Principal component and volume of interest analyses in depressed patients imaged by ^{99m}Tc-HMPAO SPET: a methodological comparison

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Abstract. Previous regional cerebral blood flow (rCBF) studies on patients with unipolar major depressive disorder (MDD) have analysed clusters of voxels or single regions and yielded conflicting results, showing either higher or lower rCBF in MDD as compared to normal controls (CTR). The aim of this study was to assess rCBF distribution changes in 68 MDD patients, investigating the data set with both volume of interest (VOI) analysis and principal component analysis (PCA). The rCBF distribution in 68 MDD and 66 CTR, at rest, was compared. Technetium-99m *d*,*l*-hexamethylpropylene amine oxime single-photon emission tomography was performed and the uptake in 27 VOIs, bilaterally, was assessed using a standardising brain atlas. Data were then grouped into factors by means of PCA performed on rCBF of all 134 subjects and based on all 54 VOIs. VOI analysis showed a significant group × VOI × hemisphere interaction ($P<0.001$). rCBF in eight VOIs (in the prefrontal, temporal, occipital and central structures) differed significantly between groups at the $P<0.05$ level. PCA identified 11 anatomo-functional regions that interacted with groups ($P<0.001$). As compared to CTR, MDD rCBF was relatively higher in right associative temporo-parietal-occipital cortex ($P<0.01$) and bilaterally in prefrontal ($P<0.005$) and frontal cortex ($P<0.025$), anterior temporal cortex and central structures ($P<0.05$ and $P<0.001$ respectively). Higher rCBF in a selected group of MDD as compared to CTR at rest was found using PCA in five clusters of regions sharing close anatomical and functional relationships. At the single VOI level, all eight regions showing group differences were included in such clusters. PCA is a data-driven method for recasting VOIs to be used for group evaluation and comparison. The appearance of significant differences absent at the VOI level emphasises the value of analysing the relationships among brain regions for the investigation of psychiatric disease.

Keywords: Unipolar depression – SPET – rCBF – Principal component analysis

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Introduction

Depression is estimated to rival virtually every other known medical illness in burden of disease morbidity early in this millennium [1]. On a life-time basis, unipolar major depressive disorder (MDD) is estimated to affect about a quarter (26%) of the population [2]. MDD is a common primary idiopathic disorder characterised by the occurrence of depressive episodes (unipolar depression, DSM-IV). Associated somatic symptoms, including pains, are listed as common features [3].

Changes in cerebral blood flow (rCBF) in MDD may reflect physiological correlates of the depressive state, mood-state-independent grey matter volume and histological abnormalities [4, 5, 6] as well as patho-physiological alterations predisposing the subject to depression [7, 8, 9, 10]. Discrimination between these alternatives may be of extreme importance in differentiating primary

depression from depression secondary to neurodegenerative disorders.

In neuroimaging studies, unipolar depression has frequently been reported to be associated with reduced rCBF in prefrontal cortex (PFC) and anterior cingulate cortex [11, 12, 13], and this has been tentatively explained by an abnormal flow reduction in the grey matter volume of these regions [6, 14]. However, other studies have pointed to a higher PFC rCBF in depressed patients [15, 16, 17]. A reduced flow in depressed patients has also been found in the lateral temporal and parietal cortex [15, 18, 19, 20, 21] as well as in basal ganglia [15, 22, 23]. On the other hand, increased flow has been observed in patients with unipolar depression in the amygdala, in the mediodorsal nucleus of the thalamus and in the ventral striatal regions involved in mediating emotions [15, 24, 25]. Similar findings of higher blood flow have also been reported among MDD patients in the lateral orbital and ventrolateral PFC and in the pregenual anterior cingulate cortex [15, 18]. Overall, there is a predominance of investigations indicating rCBF reductions as compared to reports suggesting rCBF increases in depressed patients [26].

