D₂ receptor availability and has extensively been used to measure striatal functional integrity in Huntington's disease, where the most affected neuron population within that area, the medium spiny neurons, express these receptors (Ginovart *et al.*, 1997; Antonini *et al.*, 1998; Andrews *et al.*, 1999; Pavese *et al.*, 2003).

¹¹C-(R)-PK11195 (PK) PET binds selectively to peripheral benzodiazepine receptors on activated microglia. Recently, two studies from our group have detected significant microglial activation in the striatum and cortical regions of symptomatic Huntington's disease patients (Pavese *et al.*, 2006) and premanifest Huntington's disease gene carriers (Tai *et al.*, 2007) and reported an inverse correlation between increasing striatal PK binding and

decreasing striatal dopamine D₂ binding, as measured with RAC PET.

In the present study, we hypothesized that dysfunction in the hypothalamic postsynaptic dopaminergic system, including the dopaminergic hypothalamohypophysial pathway, and microglia activation would be evident in both symptomatic and premanifest Huntington's disease subjects and may account for some of the clinical features listed above.

The functionality of D₂ receptors was assessed *in vivo* by using RAC PET, while PK PET was used to assess microglia activation.

Methods

Subjects

Nine clinically diagnosed symptomatic Huntington's disease patients (four males, five females; age = 46.8 ± 4.7 years; mean ± SD) and 10 premanifest Huntington's disease gene carriers (5 males, 5 females; age = 41.9 ± 8.2 years, mean ± SD) were studied.

For the patients in the symptomatic Huntington's disease group (Table 1), the disease duration ranged from 3 to 10 years, with a mean of 7.25 ± 4.5 years (mean ± SD) and they had an age of symptomatic onset of 40 ± 5.3 years (mean ± SD). All had an expanded CAG repeat in exon 1 of the gene that codes for Huntington's disease on chromosome 4. Three symptomatic Huntington's disease patients had genetic test reported as >36, whereas the exact length of the CAG repeat was available for the remaining symptomatic Huntington's disease patients. The symptomatic Huntington's disease patients' motor signs were rated using the Unified Huntington's Disease Rating Scale motor score by an independent investigator who was blinded to the PET data. Total functional capacity (TFC) scores for the patients, similarly assessed by an independent investigator, ranged from 3 to 13 (7.25 ± 3.3, mean ± SD). The only subject on medication was Subject 9 in patients' group, who was on the antidepressant fluoxetine on stable doses.

For the premanifest Huntington's disease group (Table 2), the number of CAG repeats ranged from 39 to 48, with a mean of 43.4 ± 6.5 (mean ± SD) and a 10 year probability of developing

Table 1 Characteristics of symptomatic Huntington's disease patients

Subject	Sex	Age in years	CAG repeats	CAG index ^a	UHDRS motor score ^b	TFC score	Disease duration (years) ^c	Treatment
1	M	44	41	242	3	3	7	No
2	M	44	>36	NA	4	13	6	No
3	M	47	45	446.5	4	9	9	No
4	M	49	44	416.5	31	5	8	No
5	F	39	>36	NA	30	NA	6	No
6	F	43	51	666.5	28	4	NA	No
7	F	49	42	318.5	14	9	10	No
8	F	52	>36	NA	16	9	9	No
9	F	54	43	405	12	6	3	Yes (Fluoxetine)

^aCAG index = age × (CAG repeats length – 35.5) (Penney *et al.*, 1997). ^bUHDRS motor score: Range (Best = 0, Worst = 124).

UHDRS = Unified Huntington's Disease Rating Scale (Huntington Study Group, 1996). ^cDisease duration measured from the time where the subject could remember his/her first symptoms.

NA = not available.

Table 2 Characteristics of premanifest Huntington's disease gene carriers

Subject	Sex	Age in years	CAG repeats	CAG index ^a	p10 years ^b
1	M	37	48	462.5	0.91
2	M	41	44	348.5	0.73
3	M	41	43	307.5	0.57
4	M	45	40	202.5	0.19
5	M	46	41	253	0.37
6	F	32	46	336	0.65
7	F	33	47	379.5	0.80
8	F	40	46	420	0.87
9	F	43	39	150.5	0.08
10	F	61	40	274.5	0.61

^aCAG index = age × (CAG repeats length – 35.5) (Penney *et al.*, 1997). ^bProbability of developing Huntington's disease in the following 10 years. Taken from age-CAG repeats correlation tables (Langbehn *et al.*, 2004).

the disease [derived from Langbehn *et al.* (2004), based on subject's age and CAG repeat length] ranging from 0.08 to 0.91, with a mean of 0.58 ± 0.56 (mean \pm SD).

