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Towards mapping the brain connectome in depression: Functional connectivity by perfusion SPECT

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ABSTRACT

Several studies have demonstrated altered brain functional connectivity in the resting state in depression. However, no study has investigated interregional networking in patients with persistent depressive disorder (PDD). The aim of this study was to assess differences in brain perfusion distribution and connectivity between large groups of patients and healthy controls. Participants comprised 91 patients with PDD and 65 age- and sex-matched healthy controls. Resting state perfusion was investigated by single photon emission computed tomography, and group differences were assessed by Statistical Parametric Mapping. Brain connectivity was explored through a voxel-wise interregional correlation analysis using as covariate of interest the normalized values of clusters of voxels in which perfusion differences were found in group analysis. Significantly increased regional brain perfusion distribution covering a large part of the cerebellum was observed in patients as compared with controls. Patients showed a significant negative functional connectivity between the cerebellar cluster and caudate, bilaterally. This study demonstrated inverse relative perfusion between the cerebellum and the caudate in PDD. Functional uncoupling may be associated with a dysregulation between the role of the cerebellum in action control and of the caudate in action selection, initiation and decision making in the patients. The potential impact of the resting state condition and the possibility of mitochondrial impairment are discussed.

1. Introduction

A general consensus about the regional cerebral blood flow (rCBF) and the metabolic changes in the brain occurring during unipolar depression has not yet been reached despite extensive investigations. Studies of patients with depression using single photon emission computed tomography (SPECT) have had inconsistent results, showing regional decreases of rCBF, No differences, or increases in comparison with controls (Nikolaus et al., 2000).

The ability to collect large amounts of connectivity data combined with the understanding that the fundamental properties of the brain result from large-scale network topology led researchers to conceptualize the notion of the "connectome" and its related science "connectomics" (Hagmann et al., 2012). Dysconnectivity has been implicated in the etiology of many neuropsychiatric diseases (Hagmann et al., 2010). In Alzheimer´s disease (AD), brain connectome studies based on results from electroencephalography (EEG), positron emission tomography (PET) and magnetic resonance imaging (MRI) demonstrated disrupted network connectivity pattern in support of the theory that AD is a disconnection syndrome which might be the functional basis of the cognitive deficits (reviewed in Delbeuck et al., 2003; Xie and He, 2012). In this respect, factorial analysis was implemented to discriminate patients from controls resulting in a high accuracy of the method (Nobili et al., 2008; Pagani et al., 2009). However these studies investigated the correlations between regions defined *a priori*, an approach that could have resulted in missing brain networking at the cluster level.

The well-established Statistical Parametric Mapping (SPM) approach can be used to explore metabolic and perfusion connectivity with voxel-wise interregional correlation analysis (IRCA). IRCA uses a cluster of voxels as a seed to explore the reciprocal positive and negative interconnections with the rest of the brain

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(Lee et al., 2008) and has been shown to consistently reproduce resting-state networks across healthy subjects (Damoiseaux et al., 2006). Recently, this approach has been used in several neuroimaging studies in which important networks as well as abnormalities of connectivity in neurodegenerative diseases have been disclosed (Bookheimer et al., 2000; Mosconi et al., 2004; Drzezga et al., 2005; Morbelli et al., 2013). A recent PET study of prodromal AD used IRCA to demonstrate widespread metabolic disconnection that may be a possible sign of synaptic degeneration and may contribute to disease's clinical signs (Morbelli et al., 2012).

A prevalence of chronic depressive disorder of 4.6% was reported in an Australian study (Murphy and Byrne, 2012). Chronic depression was found in 29.4% of patients with depressive disorder at some time(s) during the lifetime. As compared with non-chronic depression, chronic depression is characterized by a younger age at onset, more frequent depressive episodes, and more co-morbid medical and other psychiatric conditions. A biological predisposition to chronic depression has been suggested (Murphy and Byrne, 2012). Criteria have been formulated for the new diagnosis of "Persistent Depressive Disorder (Dysthymia)" in the DSM-5, a diagnosis that represents a consolidation of DSM-IV-defined chronic major depressive disorder and dysthymic disorder (American Psychiatric Association, 2013). The criteria require that depressed mood must have been present for at least 2 years, as in the patients in the current study. Chronic depression has been associated with poorer response to antidepressant medications (Fournier et al., 2009) as well as to cognitive behavioral therapy compared with response in non-chronic major depression (Horn, 2012).