Most previous brain single-photon emission tomography (SPET) studies have evaluated and analysed rCBF using an asymmetry index or outlining the regions of interest in a manual or semi-automatic mode, building up ratios between target and reference regions. For evaluation and analysis of images obtained in this work, we adapted all SPET studies to a computerised brain atlas (CBA), originally developed by Greitz et al. [27]. The atlas automatically identified volumes of interest (VOIs), corresponding to Brodmann areas and central structures, that were submitted to statistical analysis for group comparison. In a second step, in order to investigate physiologically correlated but anatomically distributed brain regions, VOIs were subject to principal component analysis (PCA), which resulted in a reduction of the number of variables into factors. This latter way of selecting volumes to be analysed takes into account the mathematical correlations between variables, reflecting presumably the influence one neuronal system exerts over another and implicating mutual functional interactions [28].

Neuronal circuitry abnormalities in major depression have been analysed by Mayberg [29], the results suggesting that "foci of network dysfunction identified in the base-line depressed state" might be responsible for the reported variations in the pretreatment scan.

The aim of this study was to assess at rest the differences in rCBF between a group of unipolar depressed patients and a control group of normal individuals. VOIs and factors were analysed independently and the results compared.

Materials and methods

Subjects

Normal subjects. Sixty-six control subjects (CTR) were included in the study. They were 39 males (mean age±SD 45.5±17.1 years) and 27 females (mean age±SD 54.7±13.4 years). This group of subjects was specifically recruited to serve as control subjects for brain SPET investigations.

Control subjects had no personal history of physical or mental illness and the exclusion criteria also included brain trauma, cerebrovascular disorder and psychiatric disorders.

Subjects were assessed using two different neurological rating scales, the NIH-stroke scale [30] and Scandinavian Stroke Scale [31]. Psychiatric rating, based on a sub-scale of the Comprehensive Psycho-Pathological Rating Scale [32], the Montgomery-Åsberg Depression Rating Scale (MADRS) [33] and Mini Mental State Examination [34] were also performed. The results of all tests were within the range of normality.

All subjects received both oral and written information about the study from the responsible physician and signed a written informed consent. The study was approved by the ethics committee and the radiation protection committee at Karolinska Hospital.

Patients. Sixty-eight patients representing a selected group of MDD patients were included in the study. Thirty-one patients were males (mean age±SD 52.3±8.5 years) and 37 females (mean age±SD 45.8±8.5 years). Thirty-four percent of patients (n=23) were taking psychotropic medications at the time of the SPET study (serotonin re-uptake inhibitors, n=11, and tricyclic or tetracyclic agents, n=12). No patient was taking any neuroleptic agent. The remaining patients were either drug naive (n=13) or had previously been treated with antidepressant or psychotherapy without any effect. In some of them antidepressant treatment had been interrupted due to side-effects.

The patients were recruited from a hospital-affiliated psychiatric outpatient clinic accepting patients with concomitant physical symptoms (audiological, pain and/or intestinal motility symptoms), i.e. symptoms that have been found to be commonly associated with mood disorders [35, 36, 37, 38, 39, 40]. Seventy-eight percent of patients (n=53) had tinnitus and 99% (n=67) muscular stiffness and/or pain. All patients had had at least one MDD episode according to DSM-IV criteria and had a chronic depressive disorder with pronounced physical symptoms and significant impairment in social functioning. For ethical and practical reasons, SPET was performed during the chronic depressive state rather than during spikes with exacerbated mood symptoms. All subjects were outpatients and had to travel to the SPET examination without any assistance. The study was approved by the local ethics committee and all patients provided written informed consent.

Radiopharmaceutical and SPET

After 30 min rest in a tranquil place with dimmed light, 1,000 MBq (27.0 mCi) of technetium-99m *d*,*l*-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO, Ceretec, Amersham International plc, Little Chalfont, UK) was injected i.v. within 15 min from reconstitution. The radiopharmaceutical was prepared strictly according to the manufacturer's instructions. SPET brain imaging was performed using a triple-headed gamma camera (TRIAD XLT 20, Trionix Research Laboratory Inc., Twinsburg, OH, USA)

equipped with low-energy ultra-high-resolution collimators. 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 using the uniform Chang method [41]. 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.

