Cortical hypoperfusion in Parkinson's disease assessed using arterial spin labeled perfusion MRI

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ABSTRACT

Alterations in cerebral perfusion and metabolism in Parkinson's disease have been assessed in several studies, using nuclear imaging techniques and more recently magnetic resonance imaging. However, to date there is no consensus in the literature regarding the extent and the magnitude of these alterations. In this work, arterial spin labeled perfusion MRI was employed to quantify absolute cerebral blood flow in a group of early-to-moderate Parkinson's disease patients and age-matched healthy controls. Perfusion comparisons between the two groups showed that Parkinson's disease is characterized by widespread cortical hypoperfusion. Subcortically, hypoperfusion was also found in the caudate nucleus. This pattern of hypoperfusion could be related to cognitive dysfunctions that have been previously observed even at the disease early stages. The present results were obtained by means of whole brain voxel-wise comparisons of absolute perfusion values, using statistical parametric mapping, thus avoiding the potentially biased global mean normalization procedure. In addition, this work demonstrates that between-group comparison of relative perfusion values after global mean normalization, introduced artificial relative perfusion increases, where absolute perfusion was in fact preserved. This has implications for perfusion studies of other brain disorders.

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Introduction

Cerebral perfusion disturbances have been observed in a variety of neurodegenerative and psychiatric disorders. Due to the well-known phenomenon of neurovascular coupling, changes in cerebral blood flow (CBF) can be directly related to regional metabolism and neural activity (Raichle, 1998). Thus, CBF measurements could potentially become biomarkers for early disease diagnosis, and also serve to follow up disease progression and treatment response.

Cerebral glucose consumption (CMRglc) and perfusion have been previously assessed in Parkinson's disease (PD) using positron emission tomography (PET) (Eckert et al., 2007), single photon emission computed tomography (SPECT) (Amorim et al., 2007) and, more recently, perfusion magnetic resonance imaging (MRI) (Melzer et al., 2011). Previous studies with PET and SPECT have found alterations in metabolism and perfusion in PD with a pattern of relative cortical decreases concurrent with subcortical increases extending into large areas of the basal ganglia and thalamus (Eckert et al., 2007). However, recent publications have questioned the presence of such subcortical increases, arguing that they are an artifact introduced by global mean normalization of the data (Borghammer et al., 2010; Borghammer et al., 2009a; Borghammer et al., 2009b). Normalization is commonly used in PET and SPECT studies and indeed required when absolute quantification is not feasible. Even when absolute quantification can be performed, both perfusion and glucose consumption measurements from PET and SPECT have great interindividual variability with large coefficients of variation (Borghammer et al., 2008), which makes the detection of small between-group differences difficult. However, normalization to the global mean is only appropriate when there is no difference in global mean values between groups, an assumption that seems to be violated in comparisons of PD patients and healthy controls (Borghammer et al., 2010).

Although anatomical studies in experimental models of PD using 2-deoxyglucose (2DG) autoradiography have also reported increased metabolism in several subcortical and brainstem structures (see for review (Borghammer et al., 2009a; Obeso et al., 2008)), these structures are generally too small to be detected with the resolution achievable using conventional PET and SPECT cameras (Cassidy and Radda, 2005).

Arterial spin labeled (ASL) perfusion MRI (Detre et al., 1992; Williams et al., 1992) has been recently introduced as a non-invasive alternative for perfusion measurements in PD (Fernandez-Seara et al., 2009; Kamagata et al., 2011; Ma et al., 2010; Melzer et al., 2011). The ASL technique utilizes electromagnetically labeled arterial blood water as an endogenous tracer, yielding quantitative CBF measurements...
in well-characterized physiological units of ml·100 g$^{-1}$ min$^{-1}$. Recent technical advances in ASL have increased the sensitivity of the method and, as a result, absolute CBF measurements can now be obtained with high reliability in both young and elderly subjects (Xu et al., 2010). Using quantitative ASL methods, a recent study has reported absolute perfusion decreases in PD in diverse cortical areas, while subcortical perfusion appeared preserved (Melzer et al., 2011). This study, while being the largest study of perfusion in PD to date, involved a heterogeneous group of patients that also included patients with dementia.

