Eur J Nucl Med Mol Imaging (2007) 34:1071–1081
DOI 10.1007/s00259-006-0344-7

ORIGINAL ARTICLE

Identification by [^{99m}Tc]ECD SPECT of anterior cingulate hypoperfusion in progressive supranuclear palsy, in comparison with Parkinson's disease

Andrea Varrone · Marco Pagani · Elena Salvatore · Dario Salmaso · Valeria Sansone · Marianna Amboni · Flavio Nobili · Giuseppe De Michele · Alessandro Filla · Paolo Barone · Sabina Pappatà · Marco Salvatore

Received: 31 May 2006 / Accepted: 20 November 2006 / Published online: 24 January 2007

Abstract
Purpose Progressive supranuclear palsy (PSP) is an akinetic-rigid syndrome that can be difficult to differentiate from Parkinson's disease (PD), particularly at an early stage. [^{99m}Tc]ethyl cysteinate dimer (ECD) SPECT could represent a widely available tool to assist in the differential diagnosis. In this study we used voxel-based analysis and Computerised Brain Atlas (CBA)-based principal component analysis (PCA) of [^{99m}Tc]ECD SPECT data to test whether: (1) specific patterns of rCBF abnormalities can differentiate PSP from controls and PD; (2) networks of dysfunctional brain regions can be found in PSP vs controls and PD.

Methods Nine PD patients, 16 PSP patients and ten controls were studied with [^{99m}Tc]ECD SPECT using a brain-dedicated device (Ceraspect). Voxel-based analysis was performed with statistical parametric mapping. PCA was applied to volume of interest data after spatial normalisation to CBA.
Results The voxel-based analysis showed hypoperfusion of the anterior cingulate and medial frontal cortex in PSP compared with controls and PD. In PSP patients the rCBF impairment extended to the pre-supplementary motor area and prefrontal cortex, areas involved in executive function and motor networks. Compared with PSP patients, PD patients showed a mild rCBF decrease in associative visual areas which could be related to the known impairment of visuospatial function. The PCA identified three principal components differentiating PSP patients from controls and/or PD patients that included groups of cortical and subcortical brain regions with relatively decreased (cingulate cortex, prefrontal cortex and caudate) or increased (parietal cortex) rCBF, representing distinct functional networks in PSP.
Conclusion Anterior cingulate hypoperfusion seems to be an early, distinct brain abnormality in PSP as compared with PD.

Keywords Brain mapping · Progressive supranuclear palsy · Parkinson's disease · Regional blood flow · Voxel-based analysis · Principal component analysis

A. Varrone · V. Sansone · S. Pappatà · M. Salvatore
Biostructure and Bioimaging Institute, National Research Council/
Department of Biomorphological and Functional Sciences,
University Federico II,
Napoli, Italy

M. Pagani · D. Salmaso
Institute of Cognitive Sciences and Technologies,
National Research Council,
Rome and Padua, Italy

E. Salvatore · M. Amboni · G. De Michele · A. Filla · P. Barone
Department of Neurological Sciences, University Federico II,
Napoli, Italy

F. Nobili
Clinical Neurophysiology,
Department of Endocrinological and Metabolic Sciences,
University of Genoa,
Genoa, Italy

A. Varrone (✉)
Department of Clinical Neuroscience,
Psychiatry Section, R5:02 Karolinska Hospital,
17176 Stockholm, Sweden
e-mail: Andrea.Varrone@ki.se

Introduction

Progressive supranuclear palsy (PSP) is an akinetic-rigid syndrome characterised by the combination of early postural instability, supranuclear vertical gaze palsy, par-

kinsonism that does not benefit from levodopa therapy, pseudobulbar palsy and dementia [1]. Although symptoms such as postural instability with early falls and vertical gaze palsy are specific for PSP [2, 3], the differential diagnosis from Parkinson's disease (PD) can be difficult, particularly at an early stage. Williams et al. [4] have recently reported that more than 50% of the pathologically confirmed cases of PSP are characterised by early onset of postural instability and falls, supranuclear vertical gaze palsy and cognitive dysfunction, while approximately one-third of PSP patients present with asymmetrical onset, tremor and a moderate therapeutic response to levodopa and are frequently confused with PD patients.

Functional neuroimaging techniques may contribute to the diagnostic assessment of PSP. Initial [^{18}F]FDG and [^{15}O]CO_2 positron emission tomography (PET) studies reported decreased metabolism or regional cerebral blood flow (rCBF) in the frontal cortex and basal ganglia in PSP patients [5–8]. These findings have been confirmed and extended by more recent FDG-PET studies with voxel-based analysis that have provided a more specific identification of the cortical and subcortical regions selectively involved in PSP [9–11] as compared with PD [12]. These studies have shown involvement of the anterior cingulate, medial frontal cortex, motor and premotor cortex, midbrain and basal ganglia in PSP.

Potentially useful PET/single-photon emission computed tomography (SPECT) radiopharmaceuticals for the differential diagnosis between PD and PSP also include [^{11}C] diprenorphine, [^{11}C]MP4A, and dopamine transporter (DAT) or dopamine D_2 receptor ligands. Reduced striatal opioid receptors [13] and reduced thalamic acetylcholinesterase activity [14] have been found in PSP patients, but not in PD patients. However, [^{11}C]diprenorphine and [^{11}C] MP4A are not widely available tracers in routine clinical use. SPECT ligands for DAT and D_2 receptors are currently available and may contribute to the diagnosis of PSP versus PD. The loss of striatal DAT appears to be more uniform in PSP than in PD patients, and the putamen-to-caudate ratio (lower in PD than PSP) could be used as a measure to differentiate the two conditions [15, 16]. However, there could be overlap between PD and PSP, and this pattern might not be discriminative in all cases. Reduced striatal dopamine D_2 receptors have been reported in PSP patients [7, 17], but there may be overlap of receptor density values with PD in some patients [7, 18], particularly if they are treated with dopaminergic drugs [7].

Although rCBF SPECT represents a widely available nuclear medicine procedure for the study of neurodegenerative disorders, there are limited data on the study of rCBF abnormalities in PSP. A [^{99m}Tc]HMPAO SPECT study by Defebvre et al. [19] reported decreased rCBF in the frontal cortex of both PSP and PD patients, with no differentiation between the two disorders. A more recent [^{99m}Tc]ethyl cysteinate dimer (ECD) SPECT study [20] has directly compared PSP and corticobasal degeneration, reporting increased asymmetry of cortical and subcortical rCBF in corticobasal degeneration without specifying distinct changes occurring in PSP. These studies used conventional region of interest analysis and did not investigate whether rCBF changes could be detected in specific brain areas in PSP. One recent double tracer SPECT study with [^{123}I]FP-CIT/[^{99m}Tc]ECD and voxel-based analysis in parkinsonian patients [21] has reported a pattern of rCBF reduction in PSP versus PD that included the left prefrontal and supplementary motor area (SMA) cortex, left peri-insular cortex, left caudate, bilateral rostral anterior cingulate, thalamus and mesencephalon. This study lacked a control group with which PD or PSP patients could be compared and did not evaluate whether specific networks of dysfunctional brain regions were present in PSP.