Standardisation software

CBA (Applied Medical Imaging, Uppsala, Sweden) is a software tool for analysis of neuroimaging data [27, 42].

All image sets were spatially normalised into the stereotactic space of the atlas by using the global polynomial transformation [43]. This consists of translations, rotations and linear scaling along and around each of the three image axes. It also contains 18 non-linear shape-deforming parameters, which makes it possible to individualise the shape of the brain. In this study the fully automatic method was systematically implemented, in which CBA was fitted to all scan maximising the similarities with a reference one [44].

For evaluation and statistical analysis of the reformatted data sets, 27 VOIs, bilaterally, were selected. These regions corresponded to Brodmann areas (B) and numeration, in prefrontal (B9, B10, B46), frontal (B4, B6, B8, B44, B45), parietal (B1–3, B5, B7, B39, B40) and temporal (B21, B37, B38) cortex. Four regions, representing primary and associative auditory cortex (B22, B41, B42, B52), were merged into a single VOI. The remaining regions corresponded to cingulate (B24, B31, B32) and occipital (B17, B18, B19) cortex as well as putamen, nucleus caudatus, thalamus and hippocampus. In order to obtain a set of normalised relative flow data, a scaling factor was computed by averaging the brain voxel data and setting the global brain average to a predefined value. Before averaging the voxel data, a fixed counts/voxel threshold was selected to include in the normalisation process the 30% of all brain voxels with the highest counts [45]. The normalised value was set to 50 "uptake units" and all rCBF values of this work were related to this value.

Data analysis

After adaptation and definitions of VOIs using CBA, the ^{99m}Tc-HMPAO uptake data of all subjects were exported to a statistical package [46] for subsequent statistical analysis.

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

PCA was performed on all 134 subjects and based on all 54 VOIs in order to assess mathematical relationships characterising distributed neurophysiological connections among brain regions. The analysis was totally data-led and was independent of any model or established hypothesis.

PCA involves a mathematical procedure that transforms consecutively a number of (possibly) correlated variables into a (smaller) number of un-correlated factors called principal components, providing a unique solution. The first principal component accounts for the highest percentage of the global variability between the data, and each succeeding component accounts for the remaining variability in a descending scale. The number of factors to be extracted was determined after examining both eigenvalue and "Scree Plot" [47]. Given the large number of variables and to obtain a meaningful reduction of data, an eigenvalue greater than 1.2 was selected. Variables with a factor loading greater than 0.5 were extracted for each factor. The factors so obtained were then used to compare controls' and patients' rCBF.

ANOVA was used to test statistical significance of flow differences, considering groups and gender as independent between-subject variables. All means were weighed according to the VOI volume at both VOI and factor level.

Stepwise discriminant analysis was performed to estimate the relationship between groupings performed according to either previous clinical diagnosis or CBF distribution as determined by PCA.

Wilks' lambda for the discriminant function was assessed to test the statistical significance. Jackknifed classification matrix was used as a form of cross-validation.

Results

Demographic, whole brain and hemispheric data of both groups are shown in Table 1. No significant difference was found in the frequency distribution between groups and gender. When age was taken into account, there was a statistically significant interaction between groups and gender [$F(2,130)=12.72$, $P<0.001$]. Therefore, all subsequent ANOVA were co-varied for age.

In both VOI and factors analyses, there was a significant gender difference ($P<0.001$), but gender did not interact with groups in either of the two comparisons. Statistical analysis did not show any effect of medication on CBF.

The ANCOVA performed on the 54 VOIs showed significant hemisphere × group [$F(1,129)=9.56$; $P<0.005$] and VOI × hemisphere × group [$F(26,3354)=2.81$; $P<0.001$] interactions. We therefore performed 27 ANCOVA at the single VOI level. Group differences were found in eight VOIs belonging to the prefrontal, occipital and temporal cortex and to the central structures (Table 2).