Nine (all men; age = 41.2 ± 5.5 years; mean \pm SD) and 10 (8 men, 2 women; age = 56.7 ± 11.7 years; mean \pm SD) normal subjects, all in good health, served as controls for the RAC PET and PK PET studies, respectively.

None of these individuals was on any medication. Smoking, consumption of alcohol, coffee and other caffeinated beverages were not allowed at least 12 h before scanning. Eating and drinking was not allowed for 8 h before the scan.

Scanning

Each symptomatic Huntington's disease patient and premanifest Huntington's disease gene carrier underwent an RAC PET and a PK PET scan, no >2 months apart. Similarly, normal controls from each group had a PET scan with either RAC or PK tracer. All subjects from all groups had a volumetric MRI for co-registration purposes and volume analysis, performed on the same day with one of the PET scans.

PET scanning procedure

The subjects from all groups were scanned using an ECAT EXACT HR++ (CTI/Siemens 966; Siemens, Knoxville, TN) PET tomography scanner that has 23.4 cm total axial field of view. The camera of tomography has a transaxial spatial resolution of 4.8 ± 0.2 mm and an axial resolution of 5.6 ± 0.5 mm after image reconstruction (Spinks *et al.*, 2000). This high resolution, high sensitivity tomograph permits unprecedented interrogation of the function of small volume structures.

The mean tracer dose for RAC was 185 MBq (ranging from 182 to 190 MBq) and that for PK was 294 MBq (ranging from 292 to 300 MBq). Scanning began 30 s before tracer infusion (administered IV), generating 20 time frames over 65 min.

The study received ethical approval from the Ethics Committee of Hammersmith, Queen Charlotte's and Chelsea and Acton Hospitals. Permission to administer RAC and PK was obtained from the Administration of Radioactive Substances Advisory Committee (ARSAC) of the Department of Health, United Kingdom. All subjects gave informed written consent in accordance with the

Declaration of Helsinki (International Committee of Medical Journal Editors, 1991). RAC and PK tracers were fully supplied by Hammersmith Imanet plc, London, UK.

DATA analysis

Data for both RAC and PK PET were analysed using region of interest (ROI) approach. Volume of interest (VOI) analysis was also performed on individual subjects MRIs and statistical parametric mapping (SPM) analysis was used in RAC PET. Moreover, an investigation for correlations between PET and MRI findings and subject characteristics was performed.

ROI analysis

ROI image analysis was performed using ANALYZE software (version 8.0b, BRU, Mayo Foundation, Rochester, MN, USA) on a Sun Sparc Ultra 10 workstation (Sun Microsystems Inc., Santa Clara, CA, USA). Parametric images of RAC binding potentials (BPs) were generated from the dynamic RAC scans, using a basis function implementation of the simplified reference region compartmental model, with the cerebellum as the reference tissue (Gunn *et al.*, 1997). In order to obtain parametric images of PK, a simplified reference tissue model was used. We performed cluster analysis in order to identify a cluster of voxels having non-specific PK uptake and thus to provide a tissue input function, as it has been previously described (Banati, 2002; Pavese *et al.*, 2006; Tai *et al.*, 2007). The RAC and PK BPs, for each subject, were anatomically coregistered and resliced to the corresponding volumetric T1-weighted MRI. This was done using the Mutual Information Registration algorithm in the SPM2 software package (Wellcome Department of Cognitive Neuroscience, Institute of Neurology, London, UK) implemented in Matlab5.

The resolution of PET scan is sufficient to allow right and left hypothalamus to be traced and BP values for the left and right hypothalamus were obtained by defining ROIs on the individual MR images that were subsequently used to sample the parametric images. For each patient, the left to right averaged hypothalamic BPs were calculated. In addition, we have taken BPs, defining ROIs, for caudate nucleus and putamen using the same method, for correlation purposes.