Multivariate spatial covariance methods were proposed in early studies of PD and parkinsonism [22, 23], and more recently principal component analysis (PCA)-based methodologies have been implemented in PD [24]. Both voxel-based analysis with statistical parametric mapping (SPM) and PCA are established methods to investigate independently functional abnormalities of the brain. In this [^{99m}Tc] ECD SPECT study we aimed to combine the two methods to evaluate whether: (1) specific patterns of rCBF abnormalities can differentiate PSP from controls and PD at the voxel level with SPM; (2) networks of dysfunctional brain regions can be found in PSP versus controls and PD with PCA. The findings from both analyses could be complementary in highlighting distinct rCBF abnormalities and specific dysfunctional networks associated with PSP.

Materials and methods

Subjects

Among consecutive patients referred to the Department of Neurological Sciences of the University Federico II of Napoli for evaluation of parkinsonism, 25 were included in this study. Patients gave their informed consent prior to inclusion in the study. They underwent clinical evaluation in the off-state, including the Unified Parkinson's Disease Rating Scale (UPDRS). Nine patients satisfying the clinical criteria for PD according to the UK PD Brain Bank [25] were included. They were compared with 16 patients diagnosed as having probable or possible PSP according to the NINDS-SPSP criteria [2, 3]. Patients did not have a history of stroke or psychiatric disorders and did not show severe white matter ischaemic lesions at magnetic resonance imaging. Demographic and clinical data of PD and PSP patients are reported in Table 1.

Table 1 Demographic and clinical data of patients with PD and PSP

Group	Gender	Age (yr) (mean±SD)	Disease duration (yr) (mean±SD)	UPDRS[a] (mean±SD)	More affected side[b]	Diagnosis[c]	Cognitive impairment[d]	Gaze palsy[e]	Bulbar signs[e]
PD	6M/3F	64±6	2.2±1.7	22.3±11.7	2R/7L	–	–	–	–
PSP	9M/7F	67±6	3.1±1.4	43.7±20.4	3R/5L/8B	Possible (4)/ probable (12)	− (3) / + (5) / ++ (5) / +++ (3)	+ (12) / SVS (4)	+ (14)/− (2)

[a] *UPDRS* Unified Parkinson's disease rating scale
[b] *R* right, *L* left, *B* bilateral
[c] Diagnosis of probable or possible PSP according to NINDS-SPSP criteria; number of patients is shown in parentheses
[d] Cognitive impairment was evaluated on clinical assessment or MMSE when available: − = absent; + = mild; ++ = moderate; +++ = severe; number of patients is shown in parentheses; none of the PD patients had clinical evidence of cognitive deterioration
[e] Gaze palsy and bulbar signs: + = present; − = absent; *SVS* slow vertical saccades; number of patients is shown in parentheses

Ten subjects (four males, six females, age 59±16 years) served as controls. They were otherwise healthy except that in three of them the SPECT study was done to evaluate functional integrity of rCBF in headache (two) or peripheral neuropathy (one) and was found to be normal. Subjects with headache had been free of any episodes for more than 1 week prior to the SPECT study. Four control subjects were studied at the University Federico II of Napoli. Six subjects were recruited at the Unit of Clinical Neurophysiology of the University of Genova and belonged to a database of 37 healthy control subjects aged from 52 to 78 years who underwent brain SPECT examination with [^{99m}Tc]ECD (six) and [^{99m}Tc]HMPAO (31). These subjects were carefully screened and found to be healthy based on medical history, clinical examination, MMSE score, laboratory test, urinalysis and negative history for neuropsychiatric disorders.

Methods

SPECT studies

All subjects were studied with [^{99m}Tc]ECD (Neurolite Bristol-Myers Squibb Medical Imaging). The radiopharmaceutical was prepared according to the manufacturer's guidelines. Subjects received an intravenous injection of 740–1,110 MBq of the tracer in a dimly lit room with the eyes closed. Dopaminergic drugs were withdrawn for at least 12 h before the injection. Patients were allowed to take their medications only 15 min after tracer injection, if needed to improve their comfort during performance of the SPECT acquisition.

SPECT studies were performed 40 min after the injection using a brain-dedicated camera equipped with a circular low-energy high-resolution collimator (Ceraspect, Digital Scintigraphics, D.S.I., Waltham, MA, USA). Studies were acquired for 30 min in step-and-shoot mode (120 steps, 3° steps, 15 s per step) using a symmetrical window of 20% centered around 140 keV and a 128×128 matrix. Images were reconstructed using a bidimensional Butterworth filter (cut-off 0.9 cm^{-1}, order 10) and were corrected for attenuation assuming uniform attenuation within the skull, applying a zero order attenuation factor (μ 0.12 cm^{-1}) and Chang's algorithm. Sixty-four transaxial slices of 1.67×1.67×1.67 mm were obtained. No scatter correction was applied and no attempt was made to obtain quantitative measures of rCBF. The ten control subjects from the two centres were studied using the same SPECT camera and the same injection and acquisition protocols.

Voxel-based analysis

Voxel-based analysis was performed using the 2000 version of SPM (SPM2, Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College London, UK) implemented in Matlab 6.1 on a Windows XP PC workstation. Images of relative tracer distribution were spatially normalised in the stereotactic Montreal Neurological Institute (MNI) space to a predefined SPECT template available in SPM2 (voxel size: 2×2×2 mm), using a 16-parameter affine (non-linear) transformation. After normalisation, images were smoothed with a Gaussian filter (12 mm) to account for individual gyral differences and brain anatomy. Images were globally normalised using proportional scaling [21, 26] to remove confounding effects due to global CBF changes, with a threshold masking of 0.8.

The SPM analysis was performed with a single-subject condition and covariate model. Sex and age were modelled as nuisance variables, and disease groups were modelled as different conditions. Significant differences between the groups were set at the threshold of $p<0.001$ ($p<0.005$ when specified) for voxel height, uncorrected, and $p<0.05$ for cluster extent, after Bonferroni's correction for multiple comparisons. Spatial coordinates of the SPM results were reported in the MNI space.

Principal component analysis

Computerised Brain Atlas (CBA, Applied Medical Imaging, Uppsala, Sweden) is a software tool for analysis of neuro-

imaging data [27]. All image sets were spatially normalised into the stereotactic space of the atlas by using the global polynomial transformation [28]. The procedure consists of translations, rotations and linear scaling along and around each of the three image axes, plus 18 non-linear shape-deforming parameters, which makes it possible to individualise the shape of the brain.