PCA, performed on the 54 VOIs of the 134 subjects, resulted in 11 factors. These orthogonal and un-correlated factors explained 76% of the total data variance. Only two out of the 54 VOIs were excluded from the final solutions.

Overall analysis showed a group × factor interaction [$F(10,1290)=4.57$, $P<0.001$]. Such interaction demonstrated that unipolar depression preferentially affected blood flow distribution in some of the functional regions under study.

As compared to CTR, MDD rCBF was relatively higher in right associative temporo-parietal-occipital cortex ($P<0.01$) and bilaterally in prefrontal ($P<0.005$) and frontal cortex ($P<0.025$), anterior temporal cortex and central structures ($P<0.05$ and $P<0.001$ respectively;

Table 1. Demographic and mean CBF data relative to controls and patients for whole brain, hemispheres and gender

	CTR		MDD		CTR + MDD
	M (*n*=39)	F (*n*=27)	M (*n*=31)	F (*n*=37)	
Mean age	45.5	54.7	52.3	46.1	
Whole CBF	44.2		44.6		
R. hem. CBF	44.5		45.0		
L. hem. CBF	44.0		44.2		
Male rCBF					44.2
Female rCBF					44.6

Mean CBF values are normalised to 50 CTR, Normal controls; MDD, patients with major depressive disorder; M, males; F, females; R, right; L, left

Table 2. Relative rCBF mean values for groups, hemispheres and VOIs. *F* and *P* values are relative to the single ANCOVA analyses in which groups and hemispheres are considered

VOIs	Groups					Right Hem		Left Hem		Hem×Group	
	All Mean	CTR Mean	MDD Mean	*F*(1, 129)	*P*<	CTR Mean	MDD Mean	CTR Mean	MDD Mean	*F*(1, 129)	*P*<
BSE	42.1	42.2	42.1			42.2	42.0	42.1	42.3		
B04	44.1	44.1	44.2			43.9	44.3	44.2	44.2		
B05	48.2	48.2	48.2			48.9	48.9	47.6	47.5		
B06	47.0	46.9	47.1			46.2	46.5	47.6	47.7		
B07	45.8	45.9	45.7			45.9	45.7	45.9	45.7		
B08	47.9	47.7	48.1	4.89	0.05	48.0	48.6	47.3	47.5	3.94	0.05
B09	47.2	46.9	47.5	8.64	0.005	47.2	47.9	46.6	47.0		
B10	42.5	42.1	42.9	9.06	0.005	42.3	43.1	42.0	42.7		
B44	44.9	44.7	45.1			45.4	46.1	44.1	44.2		
B45	44.8	44.7	45.0			44.6	45.3	44.7	44.6		
B46	42.5	42.3	42.8			43.3	44.4	41.2	41.1	15.86	0.001
B17	47.3	47.0	47.5			46.4	47.4	47.6	47.7	12.00	0.001
B18	40.9	40.9	40.9			40.1	40.6	41.8	41.3	11.19	0.001
B19	40.7	40.4	41.0	3.98	0.05	41.3	42.2	39.6	39.8	6.63	0.025
B24	41.0	41.2	40.8			42.4	42.0	39.9	39.6		
B31	49.6	49.8	49.5			49.7	49.4	49.8	49.6		
B32	48.6	48.9	48.4			49.2	48.7	48.5	48.1		
AUD	43.9	43.8	44.1			44.8	45.0	42.8	43.2		
B21	40.8	40.5	41.1			42.8	43.6	38.2	38.6		
B37	42.5	42.3	42.6			42.6	43.2	42.1	42.0	6.61	0.025
B38	37.9	37.5	38.4	6.97	0.01	38.1	39.1	36.8	37.6		
B39	41.1	40.9	41.3			41.2	41.8	40.6	40.8		
B40	42.6	42.4	42.7			41.9	42.7	42.9	42.7	8.68	0.005
Cd	43.0	42.4	43.6	8.74	0.005	42.1	43.1	42.6	44.1		
Hippo	42.4	42.3	42.5			42.8	43.2	41.7	41.8		
Pt	49.5	48.9	50.1	14.47	0.001	48.7	49.8	49.2	50.4		
Th	49.9	49.3	50.6	11.82	0.001	48.9	50.3	49.7	50.9		