In the current study, an optimized ASL technique (Fernandez-Seara et al., 2008a) has been used to quantify absolute CBF in a homogeneous group of early-to-moderate PD patients and age-matched healthy controls. We hypothesized that perfusion alterations in PD would consist in cortical decreases and that those alterations could be detected using statistical parametric mapping analysis with this technique. In addition, comparisons of relative CBF values were also carried out to evaluate the effect of global mean normalization.

Materials and methods

Subjects

Twenty-five early to moderate stage PD patients without dementia and thirty-four healthy age-matched controls participated in the study, approved by the Ethics Research Committee of the University of Navarra, after signing a written informed consent. The healthy volunteers had no past or present history of neurological or psychiatric disorders. The patients had been diagnosed with idiopathic Parkinson’s disease according to the United Kingdom Parkinson’s Disease Society Brain Bank criteria (Hughes et al., 1992). The diagnosis was confirmed by a neurologist, specialized in movement disorders. They were staged according to the Hoehn and Yahr scale and their motor symptoms were evaluated during the ON state, under medication, using the motor examination score of the Unified Parkinson’s Disease Rating Scale part III (motor UPDRS) (Martinez-Martin et al., 1994). To assess cognitive impairment, the Mini Mental State Examination (MMSE) was used in the patient group (Folstein et al., 1975). Only 2 patients showed mild cognitive impairment as determined by their scores in the test. At the time of the study, the patients were medicated as follows (medication (number of patients)): dopamine receptor agonist (3); agonist + monoamine oxidase B (MAO-B) inhibitor (2); levodopa (1); levodopa + agonist (8); levodopa + MAO-B inhibitor (2); levo-dopa + agonist + MAO-B inhibitor (7); levodopa + agonist + amantadine (1); levodopa + agonist + amantadine + MAO-B inhibitor (1). Patients were scanned under medication in ON state. See Table 1 for detailed information on the subjects.

Scanning protocol

Studies were performed on a 3 T Trio TIM (Siemens AG, Erlangen, Germany) using a 12-channel head array. Subjects were instructed to remain still with eyes open during the scanning session. T1-weighted anatomical images were acquired with a MPRAGE sequence, with the following imaging parameters: resolution = 1 mm isotropic, FOV = 192 × 256 mm$^2$, matrix = 192 × 256, 160 axial slices, TR/TE/TI = 1620/3.87/950 ms, flip angle = 15°.

Perfusion MRI data were acquired using a pseudo-continuous ASL (PCASL) technique with a background-suppressed 3D GRASE read-out sequence, modified to achieve late inflow delay (Fernandez-Seara et al., 2008b), with imaging parameters: resolution = 4 × 4 × 7 mm$^3$, FOV = 250 × 188 × 112 mm$^3$, 16 nominal partitions with 13% oversampling, 5/8 partial Fourier, measured partitions = 11, matrix size = 64 × 49, BW = 2790 Hz/pixel, GE spacing = 0.4 ms (with ramp sampling), SE spacing = 26 ms, read-out time = 270 ms, TE = 55.7 ms and TR = 3.5 s. Two non-selective inversion pulses (15.35 ms duration and 220 mG amplitude) were added for background suppression (BS) with inversion times TI1 = 1800 ms; TI2 = 500 ms. The PCASL pulse consisted of 1536 selective radio-frequency (RF) pulses (Hanning window, peak B1 = 53 mG, duration = 500 μs and G = 0.6 G/cm, labeling duration = 1600 ms, post-labeling delay = 1530 ms). For the control, the RF phase alternated from 0 to 180°. Bipolar gradients (b = 5 s/mm$^2$) were added to suppress intravascular signal. The inversion plane was offset 8 cm from the center of the FOV in the head-foot direction, so that it was located at the base of the cerebellum to achieve good labeling efficiency. The imaging slab covered the cerebrum and the superior part of the cerebellum. 50 label/control pairs were acquired in a scan time of 6 min. A short scan of 5 label/control pairs was performed using the same sequence without background suppression to acquire control images needed for calculation of CBF. Raw data were saved and reconstructed off-line.