For evaluation and statistical analysis of the reformatted data sets, 13 volumes of interest (VOIs) were selected in each hemisphere, in order to cover the cortical and subcortical brain structures possibly involved in movement disorders on the basis of current literature [11, 21]. The set of VOIs included cortical regions corresponding to Brodmann areas (BA) 9, 10, 11, 47 (prefrontal and orbitofrontal cortex), 23 (posterior part of the cingulate gyrus), 24, 32 (anterior part of the cingulate gyrus), 7, 39 and 40 (parietal cortex) and deep grey nuclei (caudate, putamen, thalamus) of both hemispheres. Brain VOIs covering the highest and lowest cortical and brainstem regions were not examined because, given the axial field of view of the Ceraspect camera, the entire brain was not included in all subjects.

The brain activity was normalised to a global preset value of 50 uptake units. In one control the spatial normalisation of the brain with CBA was not satisfactory and this subject was not included in the PCA.

CBF data were submitted to analysis of variance (ANOVA) in two steps: the first one by considering only individual VOIs and the second one by using the principal components as identified by PCA.

PCA was performed considering all 13 VOIs for each hemisphere and all 34 subjects. PCA takes a set of correlated variables and clusters them into common factors, or principal components (PCs), such that variables within each factor are highly correlated, but factors are uncorrelated. PCs can be treated as new variables and their values computed for each case. The values in which PCs are expressed are known as factor scores or component scores (CSs). For practical purposes it is preferable, however, to use an imperfect estimate (coarse component scores, CCSs) generated by summing all VOIs selected as the most representative of a given factor. An advantage to using CCSs is that they are more easily interpreted than CSs and can also be compared between studies [29]. CCS values were then standardised, placing raw data on a 0–1 scale. The number of factors was determined by the number of eigenvalues greater than 1. We considered the variables representative of a factor to be those with factor loadings greater than 0.5 (absolute value).

Data were analysed through ANOVA considering groups as between subjects and VOIs or PC scores as within subjects. Post hoc tests were performed with Tukey HSD test. Statistical significance was set at $p<0.05$.

Results

There were no differences in age and disease duration between PD and PSP patients. PSP patients were clinically more severely impaired than PD patients on the basis of UPDRS assessment ($p=0.009$ by two-tailed unpaired t test (Table 1), as expected from the different disease course.

SPM2 analysis

PSP patients were compared with the control group and the PD group (Table 2) to highlight those brain regions with a relative rCBF decrease (PSP < controls and PSP < PD) or increase (PSP > controls and PSP > PD). In PSP patients there was a relative rCBF decrease in the right anterior cingulate (BA32) and the right medial frontal gyrus/pre-supplementary motor area (pre-SMA, BA6), extending to the middle frontal gyrus (BA6/9) bilaterally ($p<0.005$), compared with controls (Fig. 1), and in the anterior cingulate (BA32/24) bilaterally and the left medial frontal gyrus (BA9), compared with PD patients (Fig. 2). Relative rCBF increase in PSP patients was found in the middle temporal gyrus (BA22), the fusiform gyrus (BA20) and the caudate tail of the left hemisphere, compared with controls, and in the left middle occipital gyrus (BA37) and the cuneus (BA18) bilaterally ($p<0.005$), compared with PD patients. No differences were found between PD patients and controls (PD < controls and PD > controls).

VOI and PCA analyses

An overall ANOVA was performed considering as variables VOIs (13) and hemispheres. This analysis showed a significant VOI × group [$F(24,372)=3.554$, $p<0.001$] interaction but no hemisphere × group or VOI × hemisphere × group interactions. Therefore ANOVA for single VOIs was performed averaging, for each VOI, left and right data.

ANOVA for single VOIs revealed the following significant effects: anterior cingulate gyrus (BA24) [$F(2,31)=14.124$, $p<0.001$], anterior cingulate gyrus (BA32) [$F(2,31)=13.368$, $p<0.001$], parietal cortex (BA40) [$F(2,31)=6.012$, $p<0.01$)], posterior cingulate gyrus (BA23) [$F(2,31)=4.871$, $p<0.05$)]. As compared with controls, PSP patients had relatively lower rCBF in the anterior cingulate (BA24, BA32) and the posterior cingulate (BA23) gyrus and higher rCBF in the parietal cortex (BA40). As compared with PD patients, they had relatively lower rCBF in the anterior cingulate gyrus (BA24 and BA32) and higher rCBF in the parietal cortex (BA40). No significant differences were found between controls and PD patients.

PCA identified seven factors including cortical areas and deep brain nuclei (Table 3) explaining 81% of the total

Table 2 Results of SPM analysis examining relative rCBF changes in PSP patients vs controls or PD patients

Contrast	BA	Region	Coordinates (mm)			*Z* score
			x	*y*	*z*	
PSP < controls						
Cluster level $p<0.001$, $k=1,230$	32	R anterior cingulate	4	24	30	4.74
	6	R medial frontal gyrus/ pre-SMA	4	12	50	3.54
Cluster level $p=0.062$, $k=366$	9	R middle frontal gyrus	30	18	36	4.07
PSP < PD						
Cluster level $p=0.004$, $k=783$	32	R anterior cingulate	2	28	30	3.85
	24	R anterior cingulate	4	30	26	3.82
	32	L anterior cingulate	−2	22	36	3.96
	9	L medial frontal gyrus	−2	44	24	3.35
PSP > controls						
Cluster-level $p=0.002$, $k=722$	22	L middle temporal gyrus	−46	−40	−2	4.25
	20	L fusiform gyrus	−46	−30	−20	3.83
		L Caudate tail	−34	−26	−8	3.62
PSP > PD, $p<0.005$						
Cluster level $p=0.028$, $k=1,224$	37	L middle occipital gyrus	−36	−66	−2	4.0
	18	R cuneus	16	−78	18	3.24
	18	L cuneus	−16	−82	14	3.62

Significance refers to the uncorrected $p<0.001$, unless otherwise reported. For each contrast, *p* values of relevant clusters corrected for multiple comparisons along with the number of voxels (*k*) are reported.
BA Brodmann area; coordinates are reported in MNI space; *R* right, *L* left

variance. Three of the seven PCs revealed a significant group effect (Fig. 3). The first such component (PC1) included the anterior cingulate gyrus (BA24) and the posterior cingulate gyrus (BA23) of both hemispheres and the left caudate. This PC (Table 3) differentiated PSP from controls ($p<0.001$) and from PD ($p<0.05$). PSP patients showed a relatively decreased rCBF compared with the other two groups. The second factor discriminating between groups (PC2) included the parietal cortex (BA39 and BA40) of both hemispheres (Table 3). In this PC there was a significant PSP versus PD group effect ($p<0.05$), while the PSP versus control comparison did not reach statistical significance ($p=0.054$). In this PC, PSP patients showed a relatively higher rCBF than PD patients. The third factor (PC6) included the anterior cingulate gyrus (BA32) and the prefrontal cortex (BA9) of both hemispheres (Table 3). This PC differentiated PSP from controls ($p<0.05$) and PD ($p<0.01$). In PSP patients rCBF was relatively lower than in controls and PD patients.