Mean CBF values are normalised to 50
B, Brodmann area; CTR, normal controls; MDD, patients with major depressive disorder; R, right; L, left; Hem, hemisphere; SE, 1+2+3; AUD, 22+41+42+52; Pt, putamen; Th, thalamus; Cd, nucleus caudatus; Hippo, hippocampus

Table 3 and Fig. 1). In both CTR and MDD groups, the highest rCBF was found in central structures and the lowest in anterior temporal cortex (Table 3).

Forward stepwise discriminant analysis, performed on all factors for which group differences were significant, identified the central structures as the most useful variable in discriminating between CTR and MDD. Considering the results of the jackknifed classification matrix, in almost two-thirds of individuals (accuracy =65%) rCBF groupings and clinical diagnosis were concordant (Wilks' lambda =0.893; $P<0.001$), with a sensitivity of 68% and a specificity of 62% (Table 4).

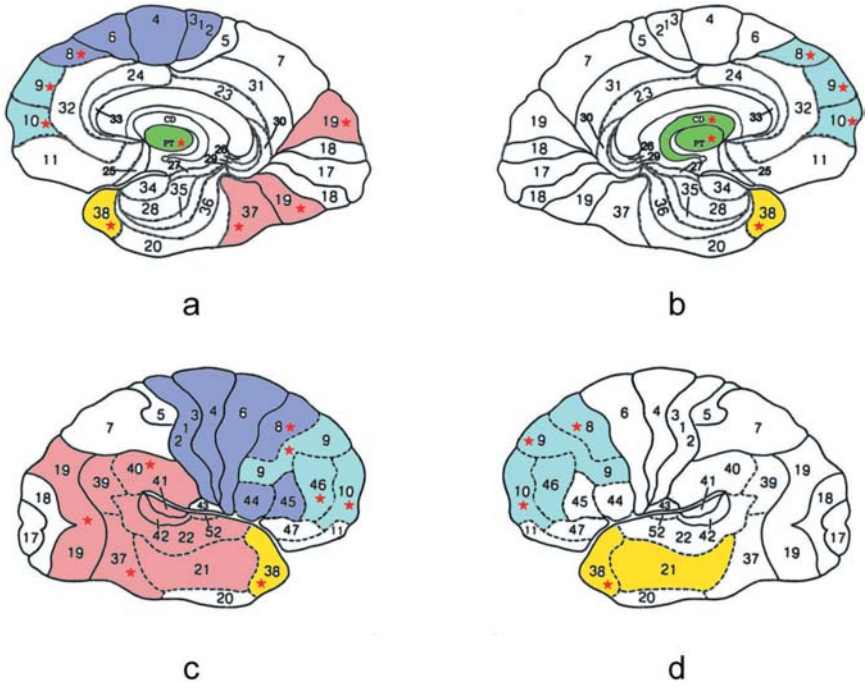

Fig. 1a–d. Representation of medial and lateral aspects of hemispheres depicting the five factors for which there was a significant group effect. *Red stars* represent brain regions significant at the VOI level which were included in significant factors. **a** Right medial aspect; **b** left medial aspect; **c** right lateral aspect; **d** left lateral aspect

Table 3. Factorial grouping of VOIs following PCA on all 134 subjects. *F* and *P* values are relative to group differences