Data processing and analysis

Data analysis was performed using SPM (version 8, Wellcome Trust Center for Neuroimaging, University College London, UK) and custom scripts in Matlab (Mathworks, MA, USA).

Voxel-based morphometry

The T1-weighted anatomical images were segmented, using a unified segmentation procedure (Ashburner and Friston, 2005) and a study-specific gray matter template was generated using the Diffeomorphic Anatomical Registration through Exponentiated Lie algebra (DARTEL) algorithm (Ashburner, 2007). The individual gray matter images were normalized to this template and modulated, preserving the total amount of signal from each region in the image. The modulated normalized grey matter images were mapped into MNI space using an affine transformation and spatially smoothed with an 8 mm full-width at half-maximum Gaussian kernel. Total intracranial volume of each subject was computed by adding voxels in the segmented gray and white matter and cerebrospinal fluid images.

Voxel-wise comparison of gray matter volume between patient and control groups was carried out using a two-sample t-test (unequal variances), with age, sex and intra-cranial volume as covariates of no-interest. A cluster-corrected p value of 0.05 was employed, determined using a non-stationarity correction toolbox (Hayasaka et al., 2004).

Perfusion data

Raw ASL images acquired with BS and images acquired without BS were realigned and co-registered to the anatomical dataset. Forty-nine perfusion weighted images were obtained by pair-wise subtraction of the background-suppressed label and control images, after discarding the first pair, because the signal had not reached the steady state. Perfusion weighted images identified as outliers were eliminated from the time series and replaced by the average of the two adjacent images. A perfusion image was considered an outlier when the global perfusion signal differed from the mean of the perfusion image series by ± 2 standard deviations (Wang et al., 2008). A mask was generated.
from the unsuppressed mean control image, using a brain extraction tool in MRcron (www.mrcron.com) and manual adjustment when needed. This mask was applied to the mean perfusion weighted image to remove out-of-brain voxels. Subsequently, a CBF map was computed from the masked mean perfusion weighted image, based on a single compartment ASL perfusion model, as described in Wang et al. (2005). A whole brain mean CBF value was calculated for each subject by averaging CBF voxel values. Differences in whole brain mean CBF between patient and control groups were assessed by means of a linear regression analysis with group, age and sex as independent variables.

The CBF maps were normalized to the study-specific gray matter template previously generated, and mapped into MNI space using an affine transformation, re-sliced to an isotropic voxel size of 2 mm and spatially smoothed using an 6 mm full-width at half-maximum Gaussian kernel.

Whole brain voxel-wise statistical analysis of the CBF data was performed using a two-sample t-test (unequal variances), with age and sex as covariates of no-interest. A gray matter mask generated from the gray matter template, including voxels with a probability of being gray matter larger than 0.5%, was applied. Coverage of the cerebellum was variable from subject to subject, so this region was excluded from the analysis using an additional mask. Areas of perfusion change between patient and control groups were identified using a cluster-corrected p value < 0.05, determined using a non-stationarity correction toolbox (Hayasaka et al., 2004). An exploratory analysis was also carried out with a significance value of p < 0.005, uncorrected for multiple comparisons and a minimum cluster size of 30. Anatomical labeling was performed using the SPM Anatomy toolbox (Eickhoff et al., 2005) and the Talairach Daemon (Lancaster et al., 2000).

In order to determine the magnitude of the perfusion differences between patients and controls in anatomical structures, spherical regions of interest (ROIs) (radius = 3 mm) were defined from the thresholded SPM (t) map, centered in every local maximum. CBF values in these ROIs were extracted from the normalized CBF images for each subject. Individual CBF values were averaged to obtain group means. Percentage CBF difference was computed as the difference in group means divided by the mean CBF in the control group.