Discussion

This [^{99m}Tc]ECD SPECT study investigated the relative impairment of rCBF in PSP patients compared with PD patients and controls. Patients with a recent onset (average 2–3 years) of parkinsonism were included in the study to assess the capability of the technique to identify early rCBF changes in PSP and PD patients. Voxel-based analysis with SPM2 was performed on the SPECT data to highlight discrete brain regions showing relative rCBF changes. PCA was performed to evaluate whether networks of brain regions differentially affected in PSP and PD could be identified. PCA was performed on predefined VOIs rather than at the voxel level owing to the small number of available scans.

SPM analysis

The voxel-based analysis demonstrated a reduction in rCBF in the anterior cingulate cortex of PSP patients, extending to the pre-SMA and to the middle frontal cortex bilaterally. The finding of anterior cingulate impairment in PSP has already been reported in previous FDG-PET studies using voxel-based analysis in comparison with control subjects [9, 10, 30], patients with Alzheimer's disease (AD) [10], patients with corticobasal degeneration [30] and PD patients [12], as well as in a recent [^{123}I]FP-CIT/[^{99m}Tc] ECD SPECT study using the same voxel-based approach [21]. The anterior cingulate is a cortical area implicated in the executive control of conscious actions [31] and has been reported to be activated in Stroop and Stroop-like tasks [32, 33]. There is a functional subdivision of the anterior cingulate cortex. According to Devinsky et al. [34] a more ventral subdivision corresponds to the affective component, including BA 25, 33 and rostral 24, and has connections to the amygdala, nucleus accumbens, orbitofrontal cortex and autonomic brainstem nuclei. The cognitive component corresponds to a more dorsal subdivision, including caudal areas 24′ and 32′ and the cingulate motor area, and has connections with the parietal cortex, posterior cingulate, SMA and dorsolateral prefrontal cortex [35]. The most posterior part of the cingulate cortex (the caudal cingulate zone, or cingulate motor area) projects to the spinal cord and red nucleus and has premotor functions. The

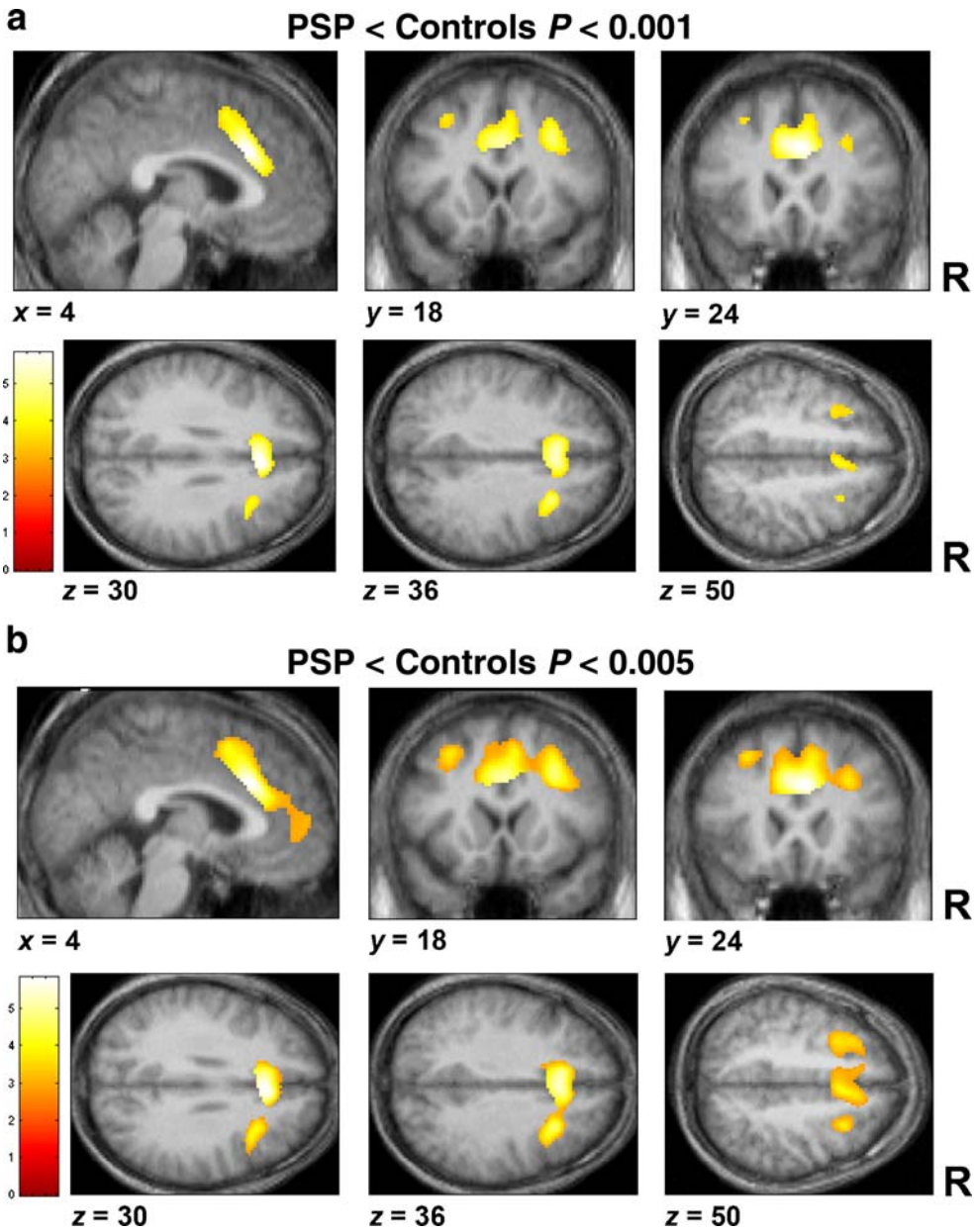

Fig. 1 Statistical parametric maps showing the regions of significant decrease in rCBF in PSP patients compared with controls, obtained by SPM2 analysis and overlaid on an average T1-weighted MR scan from ten healthy controls. *R* refers to the right side of the brain; *x*, *y* and *z* refer to the corresponding level of the sagittal, coronal and axial planes. Results are shown at the threshold of $p<0.001$ (**a**) and $p<0.005$ (**b**) to demonstrate the relative impairment of right anterior cingulate cortex, right pre-SMA and middle frontal cortex bilaterally. At $p<0.005$, there was a single significant cluster ($p<0.001$, $k=3,974$) that included the right anterior cingulate (BA32; $x,y,z=4,24,30$; Z score=4.74), the right medial frontal gyrus/pre-SMA (BA6, $x,y,z=4,12,50$; Z score=3.54), the right middle frontal gyrus (BA9; $x,y,z=30,18,36$; Z score=4.07) and the left middle frontal gyrus (BA6; $x,y,z=-30,12,48$; Z score=3.41)