The implication of an emerging consensus that most major psychiatric disorders do not arise from isolated dysfunction in one or a few brain regions, but rather from disturbed interactions within and between distributed neural circuits, is that *psychiatric disorders are disorders of brain connectivity* (Fornito and Bullmore, 2012). On the other hand functional neuroimaging studies investigating resting state functional connectivity in depression have been mainly performed by functional MRI (fMRI) and often implicate the default mode network, including prefrontal and anterior cingulate cortex and precuneus (Sheline et al., 2010; Leibenluft and Pine, 2013) as well as the cortico-limbic mood regulating circuit including prefrontal, amygdala, pallidostriatal and thalamic regions. However, these investigations were performed in groups of patients and normal controls not exceeding 32 individuals and, to the best of our knowledge, no SPECT studies have been performed so far to disclose the resting state perfusion networks in major depression.

The aim of the study was to identify the differences in the rCBF distribution as measured by SPECT between large groups of patients with longstanding depression and healthy controls, and to investigate functional connectivity arising from the affected regions under the assumption that of regions with limbic valence will be involved.

2. Methods

2.1. Subjects

2.1.1. Patients

The patients comprised 91 depressed patients with a mean ($\pm$S.D.) age of 48 ± 11 years, of whom 52 were females (57%). All patients had a chronic type of unipolar depressive disorder that fulfilled DSM-5 criteria for persistent depressive disorder (PDD). PDD criteria include depressed mood for most of the day on most days for at least 2 years (American Psychiatric Association, 2013). All patients attended a tertiary psychiatric out-patient unit for clients with any type of audiological symptom. Thirteen of them had early severe hearing impairment. Other patients with PDD were also referred to this unit, and thus there were no audiological symptoms (unilateral hearing impairment, tinnitus or hyperacusia) in 14 patients. Most patients also had other somatic symptoms (mild ocular/visual or muscular pain symptoms) that also are common in unipolar depression (Gardner et al., 2003). Although cognition was objectively assessed only in some patients of the investigated sample, none of the patients had any clinical sign of cognitive impairment, but did have fluctuating non-progressive cognitive symptoms.

Sixty-two patients (68%) were unmedicated, mostly because of a lack of effect of antidepressant medications. The remaining 29 patients were medicated with various agents (11 patients with citalopram, 9 fluoxetine, 1 sertraline, 4 tricyclics (clomipramine or trimipramine), 2 anxiolytics only, 2 neuroleptics alone and 1 in combination with citalopram). Substance abuse was excluded by interview in all patients.

At the time of the SPECT examinations, 66 patients completed the self-rated 9-item version of the Montgomery–Åsberg Depression Rating Scale (MADRS-S) (Montgomery and Åsberg, 1979). Sixty-two patients completed the Cognitive Failures Questionnaire (CFQ), which measures the capacity for attentiveness and self-monitoring in everyday life (Broadbent et al., 1982). It was not possible to obtain MADRS-S or CFQ ratings in all patients since some patients postponed the out-patient SPECT scanning beyond the time for which the ratings were considered as current (1 month). However, rating scores obtained at several times in most of them almost always showed similar results, attesting to the chronicity of mood and cognitive symptoms in the patients.

2.1.2. Healthy controls

There were 65 healthy subjects with a mean ($\pm$S.D.) age 49 ± 15, of whom 37 were females (56%). Healthy comparison subjects were specifically recruited among the friends and spouses of stroke patients hospitalized at the Neurology Karolinska Hospital to serve as controls without undergoing any other nuclear medicine examination for diagnostic purposes. Exclusion criteria were presence of major systemic illness, major vision disturbances, psychiatric illnesses, epilepsy, head trauma, Parkinsonism, previous stroke or transient ischemic attack, and brain masses as well as the current use of benzodiazepines and antidepressants. A psychiatric interview was performed. Normal results were obtained on the MADRS and Mini Mental State Examination (MMSE) (Folstein et al., 1975).