In addition to the whole brain voxel-wise comparison, a region of interest (ROI) statistical analysis was also carried out to further examine perfusion changes in subcortical structures. CBF values were measured in the basal ganglia (caudate nucleus, putamen and external and internal globus pallidus) and thalamus, in unilateral ROIs that comprised the full extent of each structure as defined by the masks in the WFU PickAtlas toolbox (Maldjian et al., 2003). Unilateral CBF values extracted from patients with predominant left body side affection were transposed to the other hemisphere, to simulate that all patients had right-sided symptoms, thus avoiding potential confounds of asymmetric brain affection. Significant differences in CBF between patient and control groups were assessed in a linear regression analysis with group, age and sex as independent variables, with a Bonferroni corrected p value of 0.05. The ROI statistical tests were carried out using SPSS 15.

Finally, in order to evaluate the effects of normalizing the data to the global mean, a second whole brain voxel-wise statistical analysis of the CBF data was performed using global mean normalization, implemented as proportional scaling in SPM.

**Results**

**Perfusion data**

**CBF quantification**

The calculated whole brain mean CBF in the control group was $38.9 \pm 6.2 \text{ mL} \cdot 100\text{g}^{-1} \text{min}^{-1}$ (mean ± standard deviation). In the patient group, the mean CBF was $36.9 \pm 6.5$, a value reduced by 5.1% with respect to the control group. However, the linear regression analysis showed that group was not a significant predictor of whole brain mean CBF ($p = 0.196$).

Parkinson’s disease related perfusion deficits

The voxel-wise analysis of absolute CBF maps revealed areas of hypoperfusion in the cortex of PD patients. These areas are listed in **Table 2** and depicted in Fig. 1. Bilateral perfusion decreases were found in frontal, parietal and occipital areas. In the frontal lobe, perfusion was reduced in the superior and middle frontal gyri as well as in the precentral gyrus, affecting Brodmann areas (BA) 6, 8 and 9. Area 6 was affected both on the lateral surface of the brain, which functionally corresponds to the premotor cortex (Matelli et al., 2004), as well as in the medial wall of the hemisphere, where the region of hypoperfusion was located rostral to the level of the anterior commissure, corresponding to the pre-supplementary motor area (pre-SMA) (Picard and Strick, 2001; Zilles et al., 1996). In contrast, perfusion was...

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**Table 2**

Areas of perfusion deficit in PD patients (non-stationarity corrected cluster p value < 0.05). Local maxima are shown in MNI coordinates (in mm), BA: Brodmann area, p unc.: uncorrected voxel p value. % CBF decrease: CBF decrease in patients relative to controls, computed in a spherical ROI (radius = 3 mm) centered in the coordinates of the local maximum.