region of the anterior cingulate cortex immediately anterior to this (posterior rostral cingulate zone) has been found to be activated in studies using Stroop-like tasks and in studies involving cognitive tasks in non-psychiatric subjects [36]. The portion of the anterior cingulate (BA32) highlighted in the present study by SPM analysis corresponds to this posterior rostral cingulate zone, which is mainly activated in executive tasks. Thus, the anterior cingulate impairment likely underlies the executive dysfunction frequently observed in PSP patients [10]. This is also suggested by the fact that the rCBF impairment observed in PSP also extended to pre-SMA (BA6) and the middle frontal gyrus (BA9), areas involved in executive function and motor networks. In addition, word generation studies in normal subjects have shown activation of BA32 and of BA6 (SMA), with activation peaks at average Talairach coordinates ($x,y,z=3,15,46$) very close to those found in the present study [35]. However, anterior cingulate impairment might not be related only to executive dysfunction. The stereotactic coordinates corresponding to the anterior cingulate found in the present study also overlap with those found ($x,y,z=0,2,38$) in apathetic AD patients, in an SPM SPECT study [37]. PSP patients also have behavioural symptoms including apathy, and we cannot exclude the possibility that part of the anterior cingulate impairment is related to the presence of apathy since patients were not tested specifically for this symptom. It is not well known whether the involvement of the anterior cingulate cortex in PSP represent a primary neuropathological abnormality or a secondary process due to functional disconnection with

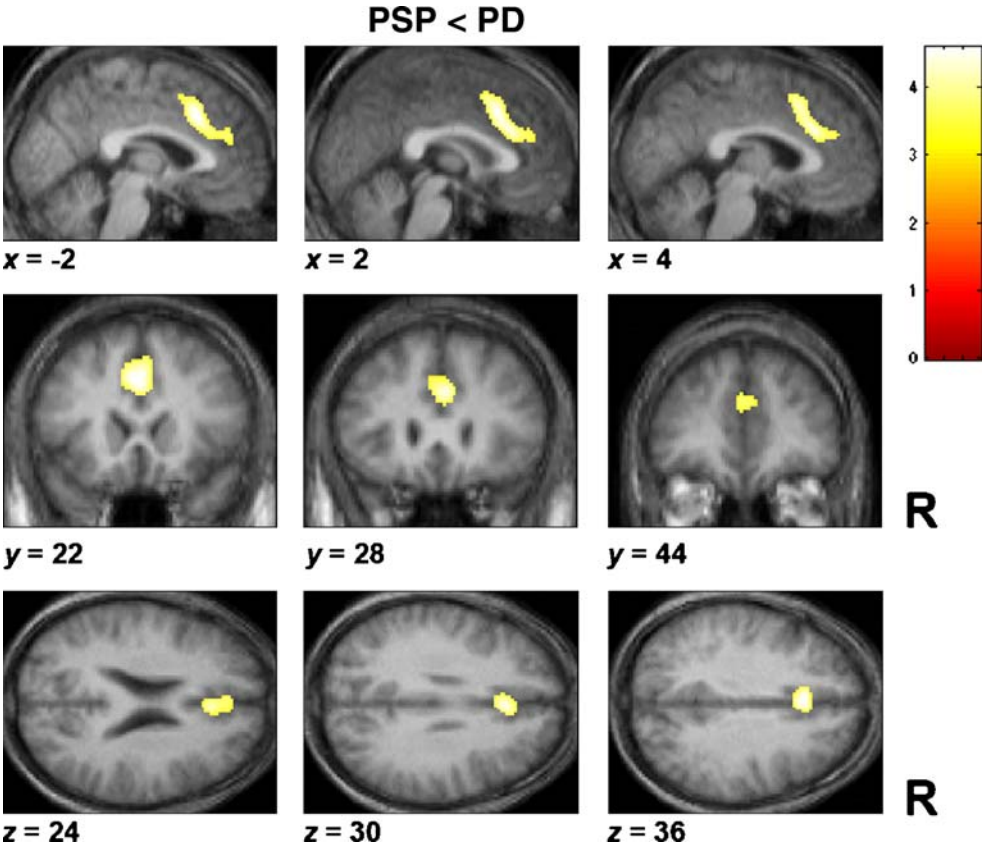

Fig. 2 Statistical parametric maps showing the regions of significant decrease in rCBF in PSP compared with PD patients, obtained by SPM2 analysis, overlaid on an average T1-weighted MR scan from ten healthy controls. *R* refers to right side of the brain; *x*, *y* and *z* refer to the corresponding level of the sagittal, coronal and axial planes. Results are shown at the threshold of $p<0.001$ and show the relative impairment of the anterior cingulate cortex

remote regions. A PET study with both [^{11}C]flumazenil and [^{18}F]FDG in PSP patients demonstrated a 20% reduction of central benzodiazepine receptors in the anterior cingulate gyrus, where glucose metabolic rates also showed the greatest reduction, suggesting that both the loss of intrinsic neurons containing benzodiazepine receptors and the deafferentation from distant brain regions could contribute to the cortical hypometabolism in PSP [38].

PSP patients showed a mild relative increase in rCBF in the visual associative cortex (bilateral cuneus, BA18, and left occipital gyrus, BA37) compared with PD patients. These findings are consistent with previous studies that have reported a relative decrease in metabolism or rCBF in associative visual areas of PD patients [12, 39] and could relate to the known impairment of visuospatial function in PD.

Principal component analysis

Multivariate analysis aims to reduce the dimensionality of the data matrix and identify a small number of components that best explain the observed variance-covariance. The statistical approach utilised in this study introduces regional

Table 3 Coarse component score (CCSs): means and SDs for each principal component (PC) and group. CCSs were standardised on the range

PC	VOIs with high loadings on the PC	Controls 1		PD 2		PSP 3		Group effect	Significant comparisons, $p<0.05$
		Mean	SD	Mean	SD	Mean	SD	$F(2,31)$ and p value	
PC1	BA23R, BA23L, BA24R, BA24L, CDL	0.725	0.205	0.530	0.140	0.295	0.256	$F=11.64$, $p<0.001$	1/3, 2/3
PC2	BA39R, BA39L, BA40R, BA40L	0.354	0.192	0.342	0.296	0.598	0.234	$F=4.531$, $p=0.019$	2/3
PC3	BA11R, BA11L, BA47R, BA47L	0.598	0.262	0.589	0.313	0.557	0.236		
PC4	BA07R, BA07L, THR, THL	0.523	0.301	0.652	0.189	0.405	0.294		
PC5	BA10R, BA10L, PTL	0.479	0.236	0.459	0.144	0.422	0.251		
PC6	BA09R, BA09L, BA32R, BA32L	0.629	0.126	0.680	0.161	0.377	0.301	$F=6.083$, $p=0.006$	1/3, 2/3
PC7	CDR, PTR	0.657	0.270	0.620	0.141	0.574	0.224		

Results of ANOVA and Tukey HSD test for multiple comparisons are reported
BA Brodmann area, *CD* caudate, *TH* thalamus, *PT* putamen, *R* right, *L* left