ROIs were traced around caudate nucleus and putamen in all corresponding MRI slices where they could be seen. In order to draw the hypothalamus, we used Talairach and Tournoux (1988) stereotaxic atlas in combination with Duvernoy (1999) three-dimensional sectional atlas. Hypothalamus is located in ventral diencephalon and is lateral to the third ventricle, medial to internal capsule and between optic chiasm rostrally and mamillary bodies caudally. We used a sagittal orientation for tracing the hypothalamus. We drew the region, defining as an anterior border the anterior commissure and lamina terminalis; as a superior border the interventricular foramen and interthalamic adhesion; as a posterior border the posterior tuber and mamillary body and as an inferior border the optic chiasm, the optic nerve and the median eminence and infundibular recess. We used the sagittal orientation because it gave us more space for tracing this region. The second step was to make corrections using the coronal orientation. In this orientation we used as borders superiorly the fornix and the anterior commissure; inferiorly the optic chiasm and the suprachiasmatic recess and laterally the ventral pallidum and the anterior perforated substance.

VOI analysis

VOI image analysis was also employed in order to detect in the hypothalamic area extracted of the premanifest and symptomatic Huntington's disease subjects, possible grey matter volume loss, that may interfere with the interpretation of ROI findings. VOI image analysis was performed, using ANALYZE software, on the MRIs acquired from all groups (RAC normal controls, PK normal controls, premanifest Huntington's disease gene carriers and symptomatic Huntington's disease patients). The total hypothalamic volume (in mm³) extracted was calculated by sampling the sum of all slices taken on each individual subjects MRI, after the time of coregistration, meaning that the hypothalamic structural VOI and functional ROI were equal in dimensions.

SPM analysis

SPM2 software package implemented in Matlab5 was used in order to localize the presence and level of significant changes in D₂ receptor availability in parametric images of RAC BP at a voxel level. We used this method specifically to investigate possible hypothalamic decreases in RAC binding in symptomatic Huntington's disease patients and premanifest Huntington's disease gene carriers when compared with normal controls. In order to limit the area of interest and comparison, a hypothalamic mask was created, using ANALYZE software. This area represented a box (total of 439 voxels) that included all the sections where hypothalamus is visible.

The individual PET parametric images were spatially transformed to a normal RAC template already created in Montreal Neurological Institute (MNI) space with the SPM software (Meyer *et al.*, 1999). Then, they were 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 ratio for the statistical analysis. After the application of hypothalamic mask, comparisons were made across its voxel regions.

A between-group comparison of the findings of the 9 symptomatic Huntington's disease patients, 10 premanifest Huntington's disease gene carriers, and 10 normal controls was made. The contrasts were used to derive Z-scores on a voxel basis, using the general linear model (Friston *et al.*, 1995).

Statistical analysis

Statistical analyses of PET results and clinical data were performed using InStat (version 3.0b for Macintosh, University of Medicine and Dentistry, NJ, USA) and Excel (Microsoft Office 2004 for Macintosh, Microsoft Inc, Richmond, USA). Regarding mean comparisons and *P* significance, the non-parametric two-tailed *P*-value test from Mann–Whitney was used. In order to investigate correlations between PET results and clinical data, the non-parametric correlation (Spearman *r*) was applied.

Results

ROI analysis

Hypothalamic binding

¹¹C-raclopride. In the symptomatic Huntington's disease group, a significant decrease in mean hypothalamic RAC BP was observed in comparison with the group of normal controls (0.28 ± 0.02 versus 0.39 ± 0.01; mean ± SE; *P* = 0.0012). In the premanifest Huntington's disease group, the mean hypothalamic RAC BP was also significantly decreased as compared with the group of normal controls (0.31 ± 0.02 versus 0.39 ± 0.01; mean ± SE; *P* = 0.0143). There were no significant differences when the BP values for the symptomatic Huntington's disease patients were compared with the BP values for the premanifest Huntington's disease gene carriers (0.28 ± 0.02 versus 0.31 ± 0.02; mean ± SE; *P* > 0.1) (Table 3).

Table 3 ROI Analysis: Hypothalamic, caudate, putamen and striatal RAC BP in symptomatic Huntington's disease (HD) patients, premanifest Huntington's disease (pHD) gene carriers and normal controls