<table>
<thead>
<tr>
<th>Cluster size</th>
<th>Region (BA)</th>
<th>MNI coordinates x, y, z</th>
<th>T</th>
<th>p unc.</th>
<th>% CBF decrease</th>
</tr>
</thead>
<tbody>
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<td>9328</td>
<td>Left lingual gyrus (18)</td>
<td>–2 –94 –12</td>
<td>3.63</td>
<td>0.0005</td>
<td>36.35</td>
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<td></td>
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<td>0.0005</td>
<td>41.37</td>
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<td></td>
<td>Right middle occipital gyrus</td>
<td>36 –84 22</td>
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<td>19.18</td>
</tr>
<tr>
<td></td>
<td>Left precuneus, superior parietal lobule (7A)</td>
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<tr>
<td></td>
<td>Right postcentral gyrus</td>
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<td>3.38</td>
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<td>27.48</td>
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<tr>
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<td>Right superior occipital gyrus</td>
<td>18 –84 20</td>
<td>3.33</td>
<td>0.001</td>
<td>22.69</td>
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<tr>
<td></td>
<td>Left precuneus, superior parietal lobule (7P)</td>
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<td>Right superior occipital gyrus</td>
<td>28 –80 24</td>
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<td>0.001</td>
<td>22.69</td>
</tr>
<tr>
<td></td>
<td>Left paracentral lobule</td>
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<td>3.18</td>
<td>0.001</td>
<td>25.54</td>
</tr>
<tr>
<td>3500</td>
<td>Right superior frontal gyrus (6)</td>
<td>12 4 68</td>
<td>3.30</td>
<td>0.001</td>
<td>35.93</td>
</tr>
<tr>
<td></td>
<td>Right superior frontal gyrus (6)</td>
<td>6 2 68</td>
<td>3.29</td>
<td>0.001</td>
<td>31.36</td>
</tr>
<tr>
<td></td>
<td>Right superior frontal gyrus (8)</td>
<td>34 28 56</td>
<td>3.29</td>
<td>0.001</td>
<td>33.76</td>
</tr>
<tr>
<td></td>
<td>Right superior frontal gyrus (6)</td>
<td>2 14 66</td>
<td>3.29</td>
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<td>37.94</td>
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<tr>
<td></td>
<td>Left superior frontal gyrus (6)</td>
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<td>35.13</td>
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<td></td>
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<td>3.27</td>
<td>0.001</td>
<td>35.82</td>
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<tr>
<td></td>
<td>Left precenral gyrus (44)</td>
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<td>19.03</td>
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<td></td>
<td>Left middle frontal gyrus (6)</td>
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<td>3.17</td>
<td>0.001</td>
<td>20.80</td>
</tr>
<tr>
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<td>Left superior medial gyrus (8)</td>
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<td>29.94</td>
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<td>Right middle frontal gyrus (6)</td>
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<td>3.04</td>
<td>0.002</td>
<td>22.63</td>
</tr>
<tr>
<td></td>
<td>Right middle frontal gyrus (8)</td>
<td>54 24 40</td>
<td>2.97</td>
<td>0.002</td>
<td>23.33</td>
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</table>
preserved in the caudal portion of the mesial area 6, where SMA proper has been located (Picard and Strick, 2001), and in most of area 4. Perfusion deficits extended to areas 8 and 9 in the prefrontal cortex (Petrides and Pandya, 2004). In the parietal lobe, decreases were located in the lateral superior parietal lobule and medial precuneus (BA 7), and in a small region of the postcentral gyrus. In the occipital lobe, decreases were found in the lingual, superior and middle occipital gyri, thus affecting both primary and mainly secondary visual cortices. The magnitude of the perfusion deficits ranged from 20 to 40% relative to the control group CBF. These values are in the range of perfusion decreases measured in a recent ASL study of PD (Melzer et al., 2011). The exploratory analysis using an uncorrected voxel-wise p value revealed two additional clusters of decreased perfusion in the right caudate nucleus \((k=51, [x, y, z]=[16, 6, 12], T=3.69, \text{uncorrected})\).

**Fig. 1.** Surface projection of clusters with significantly decreased absolute CBF in PD patients compared to healthy controls.

**Fig. 2.** Unthresholded T statistic maps corresponding to the contrast patients minus controls, obtained by comparison of absolute CBF (a) and relative CBF (b). Both positive and negative T values are displayed, representing increased and decreased CBF in the patient group, respectively. The value of the T statistic is shown on the color scale.
voxel p value < 0.0005, % CBF decrease = 14.28; k = 32, [x, y, z] = [12, 20, -4], T = 3.08, uncorrected voxel p value < 0.002, % CBF decrease = 14.57). The results of voxel-wise comparisons did not show any area of increased perfusion in the patients compared to the control subjects. The statistical analysis in the subcortical ROIs showed no significant differences in CBF between patient and control groups in the basal ganglia or thalamus, although there were trends of decreased CBF in both left and right caudate nuclei (for details, see Supplementary Table 1).

**Table 3**
Areas of atrophy in PD patients (non-stationarity corrected cluster p value < 0.05). Local maxima are shown in MNI coordinates (in mm). BA: Brodmann area. p unc.: uncorrected voxel p value.