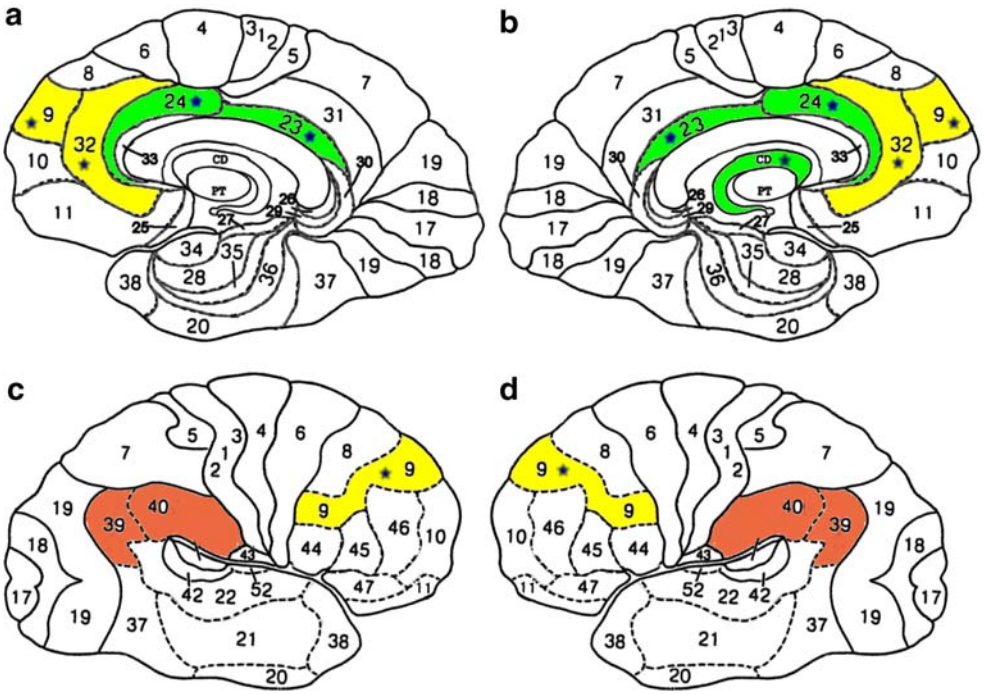

Fig. 3 Results of PCA showing the three factors (green, orange and yellow) differentiating PSP from PD and controls. Medial (*upper row*) and lateral (*lower row*) views of right (**a**, **c**) and left (**b**, **d**) hemispheres with corresponding Brodmann areas are shown. The thalamus is located deeply and is not displayed on the standard view. The star (*) inside the regions indicates significant differences between controls and PSP patients

analyses based on the assumption that correlated patterns may exist among different brain regions and that such relationships may affect rCBF distribution. PCA is used to study the correlations between a large number of variables by grouping them in "coherent subsets" (principal components). Reducing a large set of variables to a smaller one decreased the number of analyses to be made and increased statistical power.

PCA was complementary to SPM analysis, allowing the identification of three different networks that seem to be functionally affected in PSP. Two networks, represented by PC1 and PC6, share a common involvement of the cingulate cortex. One network (PC1) included the more posterior part of the anterior cingulate cortex (BA24) and extended caudally to the posterior cingulate gyrus (BA23) and to the left caudate. The relative decrease in rCBF in PSP patients was more pronounced in the anterior cingulate gyrus (BA24) than in the posterior cingulate gyrus (BA23) and the caudate. This reinforced the fact that the anterior cingulate cortex was the region showing the most severe impairment in PSP. It is interesting to note that this factor also included BA23. There are anatomical connections between the anterior (BA24) and the posterior part (BA23) of the cingulate gyrus, and the decrease in rCBF in BA24 may also have led to a functional decline in BA23 highlighted by the PCA. Decreased metabolism in the caudate has already been demonstrated in previous PET studies [9–11, 30, 40]. The basal ganglia participate in five parallel loops with the cerebral cortex, including non-motor regions involved in executive and behavioural functions, such as the anterior cingulate and the medial orbitofrontal cortices (areas 24 and 13) [41, 42]. The other network (PC6) included BA32, the most anterior part of the cingulate cortex, and extended to the dorsolateral prefrontal cortex (BA9), reinforcing the observation that cortical regions involved in executive control are functionally affected in PSP. Therefore, it seems that at least two dysfunctional networks are present in PSP which overlap partially at the level of the cognitive component of the cingulate cortex and extend to brain regions involved in executive, behavioural and motor functions. These networks seem to be specific for PSP and different from other disorders with frontal dysfunction such as frontotemporal lobe degeneration, in which the more orbital part of the frontal cortex and the more ventral part (i.e. the affective component) of the anterior cingulate are affected along with other limbic regions such as the hippocampus/amygdala and nucleus accumbens [43].

The third network showed a different functional behaviour in PSP. While the cingulate cortex was selectively impaired, parietal associative cortex, including bilateral BA39 and BA40, was functionally preserved or even hyperactive, contrasting with other dementing disorders such as AD, in which the retrosplenial posterior cingulate, the precuneus and the parietal cortex are predominantly affected [10, 44].

Overall, these results are in agreement with the findings reported by Eckert et al. [11] in a recent FDG-PET study of a large cohort of parkinsonian patients in which defining features of PSP were hypometabolism of the midline frontal cortex and supporting features of PSP were hypermetabolism of the parietal cortex and hypometabolism of the

caudate nucleus, and with the findings reported by Van Laere et al. using [^{99m}Tc]ECD SPECT [21].

Additional remarks

Some additional issues should be discussed with regard to the interpretation of the results from this study.

PD and PSP patients were recruited on the basis of comparable disease duration, since we were interested in studying patients at a relatively early stage of disease. Consequently, because of their disease course, patients differed with respect to severity of disease assessed with UPDRS. From a scanning perspective this could have potentially affected the degree of functional changes in the brain and the detection of differences between the groups. However, the voxel-based analysis restricted to those patients with comparable disease severity (data not shown) yielded essentially the same results; thus we reasonably exclude a major impact of different disease severity on the brain abnormalities detected.

Patients were diagnosed as having possible or probable PSP according to the NINDS-SPSP diagnostic criteria; a diagnosis of definite PSP requires autopsy, which was not available for any of the patients. It has been reported [45] that the NINDS-SPSP criteria have a specificity of 98.5% and a positive predictive value (PPV) of 96%, even for the category of probable PSP alone (specificity and PPV for probable PSP were 100%), with a relatively good sensitivity (50–75%). One of the concerns in applying these criteria is the possibility of underdiagnosis, but we can assume that the specificity of the clinical diagnosis of PSP in our study would have been similar to that previously reported and would not have affected the results significantly. Furthermore, patients were followed up to 2 years after the initial evaluation and the diagnosis of PSP was confirmed in all cases.