	RAC BP		
	Normal controls (<i>n</i> = 9)	Premanifest Huntington's disease gene carriers (<i>n</i> = 10)	Symptomatic Huntington's disease patients (<i>n</i> = 9)
Hypothalamus	0.39 ± 0.01 ^a	0.31 ± 0.02 pHD versus NC: <i>P</i> = 0.0143**	0.28 ± 0.02 HD versus NC: <i>P</i> = 0.0012 HD versus pHD: <i>P</i> > 0.1
Caudate	2.89 ± 0.04	2.14 ± 0.19 pHD versus NC: <i>P</i> = 0.0101	1.45 ± 0.12 HD versus NC: <i>P</i> < 0.0001 HD versus pHD: <i>P</i> = 0.0054
Putamen	3.26 ± 0.10	2.24 ± 0.20 pHD versus NC: <i>P</i> = 0.0041	1.53 ± 0.11 HD versus NC: <i>P</i> < 0.0001 HD versus pHD: <i>P</i> = 0.0113
Striatum	3.23 ± 0.09	2.21 ± 0.20 pHD versus NC: <i>P</i> = 0.0029	1.43 ± 0.11 HD versus NC: <i>P</i> < 0.0001 HD versus pHD: <i>P</i> = 0.0021

^amean ± SE.

**All *P*-values obtained using the two-tailed Mann–Whitney test.

NC = Normal Controls; pHD = Premanifest Huntington's disease gene carriers; HD = Symptomatic Huntington's disease patients.

Table 4 ROI Analysis: Hypothalamic, caudate, putamen and striatal PK BP in symptomatic Huntington's disease (HD) patients, premanifest Huntington's disease (pHD) gene carriers and normal controls

	PK BP		
	Normal controls (n = 10)	Premanifest Huntington's disease gene carriers (n = 10)	Symptomatic Huntington's disease patients (n = 9)
Hypothalamus	0.27 ± 0.03 ^a	0.44 ± 0.04 pHD versus NC: <i>P</i> = 0.0057**	0.47 ± 0.03 HD versus NC: <i>P</i> = 0.0008 HD versus pHD: <i>P</i> > 0.1
Caudate	0.08 ± 0.01	0.15 ± 0.02 pHD versus NC: <i>P</i> = 0.0062	0.20 ± 0.03 HD versus NC: <i>P</i> = 0.0013 HD versus pHD: <i>P</i> > 0.1
Putamen	0.18 ± 0.01	0.32 ± 0.04 pHD versus NC: <i>P</i> = 0.0031	0.34 ± 0.04 HD versus NC: <i>P</i> = 0.0060 HD versus pHD: <i>P</i> > 0.1
Striatum	0.13 ± 0.01	0.27 ± 0.04 pHD versus NC: <i>P</i> = 0.0007	0.31 ± 0.03 HD versus NC: <i>P</i> = 0.0003 HD versus pHD: <i>P</i> > 0.1

^amean ± SE.

**All *P*-values obtained using the two-tailed Mann–Whitney test.

NC = Normal Controls; pHD = Premanifest Huntington's disease gene carriers; HD = Symptomatic Huntington's disease patients.

¹¹C-(R)-PK11195. In the symptomatic Huntington's disease group, a significant increase in mean hypothalamic PK BP compared with the group of normal controls (0.47 ± 0.03 versus 0.27 ± 0.03; mean ± SE; *P* = 0.0008) was found. The mean hypothalamic PK BP in premanifest Huntington's disease group was also significantly increased compared with the group of normal controls (0.44 ± 0.04 versus 0.27 ± 0.03; mean ± SE; *P* = 0.0057). There were no significant differences when the BP values for the symptomatic Huntington's disease patients were compared with the BP values for the premanifest Huntington's disease gene carriers (0.47 ± 0.03 versus 0.44 ± 0.04; mean ± SE; *P* > 0.1) (Table 4).

Individual analysis was also performed for each subject in all groups and for both PET tracers. The normal range was defined as including values two standard deviation (SD) below or above the normal controls RAC and PK mean BP, respectively. Six out of 9 symptomatic Huntington's disease patients (67%) and 5 out of 10 premanifest Huntington's disease gene carriers (50%) had abnormally reduced hypothalamic RAC BP values (Fig. 1A). As for PK, 5 out of 9 symptomatic Huntington's disease patients (56%) and 5 out of 10 premanifest Huntington's disease gene carriers (50%) had abnormally increased hypothalamic PK BP values (Fig. 1B).

Striatal binding

Mean caudate, putamen and striatal RAC BP values were significantly lower in the symptomatic Huntington's disease patients and in the premanifest Huntington's disease gene carriers compared with the group of normal controls. Mean RAC BP values were also significantly lower in the symptomatic Huntington's disease patients when compared with those for the group of premanifest Huntington's disease gene carriers (Table 3).

Furthermore, mean caudate, putamen and striatal PK BP values were significantly higher in the symptomatic Huntington's disease patients and in the premanifest Huntington's disease gene carriers compared with the group of normal controls. The mean BP values for the symptomatic Huntington's disease patients were not significantly different from those for the premanifest Huntington's disease gene carriers (Table 4).