<table>
<thead>
<tr>
<th>Cluster size</th>
<th>Region (BA)</th>
<th>MNI coordinates x, y, z</th>
<th>T</th>
<th>p unc.</th>
</tr>
</thead>
<tbody>
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<td>12,037</td>
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<tr>
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<td>Right precentral gyrus (6)</td>
<td>26, -24, 66</td>
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<td>0.0005</td>
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<tr>
<td></td>
<td>Right precuneus, superior parietal lobule (7A)</td>
<td>12, -57, 45</td>
<td>3.64</td>
<td>0.0005</td>
</tr>
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<td></td>
<td>Left superior frontal gyrus (6)</td>
<td>0, 0, 68</td>
<td>3.54</td>
<td>0.0005</td>
</tr>
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<td>Left superior parietal lobule (7A)</td>
<td>-26, -65, 59</td>
<td>3.51</td>
<td>0.0005</td>
</tr>
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<td>Left precentral gyrus (6)</td>
<td>-29, -3, 63</td>
<td>3.44</td>
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<td>0.001</td>
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<td></td>
<td>Left precentral gyrus (6)</td>
<td>-30, -20, 60</td>
<td>3.04</td>
<td>0.002</td>
</tr>
</tbody>
</table>

**Effects of global mean normalization**

Fig. 2 shows the T statistic maps without thresholding for the contrast patients minus controls, obtained in the comparison of absolute CBF values (Fig. 2a) and relative CBF values (Fig. 2b). Note that both positive and negative T values are displayed, representing increased and decreased CBF in the patient group, respectively.

Visual inspection of the unthresholded T statistic maps revealed that global mean normalization led to the appearance of large areas of artifactual hyperperfusion in the patient group (Fig. 2b), in regions where...
absolute perfusion was in fact conserved (Fig. 2a). Increased absolute perfusion was only observed in two bilateral subcortical clusters covering a small fraction of the globus pallidus and a small fraction of the thalamus, with the local maxima located in the ventral lateral nucleus of the thalamus in the left hemisphere and in the lateral globus pallidus in the right hemisphere. Additional clusters of cortical hyperperfusion were found in the insulae and hippocampi (see Fig. 2a). Note that these perfusion increases were not significant.

Voxel-based morphometry

Gray matter atrophy in the patient group was found in frontal and parietal areas (see Table 3 and Fig. 3a). In the frontal lobe, atrophic areas were located in the superior frontal and precentral gyrus, affecting the premotor cortex, pre-supplementary and supplementary motor areas and a small region of the primary motor cortex. In the parietal lobe, atrophy was detected in the superior parietal lobule. Atrophic areas overlapped with areas of low cortical perfusion in the premotor cortex, the pre-supplementary motor area and the mesial superior parietal lobule (shown in pink in Fig. 3b).

Discussion

Assessment of absolute cerebral perfusion in PD using the ASL technique yielded a pattern of cortical perfusion deficit, affecting frontal, parietal and occipital areas. Our results largely confirm prior studies of perfusion and metabolism measuring absolute CBF or CMRglc (Berding et al., 2001; Bohnen et al., 1999; Hu et al., 2000; Mito et al., 2005). In particular, they are mostly in agreement with the recent study of Melzer et al. (2011) that evaluated perfusion using ASL in a large and heterogeneous group of PD patients, including patients with dementia. In their study however, Melzer et al. used principal component analysis, which required the a priori subtraction of the data mean. This technique yielded a Parkinson’s disease related perfusion pattern, reproducing results obtained in previous studies of PD employing network analysis (Poston and Eidelberg, 2009). Although they were able to quantify absolute perfusion in regions of interest defined by this pattern, the pattern obtained can be potentially biased by the de-meaning procedure, given the systematic group differences that exist in the global mean (Borghammer et al., 2010).