There are also other relevant aspects related to the number and type of patients studied or the technique used. We did not find significant differences between controls and PD patients. Previous voxel-based studies in PD patients performed with FDG-PET [11, 40] or rCBF-SPECT [26] have shown significant decreases in metabolism or perfusion in various cortical regions, including the prefrontal cortex, SMA and parietal association cortex, and significant increases in metabolism or rCBF in the striatum, thalamus and cerebellum. As compared with these studies, our PD group included a lower number of patients and patients with a shorter disease duration. A small sample size and the inclusion of patients at a relatively early stage of disease could account for the lack of significant rCBF changes in our PD group. In addition, in PSP we could not detect rCBF changes in the striatum, thalamus or midbrain, regions that have frequently been found to be affected with FDG-PET [9, 10, 40] and more recently also with ECD-SPECT [21]. This could have been due to a lower sensitivity of rCBF SPECT compared with FDG-PET, but also to the sample size. Further studies on a larger dataset of patients could clarify this issue.

Finally, other methodological aspects should be considered. First, the relationship of rCBF decline and cognitive performance or behavioural status in PSP patients remains speculative since the patients were not evaluated with a comprehensive neuropsychological battery. Therefore, we could not assess to what extent executive dysfunction, apathy, depression or other cognitive or behavioural variables were related to rCBF decline in the cingulate cortex. Second, atrophy correction was not performed. Different volumetric studies in PSP have reported grey or white matter changes in cortical and subcortical regions [46, 47], with only minor changes in the anterior cingulate cortex [48]. This suggests that in PSP, grey matter loss in the cingulate cortex does not contribute to a large extent to the functional changes in this region. Therefore, we believe that our findings were not the result of atrophy, but rather reflect mainly functional changes in PSP.

Conclusion

This study suggests that anterior cingulate hypoperfusion could be an early and distinct sign of PSP. Moreover, distinct functional networks of brain abnormalities that include cortical and subcortical brain regions with decreased (anterior and posterior cingulate cortex: BA24, BA32, BA23; prefrontal cortex: BA9 and caudate) or increased (parietal cortex: BA39 and BA40) rCBF are present in PSP.

Acknowledgements The authors thank the medical and technical staff of the Nuclear Medicine Department at the University of Naples for help in conducting the study. Prof. Guido Rodriguez, Prof. Alfredo Postiglione and Prof. Stig Larsson are acknowledged for their support in subject recruitment, study acquisition and image analysis. Part of this work has been presented in abstract form at the European Association of Nuclear Medicine 2005 Congress and at the XXXVIth Congress of the Italian Neurological Society.

References

1. Litvan I, Campbell G, Mangone CA, Verny M, McKee A, Chaudhuri KR, et al. Which clinical features differentiate progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome) from related disorders? A clinicopathological study. Brain 1997;120 Pt 1:65–74.
2. Litvan I, Agid Y, Jankovic J, Goetz C, Brandel JP, Lai EC, et al. Accuracy of clinical criteria for the diagnosis of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome). Neurology 1996;46:922–30.
3. Litvan I, Agid Y, Calne D, Campbell G, Dubois B, Duvoisin RC, et al. Clinical research criteria for the diagnosis of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome): report of the NINDS-SPSP international workshop. Neurology 1996;47:1–9.