The study was approved by the local Ethics and Radiation Radiation Safety Committees. All subjects gave written informed consent.

2.2. Procedures

2.2.1. SPECT

Brain imaging using SPECT was performed using a three-headed Gamma Camera (TRIAD XLT 20, Trionix Research Laboratory Inc., Twinsburg, OH, USA) equipped with low-energy ultrahigh-resolution collimators. The intrinsic spatial resolution of the camera was 10 mm at full width half maximum (FWHM). A dose of 1000 MBq (27.0 mCi) of ^{99m}Tc-D,L-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO, Ceretec; Amersham International plc, UK) was injected intravenously within 20 min from reconstitution. The projection data were acquired for 15 s per projection at 90 equal angles of a complete revolution (0–360°). Before reconstruction, the projection data were pre-processed using a 2D Hamming filter with a cut-off frequency of 2.25 cycles/cm. Sectional images were reconstructed by filtered back-projection using a Ramp filter with a cut-off frequency of 0.6 cycles/cm. During pre-processing, correction for attenuation was performed. No scatter correction was applied. Both acquisition and reconstruction were performed in 128×128 matrices with a pixel size of 2.22×2.22 mm^2 resulting in an isotropic voxel size of 2.2 mm^3.

2.2.2. Definition of perfusion changes area in PDD

Before interregional correlation analysis, the areas of perfusion changes in the 91 PDD patients were assessed with respect to the whole group of 65 controls. This preliminary step aimed at identifying regions of likely blood flow changes where additional perfusion connectivity was further evaluated by voxel-based analysis.

Group differences in brain ^{99m}Tc-HMPAO uptake were analyzed using Statistical Parametric Mapping (SPM2, Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 6.5 (Mathworks, Natick, MA, USA). SPECT data were subjected to affine and non-linear spatial normalization into the Montreal Neurological Institute (MNI) space. The spatially normalized set of images was then smoothed with a 12-mm isotropic Gaussian filter to blur individual variations in gyral anatomy and to increase the signal-to-noise ratio. Images were globally normalized to 50 using proportional scaling to remove confounding effects due to global blood flow changes, with a threshold masking of 0.8. The resulting statistical parametric maps, SPM{t}, were transformed into normal distribution (SPM{z}) units. The significance of identified regions was established at $p < 0.05$, corrected for multiple comparisons for the False Discovery Rate (FDR_{corr}) option at both peak and cluster level. Only those clusters containing more than 500 ($8 \times 8 \times 8$ voxels, i.e., $16 \times 16 \times 16$ mm^3) contiguous voxels were accepted as significant, based on the calculation of the partial volume effect resulting from the spatial resolution of the SPECT camera. Comparisons were performed by means of the 'compare populations: 1 scan/subject (ANCOVA)' option using age and gender as covariates (Nardo et al., 2011). The same model was implemented to test the CBF distribution difference between the two groups of medicated and unmedicated patients.

SPM2 co-registers the individual SPECT to the 152-brain average of the MNI (http://www.bic.mni.mcgill.ca). Because the MNI template does not completely match the Talairach brain, it is necessary to correct the SPM coordinates. The correction was achieved with a dedicated subroutine (http://imaging.mrc-cbu.cam.ac.uk/imaging/CbuImaging). Brodmann areas were then identified after importing the corrected coordinates, by means of the Talairach Deamon Database (http://www.talairach.org/). The clusters found to be significantly different in this analysis were saved and used as a single Volumetric Region of Interest (VROI) in the subsequent connectivity analysis.

2.2.3. Voxel-wise interregional correlation analysis (IRCA)

In both the patient and control groups, extracted mean regional VROI counts normalized to thalamus were used as covariates to find regions showing significant voxel-wise correlations across subjects. Following the group analysis, the finding of relative hyperperfusion in a large cerebellar region indicated that the values from whole brain or cerebellum not be used for normalization in post-hoc analysis. The choice of thalamus as the normalizing region was due to its high grey matter content resulting in a high relative CBF and to the lack in this region of any group difference in the PDD vs. controls comparison.