Anatomical reference	Included VOIs	Groups					
		CTR		MDD			
		Means	SD	Means	SD	*F*(1, 129)	*P*<
Occipital	B17R, B17L, B18R, B18L, B19L, B31R	44.2	1.9	44.3	1.4		
Prefrontal and R frontal	B8R, B9R, B9L, B10R, B10L, B45R, B46R, B46L	44.3	1.1	44.8	1.2	8.15	0.005
Superior parietal	B5R, B5L, B7R, B7L, B31L	47.6	1.7	47.5	1.8		
L temporoparietal	B37L, B39L, B40L	41.9	1.6	41.8	1.6		
Superior frontoparietal	B4L, B6R, B6L, B8L, BSEL	44.6	1.2	44.7	1.1		
Anterior cingulate	B24R, B24L, B32R, B32L	45.0	1.8	44.6	1.8		
L frontotemporal	AUDL, B45L, HippoL	43.0	1.3	43.2	1.3		
Anterior temporal	B21L, B38R, B38L	37.7	1.7	38.4	1.9	4.95	0.050
R frontoparietal	B4R, B44R, SER	45.0	1.2	45.5	1.1	5.79	0.025
R temporo-parieto-occipital	AUDR, B19R, B21R, B37R, B39R, B40R	42.4	1.3	43.1	1.1	8.34	0.005
Central structures	CdL, PtR, PtL, ThR, ThL	47.8	2.0	49.1	1.6	17.85	0.000

Mean CBF values are normalised to 50
B, Brodmann area; CTR, normal controls; MDD, patients with major depressive disorder; R, right; L, left; SE, 1+2+3; AUD, 22+41+42+52; Pt, putamen; Th, thalamus; Cd, nucleus caudatus; Hippo, hippocampus

Table 4. Results of discriminant analysis: jackknifed classification matrix

	Control	MDD	% Correct
Control	41	25	62
MDD	22	46	68
Total	63	71	65

Wilks' lambda =0.8931; *P*<0.001

Discussion

Interpretation of the findings

The results of previous investigations on rCBF in depressed patients have often been conflicting, showing either higher or lower blood flow than in normal subjects in various brain regions. These regions and/or clusters of voxels have always been analysed as variables independent from each other.

The functional role of neurons is strictly dependent on their connections. In this respect it is important to take anatomo-functional connectivity into account, highlighting the correlated patterns existing among the variables [49], and methods able to determine the degree of connectivity among brain regions have been advocated [50]. Functional and effective connectivity has been referred to to describe relationships among segregated areas. Related pathways connect distant cortical centres supporting a single function, whose union is mediated by functionally integrated systems [28].

Compared with normal controls, we found a significantly higher rCBF in the unipolar depressed group in the right fronto-temporo-parietal regions and the central structures. Regions showing significant group differences were larger when changes were analysed by factor as compared to VOI analysis. These approaches either consider brain regions as single units (VOI analysis) or take into account their mutual relationships (factor analysis). The higher rCBF in MDD was shown by both methods, with several overlaps in frontal and temporal lobes and central structures (Tables 2, 3, Fig. 1).

All eight VOIs differing significantly between MDD and CTR were included in the five significantly different factors (Fig. 1). Furthermore, the significantly different VOIs were mostly adjacent each other and belonged to the same functional systems (i.e. prefrontal-temporal limbic system and central structures), reinforcing the biological significance of the analysis. Such rCBF changes predominantly in the right fronto-temporo-parietal cortex are in accordance with the differential emotional involvement of the two hemispheres [51]. The concomitant higher rCBF in central structures (thalamus and putamen) is in conformity with the higher rCBF in the mediodorsal nucleus of the thalamus previously described in MDD [15] and with activation of such structures due to positive inference from the cortical regions.

The bilateral rCBF alterations in central structures may reflect altered activity in the ipsilateral and contralateral cortico-basal ganglia-thalamo-cortical projections suggested to be involved in the control of psychomotor behaviour [52]. On the other hand, the factor including the central structures showed the highest statistical difference among groups and was suggested by discriminant analysis to be the best group discriminator. Both of these findings highlight the importance of central structures in MDD with concomitant physical symptoms.