Artifacts introduced by global mean normalization

The results of our statistical analysis showed that CBF comparisons between patients and controls, using absolute and relative values, yielded vastly different T statistic maps. Examination of these maps revealed that global mean normalization introduced artifactual increases in perfusion in the patient group, in areas where absolute perfusion was in fact conserved, and also reduced the statistical significance of perfusion decreases. These effects are likely due to a systematic reduction in global perfusion in the patient group, where ratio normalization by smaller global values yields higher relative values, thus in areas where there is no true perfusion change between groups, relative perfusion appears to be increased in the patient group and on the other hand, in areas where there is a true disease related perfusion decrease, the difference in relative values is reduced and therefore the statistical power to detect this difference is lower. In our study, we measured a 5% decrease in global CBF in the PD patients compared to the control group, although this difference did not reach a significant level, likely due to a lack of statistical power. However, Borghammer et al. (2010) have demonstrated in meta-analyses of perfusion and metabolism studies, that global levels of CBF and CMRglc are generally reduced in PD. Our results help to clarify the controversy existing in the literature regarding the interpretation of changes in relative perfusion and metabolism in Parkinson’s disease (Borghammer et al., 2009a; Borghammer et al., 2009b; Ma et al., 2009). They also support the argument in favor of measuring physiological parameters in absolute values (Grunder, 2009). Arterial spin labeled perfusion MRI allows this absolute quantification, without requiring experimentally demanding procedures.

Cortical hypoperfusion in PD

Our results show perfusion deficits in prefrontal areas (BA 8 and 9), dorsal premotor cortex (PMd) and pre-SMA, posterior parietal cortex and parieto-occipital areas. Findings of perfusion changes in the pre-supplementary motor area in PD patients have been variable, with some authors reporting hypoperfusion (Kikuchi et al., 2001), others reporting hyperperfusion (Tang et al., 2010) and most reporting no change (Melzer et al., 2011). However, our finding is highly significant, with several maxima of hypoperfusion located in this area, which is also affected by gray matter volume loss, according to the morphometry study. Remarkably, a selective loss of pyramidal neurons has been reported in pre-SMA in postmortem brain tissue of PD patients, while all other nonprimary motor cortices were spared (MacDonald and Halliday, 2002). This finding highlights the relevance of this cortical area in the course of the disease. Anatomical and functional data suggest that pre-SMA is more like a prefrontal area than a motor area. In fact, only pre-SMA is interconnected to the prefrontal cortex, contrarily to SMA proper (Bates and Goldman-Rakic, 1993; Lu et al., 1994; Luppino et al., 1993). Functionally, activation of pre-SMA has been associated with cognitive aspects of a variety of tasks (see for review Picard and Strick, 2001). These features are also shared by the rostral portion of the PMd (Picard and Strick, 2001) which falls within the areas of hypoperfusion detected in the present work. Through its connections with the prefrontal cortex, pre-SMA may have access to the executive-control network that links dorsolateral frontal and parietal cortices (Seeley et al., 2007), areas that were also affected by perfusion deficits. Subcortically, this executive-control network is functionally coupled to the dorsal caudate nucleus, which also showed hypoperfusion. Disruption in this functional network may underlie the cognitive deficits that are already present in the early stages of the disease, affecting specific domains such as working memory, attention and visuo-spatial processing (Caballol et al., 2007; Possin, 2011). It will be of great interest in future studies to assess quantitatively specific neuropsychological functions of our PD patients, in order to determine possible correlations between these cognitive measures and specific perfusion disturbances. Why these areas show higher or earlier vulnerability as compared to other motor areas like SMA proper, or primary motor and sensory cortices, is not known. However, pathological studies on disease progression reveal that these primary cortical areas are the last ones to be affected (Braak et al., 2003).

The cortical perfusion deficit is probably related to gray matter atrophy in some areas, as shown by the overlap of atrophy and perfusion decreases found in our patient group, in the premotor and pre-supplementary motor areas in the frontal lobe, and in the left superior parietal lobule. However, the perfusion deficit is more extensive than the loss of gray matter volume. On the other hand, a few atrophic areas do not appear to be affected by reduced perfusion.