Correlation analyses were carried out in the two groups separately by the 'single-subjects covariates only' design model. Age and gender were included as confounding variables. Clusters were regarded as significant if they survived the $p < 0.05$ threshold, corrected for False Discovery Rate (FDR_{corr}) at peak and cluster levels. Notwithstanding this already conservative threshold, the stricter Family Wise Error correction (FWE_{corr}) at voxel level was also explored in all correlations, accepting the risk of false negatives due to type II statistical errors.

3. Results

The mean ($\pm$S.D.) score for the MADRS-S in the PDD patients was 24 ± 11. A score of 18 or above is considered to reflect major depression. The mean ($\pm$S.D.) score for the CFQ was 58 ± 19. Mean ($\pm$S.D.) CFQ scores of 32 ± 11 for healthy controls, and of 56 ± 16 for patients with multiple past episodes of unipolar depression, have been reported (MacQueen et al., 2002). No correlations were found between the total scores of the MADRS-S or the CFQ with the rCBF distribution in the cerebellum or the caudate.

Significantly increased rCBF distribution ($p < 0.05$ FDR_{corr}) was found in the patients as compared with the controls in a cluster covering large part of the bilateral cerebellum (Fig. 1). No significant hypoperfusion at the chosen threshold was found.

No significant differences in CBF distribution at the chosen statistical threshold or at any more liberal one (i.e., $p < 0.05$ uncorrected for multiple comparison) were found in comparisons of medicated versus unmedicated patients.

At IRCA this cluster showed a highly significant negative correlation ($p < 0.001$ FWE_{corr}) with bilateral caudatus in patients (Fig. 2).

When the same cluster was negatively correlated in the control group to relative CBF distribution in the whole brain, a huge cluster encompassing mainly fronto-parietal and subcortical regions was found to be significant at $p < 0.05$ FDR_{corr} level.

Besides the expected autocorrelation, no positive correlations were found in either group at the chosen statistical threshold ($p < 0.05$ FDR_{corr}).

4. Discussion

The results of this study including a large group of chronically depressed patients indicate a strong negative functional connectivity between the bilateral cerebellum and the bilateral caudate. Such a perfusion correlation was not found in a large group of healthy controls in which more widespread connections were found. Functional connectivity was assessed by interregional correlation analysis allowing extraction of regional independent components correlated in neural networks during resting state brain perfusion scans.

In a review article on functional connectivity in psychiatric disorders, the authors wrote "Over two decades of neuroimaging research has found that most major psychiatric disorders do not arise from isolated damage to one or a few brain regions, but rather from multiple abnormalities distributed throughout the cerebrum. These abnormalities likely have their origin in disturbed interactions between discrete and distributed neural circuits; i.e., disordered brain connectivity" (Fornito and Bullmore, 2012). A large number of functional connectivity studies using fMRI in depression have been published since 2007. The results of a study comparing resting state functional connectivity before antidepressant treatment in non-refractory and refractory depression suggested differences between these patient groups characterized by distinct functional changes in distributed brain networks (Lui et al., 2011).

In the present study, the cluster of voxels in which increased relative perfusion was observed in the patients covering a large part of the cerebellum has been used as the "node" or "seed" for the search for functionally connected regions. In *connectomics*, the brain is modeled as a graph of nodes, representing brain regions, functionally linked by edges, representing some measure of interregional structural or functional interaction. The seed concept applies to the statistical dependence between a specific region and all other recordings of the whole brain, and is undirected (Fornito and Bullmore, 2012). We found our "seed region" in the patients in the cerebellum to show a strong *negative* correlation with the bilateral caudatus.