VOI analysis

The mean hypothalamic volume traced on the MRIs was reduced in premanifest and symptomatic Huntington's disease groups when compared with normal RAC and PK control groups, but the difference did not reach statistical significance. Furthermore, no significant difference was detected in the mean hypothalamic MRI volume extracted when the two normal control groups were compared with each other and when the premanifest Huntington's disease group was compared with the symptomatic Huntington's disease group (Table 5).

Statistical parametric mapping

Voxel-by-voxel analysis of RAC BP differences between the three groups confirmed results obtained with ROI analysis. SPM categorical comparisons within the hypothalamic mask localized clusters of significant decreases in the right and left hypothalamus in the premanifest Huntington's disease (*P* < 0.05) and in the symptomatic Huntington's disease (*P* < 0.01) groups, respectively, when compared with the group of normal controls (Table 6, Fig. 2).

Correlations

Lower hypothalamic RAC BP values correlated with higher hypothalamic PK BP values in combined premanifest and

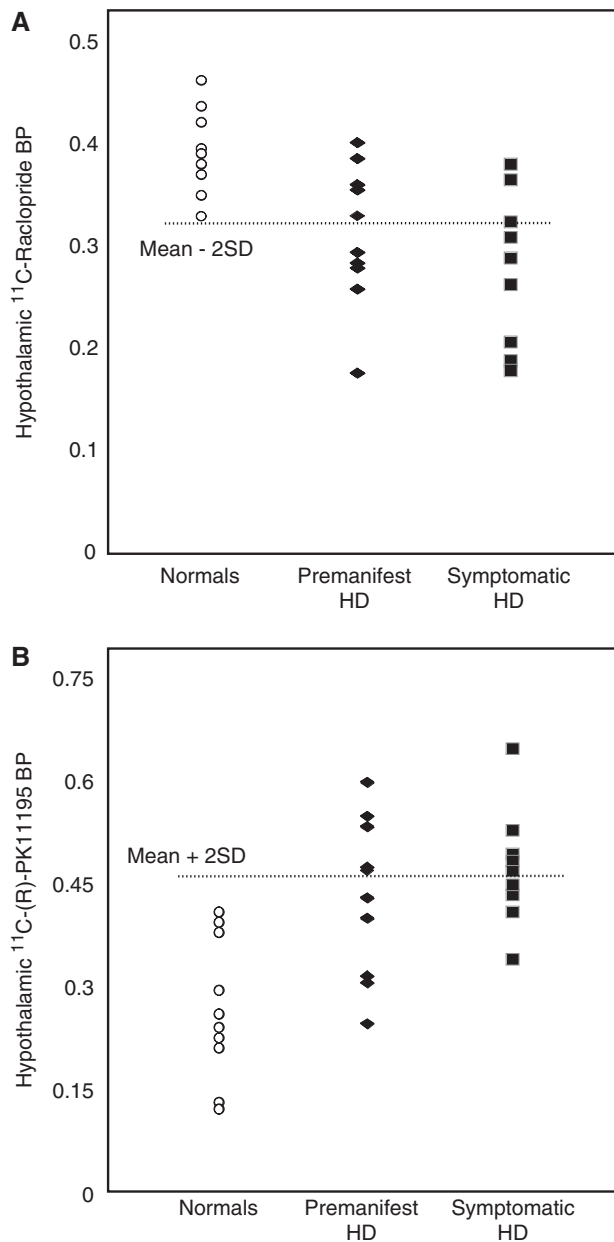


Fig. 1 Scatterplots showing individual BP values for all subjects in the three groups (normal controls, premanifest Huntington's disease gene carriers and symptomatic Huntington's disease patients) of (A) hypothalamic ^{11}C -raclopride (RAC) and (B) hypothalamic PK. Dotted line represents normal mean $- 2$ SD and normal mean $+ 2$ SD, respectively.

symptomatic Huntington's disease groups ($r = -0.6180$, $P = 0.0048$; Fig. 3). In the group of premanifest Huntington's disease gene carriers, lower individual hypothalamic RAC BP values correlated with higher CAG repeats ($r = -0.6780$, $P = 0.0312$) and marginally correlated with higher CAG index ($r = -0.5654$, $P = 0.0963$) and higher 10 year probability of Developing Huntington's disease ($r = -0.5957$, $P = 0.0734$). Hypothalamic BP values did not correlate with striatal BP values for both RAC ($r = 0.1989$, $P = 0.4142$) and PK ($r = 0.3047$, $P = 0.2047$) in

combined premanifest and symptomatic Huntington's disease groups. None of the PET findings in the hypothalamus correlated with the disease duration, the Unified Huntington's Disease Rating Scale motor scores or the TFC scores.