Morphometry studies in early to moderate stage PD patients without dementia have reported slight or no gray matter atrophy, while studies in late stage PD patients with dementia have found quite extensive gray matter loss in temporal, frontal, occipital and parietal areas (Burton et al., 2004). In our study, atrophy was not present in the occipital lobe and affected only a small area of the parietal lobe. On the other hand, cortical perfusion appeared to be decreased in most of these cortical areas, except for the temporal lobes, even though patients were at an early to moderate stage of the disease. These results suggest that functional changes precede structural changes. Thus perfusion could be a more sensitive biomarker than gray matter loss in early PD.
The etiology of the observed cortical hypometabolism remains to be elucidated. Although degeneration of the nigrostriatal pathway, with the consequent reduction of dopamine in the striatum is the better known pathological process in Parkinson’s disease, there is evidence that other nondopaminergic transmitter systems are affected early during the disease course, including serotonergic and noradrenergic systems (Obeso et al., 2010). Cholinergic denervation in primates, following lesions in the basal forebrain, has been reported to induce wide-spread hypometabolism in the cortex (Kiyosawa et al., 1989). Shimada et al. (2009) have recently shown cholinergic deficits in the cerebral cortex of PD patients without dementia, which were most significant in the medial occipital cortex. According to our data, the largest perfusion deficits are also localized in the occipital cortex. While this could be a coincidence, it could alternatively reflect an association between cholinergic dysfunction and cortical hypoperfusion. Other hypotheses have been proposed that involved deficits in other neurotransmitter systems (Borghammer et al., 2010), cortical body pathology or Alzheimer pathology (Liepert et al., 2009), although these are more likely to be relevant at the late disease stages, and in a PD population with signs of dementia (Braak et al., 2003).

Subcortical perfusion abnormalities in PD

The whole brain voxel-wise comparison of absolute CBF values revealed two clusters of decreased perfusion in the caudate nucleus of PD patients, however these clusters did not survive a cluster-corrected threshold. The ROI analysis results also supported the findings of decreased perfusion in the caudate nucleus, in agreement with Berding et al. (2001) and more recently Borghammer et al. (2010), who have also reported metabolic decreases in the caudate nucleus.

Both the whole brain voxel-wise and the ROI analyses failed to reveal significant absolute CBF changes in the other basal ganglia nuclei and thalamus. The voxel-wise comparison showed bilateral subcortical areas of increased perfusion in the PD patients, that covered a small fraction of the lateral globus pallidus and thalamus; however, these perfusion increases were not significant. 2DG autoradiographic studies in animal models of PD have consistently reported increased metabolism in the lateral globus pallidus. Pallidal hypermetabolism has also been observed in a recent high-resolution PET study of PD patients, in which the authors used reference cluster normalization methods (Borghammer et al., 2011). However, no study in PD patients has detected absolute hypermetabolism in the basal ganglia. Detection of absolute perfusion alterations in the small basal ganglia nuclei will require the acquisition of CBF maps of higher resolution and signal-to-noise ratio. Recent technical developments and the availability of high field MRI scanners will allow increased resolution and could facilitate the evaluation of perfusion in these small subcortical structures (Feinberg et al., 2009).

Influence of medication state

In our study, patients were assessed under medication in ON state. Thus an effect of dopaminergic therapy in the results cannot be discarded. Studies comparing perfusion or metabolism in PD in ON and OFF states are scarce. Berding et al. (2001) reported hypometabolism in cortex and caudate nucleus in patients ON and OFF medication compared to control subjects, with significant differences between ON and OFF located only in orbitofrontal cortex. In addition, Liepelt et al. (2009) found no correlation between daily dose of levodopa and cortical hypometabolism. This indicates that our findings are probably robust to medication state. However, the effect of dopaminergic therapy on cerebral blood flow requires further investigation and it will be the aim of future work.

Conclusions

The results of the current study contribute to identify the true pattern of perfusion alterations in Parkinson’s disease. This could have an impact on our understanding of the pathological features of the disease progression. Our results support the hypothesis that cortical perfusion and metabolism are reduced in PD, even at the early disease stages, earlier than most other previous studies have suggested. In addition, this work demonstrates that the ASL technique has enough sensitivity to detect this perfusion decreases by means of absolute CBF comparisons, without the need for normalization techniques. Normalization to a reference region requires the a priori assumption that the reference region is not affected by the disease and therefore is potentially biased. Similar problems could be affecting metabolism and perfusion assessments in other brain disorders.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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