Recent research implicates the cerebellum in emotion, language, working memory, and executive functions. The variety of observed cognitive and affective impairments in patients suffering from cerebellar lesions has been referred to as "dysmetria of thought

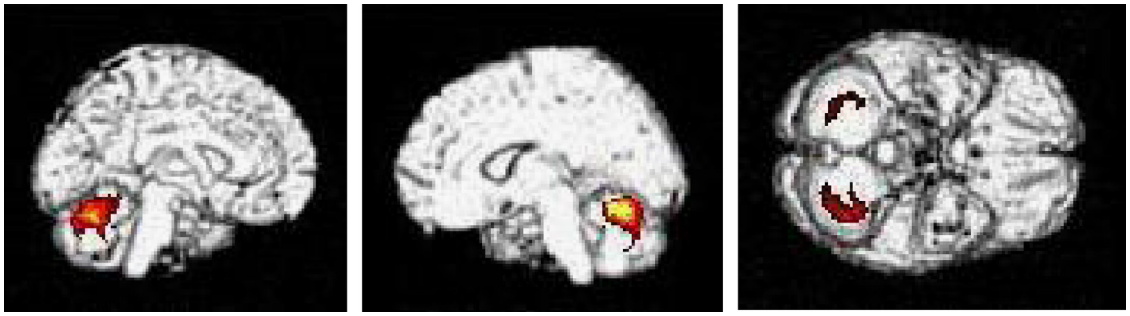

Fig. 1. Cerebellar hyperperfused cluster in patients as compared to controls. (On the left, medial aspect of left hemisphere, at the center medial aspect of right hemisphere, on the right view from below). Height significance threshold: $p < 0.05$, corrected for multiple comparisons (False Discovery Rate), at both peak and cluster levels. Figure displays regions of significant difference, color-graded in terms of z values. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

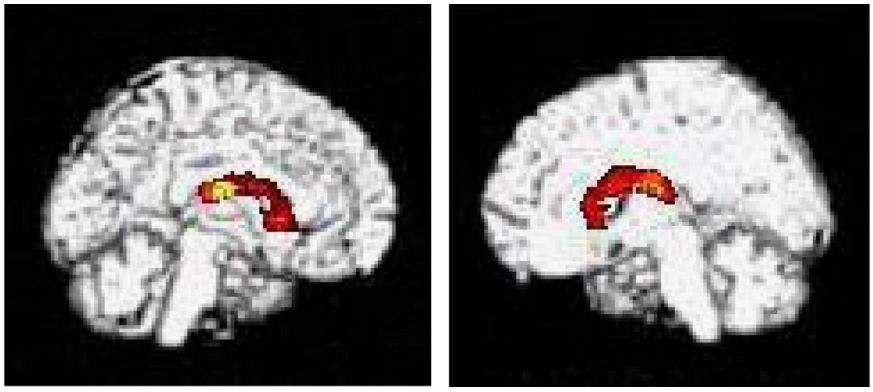

Fig. 2. Voxel-wise interregional negative correlation of bilateral caudate nuclei with the cerebellar hyperperfused cluster. (On the left, medial aspect of left hemisphere, on the right medial aspect of right hemisphere). Height significance threshold: $p < 0.001$, corrected for multiple comparisons (Family Wise Error), at both peak and cluster levels. Figure displays regions of significant difference, color-graded in terms of z values. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and emotion". The main function of the cerebellum is presumed to be an oscillation dampener that maintains function automatically around a homeostatic baseline, modulating and smoothing out performance in cognitive and emotional domains (Schmahmann, 2004; Habas et al., 2009). Patients with cerebellar lesions exhibit deficits in planning, executive control, memory and learning, and attention processing (Baillieux et al., 2008). Damage to the cerebellum causes impaired regulation of the timing of an action suggesting that the cerebellum is involved in a broad spectrum of abilities associated with voluntary action control (Grealy and Lee, 2011). In an fMRI study of healthy subjects, intrinsic connectivity networks were found between the cerebellum and sensory-motor and executive networks with, amongst other structures, the caudate (Habas et al., 2009).

Structural alterations of the cerebellum have been reported in depression including *decreased* cerebellar gray matter and total cerebellar volume correlating with severity of depression in antidepressant-nonresponsive patients (Pillay et al., 1997). Decreased volume in the bilateral cerebellar gray matter was found in those with symptoms of suicidality in geriatric depression (Hwang et al., 2010), and decreased white matter in the left cerebellum posterior lobe in early middle-aged treatment-naïve patients with depression (Zeng et al., 2012a). However, a volumetric *increase* of gray matter in the right cerebellum in early middle-aged medication-free depressed paptients has also been reported (Scheuerecker et al., 2010), as well as increased diffusivity considered to reflect fiber density in focal cerebellar areas in middle-aged depressed patients of whom more than half were in remission (Abe et al., 2010). Thus, consistent findings have not been reported for cerebellar volume in depression.