With regard to correlations between hypothalamic MRI volumes (mm^3) extracted and hypothalamic PET BP values, in the group of symptomatic Huntington's disease patients there was no correlation between MRI volumes and RAC BP values ($r = 0.5105$, $P = 0.1618$) and MRI volumes and PK BP values ($r = -0.1000$, $P = 0.8100$). Likewise, in the group of premanifest Huntington's disease gene carriers, there was no correlation between MRI volumes and RAC BP values ($r = 0.3526$, $P = 0.3129$) and MRI volumes and PK BP values ($r = -0.1879$, $P = 0.6073$).

Discussion

This is the first *in vivo* PET study showing hypothalamic dysfunctions in symptomatic Huntington's disease patients and premanifest Huntington's disease gene carriers. The findings indicate that there is a significant downregulation in postsynaptic dopamine D_2 and/or D_3 receptor binding, with evidence of increased activated microglia in symptomatic Huntington's disease patients. In this structure, similar results were observed in the premanifest Huntington's disease gene carriers, suggesting that these pathological changes occur early during the course of the disease.

Interestingly, in the premanifest Huntington's disease gene carriers, lower individual hypothalamic RAC BP values correlated with higher CAG repeats and marginally correlated with a higher 10 year probability of developing Huntington's disease. These negative correlations may indicate a relationship between the decrease in D_2 receptor availability in the hypothalamus and the clinical onset in Huntington's disease.

In the symptomatic Huntington's disease group, PET findings in the hypothalamus did not correlate with the disease duration, the Unified Huntington's Disease Rating Scale motor score or with the TFC scores. In both Huntington's disease groups, hypothalamic RAC and PK PET findings did not correlate with striatal RAC and PK PET findings.

Unified Huntington's Disease Rating Scale motor score is designed to assess motor symptoms. TFC scale measures more general activities like the capacity to maintain a usual job, the need for assistance in dealing with finance or domestic chores and the level of care needed for the patients. The lack of correlation between the clinical severity measured by these scales and the degree of hypothalamic RAC and PK binding abnormalities, together with the lack of correlation between striatal and hypothalamic RAC and PK binding abnormalities, suggests that severity of hypothalamic dysfunction occurs independent of general disease severity and progression of striatal dysfunction.

Table 5 VOI Analysis: Hypothalamic MRI volume analysis in RAC normal controls, PK normal controls, premanifest Huntington's disease gene carriers and symptomatic Huntington's disease patients

	Volume-of-interest analysis			
	Normal controls RAC (NC RAC) (n = 9)	Normal controls PK (NC PK) (n = 10)	Premanifest Huntington's disease gene carriers (pHD) (n = 10)	Symptomatic Huntington's disease patients (HD) (n = 9)
Hypothalamic volume (mm ³)	728.5 ± 96.02 ^a	70764 ± 71.79	653.52 ± 71.66	60989 ± 126.81
Volume difference (%) and P-value		NC PK versus NC RAC: -2.86% P = 0.6242**	pHD versus NC RAC: -10.29% P = 0.0947 pHD versus NC PK: -7.65% P = 0.1655	HD versus NC RAC: -16.28% P = 0.0625 HD versus NC PK: -13.81% P = 0.1128 HD versus pHD: -6.68% P = 0.4967

^amean ± SD.

**All P-values obtained using the two-tailed Mann–Whitney test.

NC = Normal Controls; pHD = Premanifest Huntington's disease gene carriers; HD = Symptomatic Huntington's disease patients.