4. Williams DR, de Silva R, Paviour DC, Pittman A, Watt HC, Kilford L, et al. Characteristics of two distinct clinical phenotypes in pathologically proven progressive supranuclear palsy: Richardson's syndrome and PSP-parkinsonism. Brain 2005;128:1247–58.
5. D'Antona R, Baron JC, Samson Y, Serdaru M, Viader F, Agid Y, et al. Subcortical dementia. Frontal cortex hypometabolism detected by positron tomography in patients with progressive supranuclear palsy. Brain 1985;108 Pt 3:785–99.
6. Foster NL, Gilman S, Berent S, Morin EM, Brown MB, Koeppe RA. Cerebral hypometabolism in progressive supranuclear palsy studied with positron emission tomography. Ann Neurol 1988;24:399–406.
7. Brooks DJ, Ibanez V, Sawle GV, Playford ED, Quinn N, Mathias CJ, et al. Striatal D2 receptor status in patients with Parkinson's disease, striatonigral degeneration, and progressive supranuclear palsy, measured with ^{11}C-raclopride and positron emission tomography. Ann Neurol 1992;31:184–92.
8. Blin J, Baron JC, Dubois B, Pillon B, Cambon H, Cambier J, et al. Positron emission tomography study in progressive supranuclear palsy. Brain hypometabolic pattern and clinicometabolic correlations. Arch Neurol 1990;47:747–52.
9. Garraux G, Salmon E, Degueldre C, Lemaire C, Laureys S, Franck G. Comparison of impaired subcortico-frontal metabolic networks in normal aging, subcortico-frontal dementia, and cortical frontal dementia. Neuroimage 1999;10:149–62.
10. Salmon E, Van der Linden MV, Franck G. Anterior cingulate and motor network metabolic impairment in progressive supranuclear palsy. Neuroimage 1997;5:173–8.
11. Eckert T, Barnes A, Dhawan V, Frucht S, Gordon MF, Feigin AS, et al. FDG PET in the differential diagnosis of parkinsonian disorders. Neuroimage 2005;26:912–21.
12. Klein RC, de Jong BM, de Vries JJ, Leenders KL. Direct comparison between regional cerebral metabolism in progressive supranuclear palsy and Parkinson's disease. Mov Disord 2005;20:1021–30.
13. Burn DJ, Rinne JO, Quinn NP, Lees AJ, Marsden CD, Brooks DJ. Striatal opioid receptor binding in Parkinson's disease, striatonigral degeneration and Steele-Richardson-Olszewski syndrome: a [^{11}C] diprenorphine PET study. Brain 1995;118 Pt 4:951–8.
14. Shinotoh H, Namba H, Yamaguchi M, Fukushi K, Nagatsuka S, Iyo M, et al. Positron emission tomographic measurement of acetylcholinesterase activity reveals differential loss of ascending cholinergic systems in Parkinson's disease and progressive supranuclear palsy. Ann Neurol 1999;46:62–9.
15. Messa C, Volonte MA, Fazio F, Zito F, Carpinelli A, d'Amico A, et al. Differential distribution of striatal [^{123}I]beta-CIT in Parkinson's disease and progressive supranuclear palsy, evaluated with single-photon emission tomography. Eur J Nucl Med 1998;25:1270–6.
16. Ilgin N, Zubieta J, Reich SG, Dannals RF, Ravert HT, Frost JJ. PET imaging of the dopamine transporter in progressive supranuclear palsy and Parkinson's disease. Neurology 1999;52:1221–6.
17. Oyanagi C, Katsumi Y, Hanakawa T, Hayashi T, Thuy DD, Hashikawa K, et al. Comparison of striatal dopamine D2 receptors in Parkinson's disease and progressive supranuclear palsy patients using [^{123}I] iodobenzofuran single-photon emission computed tomography. J Neuroimaging 2002;12:316–24.
18. Kim YJ, Ichise M, Ballinger JR, Vines D, Erami SS, Tatschida T, et al. Combination of dopamine transporter and D2 receptor SPECT in the diagnostic evaluation of PD, MSA, and PSP. Mov Disord 2002;17:303–12.
19. Defebvre L, Lecouffe P, Destee A, Houdart P, Steinling M. Tomographic measurements of regional cerebral blood flow in progressive supranuclear palsy and Parkinson's disease. Acta Neurol Scand 1995;92:235–41.
20. Zhang L, Murata Y, Ishida R, Saitoh Y, Mizusawa H, Shibuya H. Differentiating between progressive supranuclear palsy and corticobasal degeneration by brain perfusion SPET. Nucl Med Commun 2001;22:767–72.
21. Van Laere K, Casteels C, De Ceuninck L, Vanbilloen B, Maes A, Mortelmans L, et al. Dual-tracer dopamine transporter and perfusion SPECT in differential diagnosis of parkinsonism using template-based discriminant analysis. J Nucl Med 2006;47:384–92.
22. Eidelberg D, Moeller JR, Dhawan V, Sidtis JJ, Ginos JZ, Strother SC, et al. The metabolic anatomy of Parkinson's disease: complementary [^{18}F]fluorodeoxyglucose and [^{18}F]fluorodopa positron emission tomographic studies. Mov Disord 1990;5:203–13.
23. Eidelberg D, Moeller JR, Dhawan V, Spetsieris P, Takikawa S, Ishikawa T, et al. The metabolic topography of parkinsonism. J Cereb Blood Flow Metab 1994;14:783–801.
24. Lozza C, Baron JC, Eidelberg D, Mentis MJ, Carbon M, Marie RM. Executive processes in Parkinson's disease: FDG-PET and network analysis. Hum Brain Mapp 2004;22:236–45.
25. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988;51:745–52.
26. Van Laere K, Santens P, Bosman T, De Reuck J, Mortelmans L, Dierckx R. Statistical parametric mapping of ^{99m}Tc-ECD SPECT in idiopathic Parkinson's disease and multiple system atrophy with predominant parkinsonian features: correlation with clinical parameters. J Nucl Med 2004;45:933–42.
27. Greitz T, Bohm C, Holte S, Eriksson L. A computerized brain atlas: construction, anatomical content, and some applications. J Comput Assist Tomogr 1991;15:26–38.
28. Thurfjell L, Bohm C, Bengtsson E. CBA—an atlas-based software tool used to facilitate the interpretation of neuroimaging data. Comput Methods Programs Biomed 1995;47:51–71.
29. Pett M, Lackey N, Sullivan J. Making sense of factor analysis: a practical guide to understanding factor analysis for instrument development in health care research. Thousand Oaks, CA: Sage Publication, Inc.; 2003.
30. Hosaka K, Ishii K, Sakamoto S, Mori T, Sasaki M, Hirono N, et al. Voxel-based comparison of regional cerebral glucose metabolism between PSP and corticobasal degeneration. J Neurol Sci 2002;199:67–71.
31. Badgaiyan RD. Executive control, willed actions, and nonconscious processing. Hum Brain Mapp 2000;9:38–41.
32. Pardo JV, Pardo PJ, Janer KW, Raichle ME. The anterior cingulate cortex mediates processing selection in the Stroop attentional conflict paradigm. Proc Natl Acad Sci USA 1990;87:256–59.
33. Carter CS, Mintun M, Cohen JD. Interference and facilitation effects during selective attention: an $H_2{}^{15}O$ PET study of Stroop task performance. Neuroimage 1995;2:264–72.
34. Devinsky O, Morrell MJ, Vogt BA. Contributions of anterior cingulate cortex to behaviour. Brain 1995;118 Pt 1:279–306.
35. Morris J, Dolan R. Functional Neuroanatomy of human emotion. In: Frackowiak RSJ, Friston FK, Frith CD, Dolan RJ, Zeki S, Price CJ, editors. Human brain function. 2nd ed. San Diego: Elsevier Academic Press; 2004. pp 365–96.
36. Frith C, Gallagher H, Maguire E. Mechanisms of control, Chapter 18. In: Frackowiak RSJ, Friston KJ, Frith CD, Dolan RJ, Zeki S, Price CJ, editors. Human brain function. 2nd ed. San Diego: Elsevier; 2004. pp 329–62.
37. Migneco O, Benoit M, Koulibaly PM, Dygai I, Bertogliati C, Desvignes P, et al. Perfusion brain SPECT and statistical parametric mapping analysis indicate that apathy is a cingulate syndrome: a study in Alzheimer's disease and nondemented patients. Neuroimage 2001;13:896–902.
38. Foster NL, Minoshima S, Johanns J, Little R, Heumann ML, Kuhl DE, et al. PET measures of benzodiazepine receptors in progressive supranuclear palsy. Neurology 2000;54:1768–73.
39. Abe Y, Kachi T, Kato T, Arahata Y, Yamada T, Washimi Y, et al. Occipital hypoperfusion in Parkinson's disease without dementia: correlation to impaired cortical visual processing. J Neurol Neurosurg Psychiatry 2003;74:419–22.

40. Juh R, Kim J, Moon D, Choe B, Suh T. Different metabolic patterns analysis of Parkinsonism on the ^{18}F-FDG PET. Eur J Radiol 2004;51:223–33.
41. Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 1986;9:357–81.
42. Middleton FA, Strick PL. Basal ganglia output and cognition: evidence from anatomical, behavioral, and clinical studies. Brain Cogn 2000;42:183–200.
43. Franceschi M, Anchisi D, Pelati O, Zuffi M, Matarrese M, Moresco RM, et al. Glucose metabolism and serotonin receptors in the frontotemporal lobe degeneration. Ann Neurol 2005;57:216–25.
44. Minoshima S, Giordani B, Berent S, Frey KA, Foster NL, Kuhl DE. Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. Ann Neurol 1997; 42:85–94.
45. Lopez OL, Litvan I, Catt KE, Stowe R, Klunk W, Kaufer DI, et al. Accuracy of four clinical diagnostic criteria for the diagnosis of neurodegenerative dementias. Neurology 1999;53:1292–9.
46. Brenneis C, Seppi K, Schocke M, Benke T, Wenning GK, Poewe W. Voxel based morphometry reveals a distinct pattern of frontal atrophy in progressive supranuclear palsy. J Neurol Neurosurg Psychiatry 2004;75:246–9.
47. Price S, Paviour D, Scahill R, Stevens J, Rossor M, Lees A, et al. Voxel-based morphometry detects patterns of atrophy that help differentiate progressive supranuclear palsy and Parkinson's disease. Neuroimage 2004;23:663–9.
48. Cordato NJ, Duggins AJ, Halliday GM, Morris JG, Pantelis C. Clinical deficits correlate with regional cerebral atrophy in progressive supranuclear palsy. Brain 2005;128:1259–66.