A cerebellar involvement in functional networks has been reported in depression. A study of medication-naïve adults with major depression demonstrated increased cerebellar coupling with the temporal poles and decreased coupling with the frontal lobe regions and the posterior cingulate cortex compared with healthy controls (Liu et al., 2012). In another study of medication-naïve patients with major depression with previous depression episodes compared with controls, the findings suggested that the cerebellum might be considered as a node in the distributed disease-related brain network (Ma et al., 2013). Increased connections between three cerebellar ROIs were observed with cerebral regions (the anterior cingulate, the ventromedial prefrontal, and the ventrolateral prefrontal cortex) (Zeng et al., 2012b). These two latter studies were carried out by fMRI in medication-free patients with unipolar recurrent depression and investigated the cerebellar-cortical connections by systematically correlating several predefined cerebellar regions to all brain voxels. The approach of the present study was not to investigate the cerebellum *a priori* but to consider it for connectivity analysis only after it had shown significant differences between patients and controls. In this respect, the finding of a negative connectivity between the cerebellum and the caudate, not previously reported, could be due, beyond pathophysiological reasons, to differences in patients' selection and sample size, depression diagnosis, technique-driven differences and statistical trends, making a direct comparison difficult. However, the involvement of the cerebellum in disease-specific networks found in patients with different types of depression and by complementary methodologies reinforces the hypothesis of its critical role.

The caudate, or the dorsomedial striatum, has been referred to as the "cognitive associative striatum" due to its contributions to action selection and initiation, and decision-making, through integration of sensory-motor, cognitive, and motivational/emotional information in learning processes supporting goal-directed learning and action (Balleine and O'Doherty, 2010; Porcelli et al., 2012).

An involvement of the caudate in unipolar depression has been suggested in morphological and functional studies (reviewed in Pillay et al., 1998; Lorenzetti et al., 2009). Smaller caudate volume was found bilaterally in scans performed at disease inception in adolescent-onset, mostly psychotropic-naïve patients with depression, suggesting that structural deficits may be present early in depressive illness (Shad et al., 2012). Significantly smaller caudate volume was observed in elderly untreated depressed patients, with greater caudate reduction being associated with more severe depression (Butters et al., 2009). Increased right caudate rCBF (Monkul et al., 2012) and lower glucose metabolic rate in the bilateral caudate (Baxter et al., 1985) have also been reported in untreated depression.

In a resting state fMRI study of young patients with early depression and low lifetime dysfunction, most of whom were medication-free, reduced connectivity was observed between the precuneus/posterior cingulate cortex and the bilateral caudate in comparison with matched controls. No areas of increased connectivity were observed in the depressed group (Bluhm et al., 2009). These findings were interpreted to reflect a disturbance of the reward-processing function of the caudate that could be involved in the anhedonia, including difficulty in the motivation to act, that is found in depression (Bluhm et al., 2009). Unmedicated, apathetic patients with late-life depression have shown reduced connectivity in the left caudate when the right or the left nucleus accumbens is used as the seed region. Apathy is common

in late-life depression and leads to loss of interest in novel activities and planning, and a lack of effort in productive activities (Alexopoulos et al., 2013).

Regional perfusion connection analysis might reveal cortical and subcortical networks larger than those found by univariate analysis, being sensitive to the disease-induced alterations also at a sub-threshold level. In our study the identification of a cluster of voxels showing uptake differences between patients and controls suggested this region to be implicated in PDD. Perfusion changes can originate from local neuronal modifications as well as from altered input signaling from remote brain areas in which perfusion differences do not reach statistical significance. In this respect, the added value of the implemented methodology was to detect by correlation analyses sub-threshold changes that might reflect the underlying hidden perfusion network.