Functional connectivity in major depression has recently been explored by Shajahan et al [53]. They investigated pairs of structures belonging to the limbic system connected in a simple bi-variate linear mode. On the other hand, by implementing a multivariate model we were able to correlate cortical and subcortical interactions at a higher level.

Higher frontal cortex rCBF in depressed patients has previously been reported by several positron emission tomography (PET) studies [15, 16, 18, 24, 54, 55] as well as by some SPET studies investigating rCBF by means of ^{99m} Tc-HMPAO [17, 56] and xenon-133 [57]. Higher rCBF in the right as compared to the left anterofrontal, temporal and parietal cortex in untreated depression versus controls was reported in a ^{99m}Tc-HMPAO SPET study by Tutus et al [58]. In contrast to these findings, other studies have reported lower rCBF in frontal cortex, especially in its dorsolateral aspect. These obviously different and sometimes contradictory findings may be explained by a variety of heterogeneous factors such as group size, the age of subjects and the gender ratio. Furthermore, the methods of data analysis (including the use of CBA and VOIs/PCA), the selection of healthy controls (specifically recruited to serve as normal subjects) and patients (selected group of outpatients with physical symptoms), the timing of SPET (performed during the chronic depressive state in the resting state with the eyes closed), the radiopharmaceutical used and patients' medication might account for the discrepancies between the present study and some of the previous investigations.

Independently of groups, females had a significantly higher relative CBF, as also reported previously [59, 60, 61, 62, 63]. This could be due to the higher relative percentage of grey matter in women than in men [64]. However, no interaction was found between gender and groups, thus favouring a general effect of gender on CBF independently from the disease.

In PCA, VOIs were considered as independent variables and grouped into factors either containing or not containing the homologous contralateral Brodmann areas. Nevertheless, PCA confirmed the interaction between hemispheres and groups shown by the VOI analysis. In fact, the significantly higher CBF values found in MDD in factors mostly including right hemisphere VOIs (Table 3) are concordant with the higher CBF values found in the right hemisphere as a whole in MDD (Table 1).

In accordance with a recent investigation [63], in our study there was no difference in rCBF between the 23 treated and the 45 non-treated patients and the treatment did not interact with any functional region. Other studies have reported limbic and cortical CBF abnormalities in both depression [65, 66, 67, 68] and schizophrenia [69, 70, 71] which partly reversed after antidepressant or neuroleptic treatment. In all such studies, patients were scanned twice and within-subject analysis was performed showing rCBF changes associated with effective treatment. In the present study the finding of lack of interaction between medication and CBF was based on between-subject analysis, and patients had a single scan. In the absence of a "pretreatment" scan, we cannot draw any definitive conclusion on possible CBF changes following medication. Furthermore, a recent study reported persistent changes in hyperperfused limbic structures following therapy with serotonin re-uptake inhibitors,

and tentatively explained such changes an "adaptive homeostatic response necessary to maintain a recovered state" [29]. This might even apply to our group of patients in whom, on the other hand, symptoms were not relieved by medication. However, SPET was performed in the chronic phase in which patients felt fit to undergo the examination and not during pronounced mood episodes. This could have minimised possible differences due to the antidepressant treatment between treated and untreated patients.

Methodological aspects

Subjects. The outpatients included in this study suffered long-standing depression with concomitant physical symptoms (78% had tinnitus and 99% muscular stiffness and/or pain). These patients represent a subpopulation of depressed patients with chronic physical problems rather than depressed patients in the general population. On the other hand, this subgroup was found to include 16% of all depressed patients in a community study [72]. Among MDD patients, muscular pain has been reported in 67% [36], fatigue in 63% [37] and irritable bowel disease in 40% [38]. Tinnitus was found in 49% of the depressed patients as compared to 12% of normal subjects [35]. A population prevalence of 14% has been reported for chronic muscular pain [73], irritable bowel disease [74] and tinnitus [75], and of 10–19% for chronic fatigue [76].