Table 6 SPM Analysis: bilateral hypothalamic significant decreases in RAC BP in premanifest Huntington's disease gene carriers and in symptomatic Huntington's disease patients compared with the group of normal controls (uncorrected P threshold = 0.005)

z score	MNI coordinates			Talairach coordinates			Area	P-value corrected
	x	y	z	x	y	z		
Premanifest Huntington's disease gene carriers								
3.87	4.35	-2.38	-9.85	4.31	-2.72	-8.15	R hypothalamus	<0.05
3.23	-1.74	-2.00	-9.85	-1.72	-2.35	-8.17	L hypothalamus	<0.05
Symptomatic Huntington's disease patients								
4.86	4.35	-0.64	-11.59	4.31	-1.11	-9.69	R hypothalamus	<0.01
4.52	-1.74	0.23	-11.59	-1.72	-0.26	-9.74	L hypothalamus	<0.01

The lack of correlation between striatal and hypothalamic RAC and PK binding abnormalities could also suggest that the rate of degeneration of neurons bearing dopaminergic receptors and the activation of microglia and consequently the levels of neuroinflammation may be different in hypothalamic and striatal structures within the Huntington's disease brain, possibly as the result of local intrinsic factors specific to each structure.

In addition, the lack of correlation with the disease duration, the presence of hypothalamic RAC and PK BP abnormalities in premanifest Huntington's disease cases and the lack of significant differences in hypothalamic PET findings between the premanifest and symptomatic Huntington's disease group suggest that hypothalamic dysfunction may occur early in Huntington's disease and persist without further significant progression. However, longitudinal studies are needed to prove this.

The hypothalamus expresses moderate concentrations of D₂ receptors when compared with the striatum and their density appears without substantial variations across its major areas (Gurevich and Joyce, 1999). On the other hand, D₃ receptor density in hypothalamus is variable and significantly lower than that for D₂, leading to a presumption that despite the high affinity of RAC for both D₂ and D₃

receptors, our RAC PET results largely correspond to hypothalamic postsynaptic dopamine D₂ receptors.

The tuberoinfundibular dopaminergic system, originating from the A₁₂ group of dopaminergic neurons, mainly in arcuate and the adjacent periventricular nucleus, projects to the median eminence, where it regulates prolactin release (Cave *et al.*, 2005) through D₂ receptors (Gudelsky and Meltzer, 1989). Our PET findings would suggest that D₂ receptor loss or dysfunction in the tuberoinfundibular dopaminergic system may underlie the development of prolactin dysregulation in Huntington's disease that has been reported in the past (Caraceni *et al.*, 1977; Chalmers *et al.*, 1977; Hayden *et al.*, 1977; Levy *et al.*, 1979).

Similarly, a D₂ receptor loss or dysfunction in the tuberoinfundibular dopaminergic system may be the cause of the dysregulation of the hypothalamic-pituitary-gonadal axis that has previously been seen in Huntington's disease (Markianos *et al.*, 2005; Van Raamsdonk *et al.*, 2007). Furthermore, the hypothalamic-pituitary-adrenal axis has also been reported to be affected in both Huntington's disease mice and humans (Björkqvist *et al.*, 2006) and D₂ receptors in the paraventricular nucleus are known to control the regulation of oxytocin, which influences the activity of mesolimbic dopaminergic neurons in several

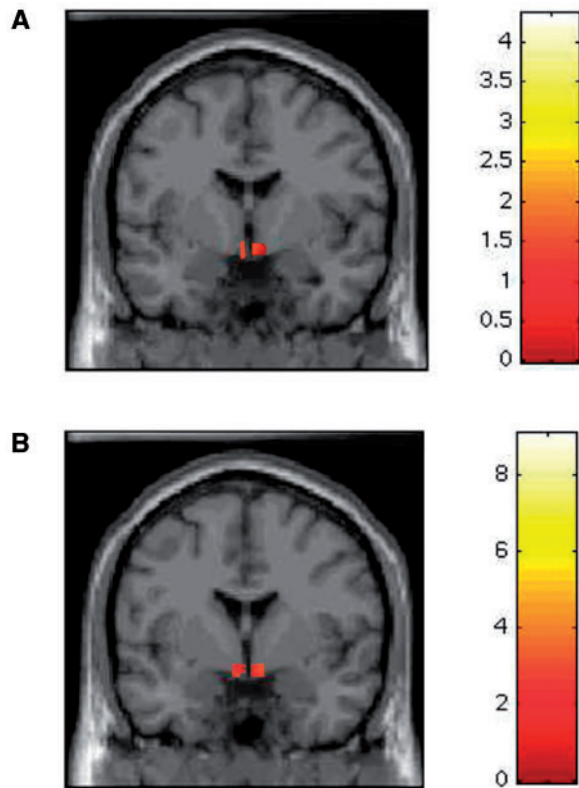


Fig. 2 Coronal sections of statistical parametric maps ($\gamma = -1$). Yellow-red areas represent voxel clusters with significant decreases in RAC binding within the hypothalamic mask in (A) premanifest Huntington disease gene carriers ($n = 10$) and in (B) symptomatic Huntington disease patients ($n = 9$) compared with a group of normal controls ($n = 9$). The colour stripe indicates z -values.

brain areas mediating the appetitive and reinforcing effects of sexual activity (Melis *et al.*, 2003; Argiolas and Melis, 2004; Succu *et al.*, 2007) that seems to be impaired in Huntington's disease (Fedoroff *et al.*, 1994).