Despite the novelty of the approach, the reliability of the study using perfusion SPECT to explore functional connectivity in depression was reinforced by the large number of investigated subjects and the high statistical reliability of the study since the sampling error decreases with increase of sample size. In the present study, 32% of patients were medicated with various drugs, mainly selective serotonin reuptake inhibitors and anxiolytics, whose use has been reported to decrease CBF (Chen et al., 2011). However, the lack of any CBF distribution group difference between medicated and unmedicated patients ruled out the possibility of any impact of medication on our results.

The CBF at rest reflects the baseline state of the brain, always active even during periods of repose. During the "resting state" in clinical and experimental studies that do not involve brain activations, each individual is actually processing information and elaborating on concepts and sensations (Papo, 2013). Our patients may have experienced the peri-injection (the 2 min preceding and the 2 min after the injection with the radioactive tracer) *psychological state* during tracer uptake differently from the controls. In reality, no "resting state" condition exists in awake individuals. Depressed patients may process the examination at a different cognitive and emotional level, experiencing a higher level of anxiety and insecurity concerning the outcome. The special conditions during radionuclide injection may also have caused concern and hypersensitivity to the external environment that was more severe in patients than in controls. Restricting body movements in a limited space for almost an hour may have been experienced as more uncomfortable in patients during the peri-injection *anticipated or sensory experienced state* due to physical pains, which are common in depression (Lépine and Briley, 2004). It cannot be excluded that the sensory experience at peri-injection in the patients might resemble the negative stimuli reported to increase cerebellum activation (Fitzgerald et al., 2008). Thus, different pathology-related psychological and physical states at the time of the SPECT procedures could also be responsible for different perfusion patterns since these states might have affected the brain, especially group-wise, resulting in the observed differences between patients and controls.

The mechanism underlying the decreased functional connectivity between the cerebellum and the caudate in our PDD patients remains to be determined. CBF, as indexed by HMPAO, is associated with the cellular content of reduced Gluthatione (GSH). Increased levels have been reported in mitochondrial disorders, considered to reflect GSH up-regulation secondary to reduced respiratory chain activity (Filosto et al., 2002). A preferential involvement of the cerebellum has been reported in secondary mitochondrial dysfunction (Montero et al., 2007). In the classic mitochondrial disorders, cerebellar involvement is frequently reported and cerebellar atrophy might even be the primary neuroradiological finding (reviewed in Lax et al., 2012). Basal ganglia structures including the caudate are also among the most frequently affected brain regions in mitochondrial disorders (Haas and Dietrich, 2004; Friedman et al., 2010) and can be combined with atrophy of the cerebellum (Delonlay et al., 2013). Impaired mitochondrial function has been suggested in depression and other psychiatric disorders (Gardner and Boles, 2011; Manji et al., 2012). The finding of high cerebellar $^{99\,m}$Tc-HMPAO uptake in chronically depressed patients as compared with controls might then also be due to local intracellular enzymatic changes associated with mitochondrial dysfunction (Gardner et al., 2008). Our findings might be speculated to reflect an inverse connectivity between cerebellum and caudate which might indicate altered signaling from the former or a failure by the latter to process information flowing into it when these structures are affected by impaired mitochondrial function. This negative functional correlation could tentatively cause a mismatch between action planning and execution, and might result in decreased attentiveness and everyday life self-monitoring, as reported by our patients as indicated by their high scores on the CFQ rating scale.

In conclusion, even if the mechanism underlying the strong negative perfusion correlation between the cerebellum and the caudate has not yet been defined, our findings imply an important role for these structures in the neurobiology of PDD and indicate that disease-related resting-state network alterations may be associated with the complex symptomatology of depression. Whole brain functional connectivity might be considered as a potential biomarker in studies that attempt to identify the various endophenotypes of unipolar depression. However, significant advances are required before functional connectivity can be used in a meaningful way at the single patient level. In the vein of the first words of the title of this study "Towards mapping", and at realizing the many pitfalls inherent in this field (Kelly et al., 2012), we end with a concluding sentence from a book of science essays (Schatz, 2011):

We scientists are working on a cathedral whose completion none of us will ever see.

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