The vast majority of our patients exhibited flight of ideas and difficulties in keeping to the topic of conversation, suggesting increased thought processes. Hence during the SPET scan, even if performed at rest, some form of "thought rumination" was possible and may have locally increased the rCBF as an exaggerated or maladaptive compensatory process [29].

Statistical analysis. As regards PCA, in the present study the solution obtained by the factor analysis performed in all 134 subjects was used since we speculated that a continuum might exist between the rCBF distribution in normal and pathological subjects. This might be especially applicable to psychiatric disorders in which clear anatomo-pathological findings have not been demonstrated to parallel the clinical status in all circumstances and in which physiological correlates of the depressive state have been suggested [4].

The statistical approach used in this study employed functional regional analyses based on the assumption that correlated patterns exist among different brain regions and that such relationships reciprocally affect the rCBF. It is worth noting that PCA does not create effects that are not present in the data, nor does it lose information. PCA is an alternative to the univariate voxel-by-voxel approach in which it is assumed that each voxel value is independent.

Standardisation software. The CBA software used in this study standardises each subject's brain in the 3D space by steps equivalent to those of statistical parametrical mapping (SPM) [77], the most widely used software in brain imaging for the purpose of group and individual comparisons. In a recent study, SPM was compared with region of interest analysis in investigating CBF differences between depressed patients and normal controls, and considerable overlap was demonstrated in the findings [78].

We evaluated the count-density signal in predefined brain regions corresponding to Brodmann areas and/or to specific anatomical regions. SPM produces statistical maps of significant changes in distribution and results are based on analysis of clusters of voxels. Comparisons between groups are performed, generating a SPM *t*-statistics map.

The present study assessed rCBF differences based on VOIs and on the results of a form of factor analysis (PCA) that served to concentrate information and suggest some form of connectivity, before examining the primary issue of interest. In this respect, CBA has the advantages of including in the analysis regions already sharing some form of "anatomo-functional" similarity (the VOIs correspond to Brodmann regions and were originally classified according to the brain cytoarchitectonics) and of producing data for further processing that contain fewer independent variables as compared to SPM.

Compared with SPM, the implemented methodology, which increases the spatial resolution of the sample by analysing entire 3D brain regions, could hide significant differences at the cluster of voxels level. On the other hand, the anatomical similarity of the voxels included in each VOI and the strong positive correlations between all VOIs included in each factor might amplify changes at both the threshold and the sub-threshold level of significance in the entire functional region.

The lack of high accuracy as assessed by discriminant analysis in grouping subjects according to either clinical diagnosis or CBF distribution might have been due to the functional trait of the disease causing a broad superimposition of the rCBF pattern with that of normal individuals. Nevertheless, this is the first study to report the use of discriminant analysis in depression, and the sensitivity of the method can be rated as fair.

Conclusion

Clusters of regions in both the neocortex and central structures sharing close anatomical and functional relationships showed higher rCBF in a selected group of unipolar depressed outpatients with concomitant audiological and other physical symptoms as compared to normal subjects. PCA grouped regions into factors according to their reciprocal rCBF positive relationships and high-

lighted significances in areas larger than those found at the VOI level. These findings confirm that functional changes occur in unipolar disorders and emphasise the value of analysing the relationships among brain regions. SPET, being the most widely available nuclear medicine technique, might play a relevant role in the diagnosis and prediction of MDD if used more extensively in research and clinical practice. Depression has been conceptualised as a multidimensional disorder affecting functionally integrated pathways [79], and its metabolic pattern is the result of adaptation to a combination of functional lesions that can sometimes be difficult to identify using conventional univariate analysis [80]. It is possible that in the near future a consensus on the rCBF pattern underlying major depression, and thus improved diagnosis by brain imaging, will be achieved. This will entail the use of multivariate analysis to elucidate the functional connections between involved brain regions, the availability of SPET cameras with increasingly high spatial resolution, the application of more sophisticated statistical analytical methods and the recognition of groups of patients who should receive the same diagnosis or treatment or be investigated with the same methods.

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