Taking the aforementioned into consideration, it seems reasonable to suggest a close relationship between hypothalamic D_2 receptor loss or dysfunction and hormonal dysregulation in Huntington's disease, although more studies are needed in order to investigate further for this relationship.

It could be argued that the interpretation of this study's findings may be confounded by the possible effect of hypothalamic atrophy in the Huntington's disease groups. However, no significant differences in the hypothalamic volumes extracted were found when the two Huntington's disease groups were compared with each other or with the groups of normal controls. Moreover, lower hypothalamic RAC BP values or higher hypothalamic PK BP values did not correlate with lower hypothalamic volumes in both Huntington's disease groups, providing further evidence that the PET findings are independent of the reductions in volume.

An interesting finding that emerged from our study is the further confirmation of the close relationship between

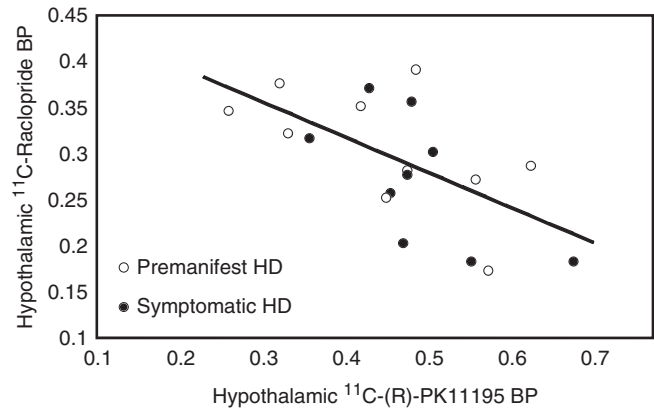


Fig. 3 Correlation between hypothalamic RAC and PK BP values ($r = -0.6180$, $P = 0.0048$) in combined premanifest and symptomatic Huntington's disease groups.

D_2 receptor dysfunction and microglia activation in Huntington's disease brain. In both symptomatic Huntington's disease patients and premanifest Huntington's disease gene carriers, lower individual hypothalamic RAC BP values correlated with higher hypothalamic PK BP values. This negative correlation, similar to that reported for striatal areas (Pavese *et al.*, 2006; Tai *et al.*, 2007), provides further evidence for the co-localization of D_2/D_3 dysfunction and increases in activated microglia, although how they relate to each other in terms of causality is not known.

The significant increase in microglia activation throughout the hypothalamus could also suggest that the pathology is widespread and may be not just confined to the D_2/D_3 receptor-bearing neurons. It is likely that the increase in microglia activation is a response to neuronal insult involving cells expressing D_2 receptors and/or cells such as orexin neurons expressing NMDA receptors that are known to be affected in Huntington's disease (Petersen *et al.*, 2005). The latter implicates the involvement of an NMDA-receptor mediated excitotoxicity (Tikka and Koistinaho, 2001), which has been suggested to play a role in the pathogenesis of Huntington's disease (Beal, 2000) and may contribute to propagate neuronal death.

Activated microglia could contribute or cause neuronal cell death in the hypothalamus by producing cytotoxic substances such as proinflammatory cytokines (TNF- α , IL-1 β , etc) and reactive oxygen species (superoxide, hydrogen peroxide, etc).

In conclusion, endocrine dysfunction, progressive weight loss, sleep disturbances, depression, sexual and autonomic dysfunction are common non-motor manifestations of Huntington's disease progression. In the past, research has mainly focused on the neurological features of Huntington's disease but the relief from these non-motor manifestations can be equally important for the patient's life.

This study highlights, through demonstrating an *in vivo* loss or dysfunction of D_2 receptors and an increase of

activated microglia, that the hypothalamus is involved in Huntington's disease.

Hypothalamic dysfunction may be partly responsible for the non-motor manifestations seen in Huntington's disease but further studies are needed to better understand the relation of the functional changes demonstrated in this study, to non-motor clinical parameters and how these can be best modified to improve patients' symptoms and signs and consequently improve their quality of life.

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