
Networking rCBF Gender Differences in Major Depression

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Abstract

Background: There is large evidence that major depressive disorder (MDD) has prevalence rates almost twice as high in females as in men. However, few studies have investigated in MDD the regional cerebral blood flow (rCBF) differences between genders. The aim of the study was to identify the influence of gender on the rCBF distribution in a group of depressed patients. This was performed by means of Volume of Interest (VOI) analysis and Principal Component Analysis (PCA), this latter exploring functional brain connectivity and transforming a number of correlated variables by clustering them into functionally uncorrelated factors

Methods: A group of 76 major depressed patients (36 males and 40 females) were investigated by ^{99m}Tc-HMPAO and SPECT. Analysis of covariance (ANCOVA) and PCA were performed on 54 VOIs. Neuropsychiatric tests (MADRS, SCID, CFQ, KSP) were also carried out to assess disease severity without finding any gender differences.

Results: VOIs analysis identified in females as compared to males a significantly higher rCBF distribution ($F(1,73)=10.875$; $p=0.002$). A significant VOI*Gender interaction was also found ($F(26,1898)=2.180$; $p=0.001$) revealing that 10 regions belonging to the frontal, temporal, parietal and occipital cortex were particularly involved in gender differences. An overall effect of gender was also found for PCA ($F(1,73)=8.814$; $p=0.004$). The significant PCs*Gender interaction ($F(12,876)=3.258$; $p<0.000$) revealed lower rCBF distribution in males as compared to females in 6 PCs. Such PCs, grouped brain regions belonging to parietal-limbic cortex (PC3; $p=0.033$), parieto-temporo-occipital cortex (PCs 8 and 9; $p=0.001$), fronto-parietal cortex (PC10;

$p=0.017$), fronto-temporal cortex (PC12; $p=0.001$) and hippocampi (PC 13; $p=0.017$). Age related hippocampal differences were found in PC13 in female only.

Conclusion: PC8 grouped two areas involved in linguistic processing, the angular and the supramarginal gyrus of the left hemispheres for which gender differences are widely accepted. PC9 with the right angular gyrus was also likely to show rCBF differences since females are known to be more bilaterally organized. Gender differences in hippocampi confirmed previous findings. However, medial prefrontal cortex (anterior cingulate) bilaterally and right dorsolateral prefrontal cortex, regions known from the existing literature to be implicated in MDD, were grouped by PCA into different PCs (PC1 and PC4, respectively) but did not show any sex difference speaking against specific gender related rCBF changes in major depression.

PCA grouping functionally connected brain regions increased the depth of the analysis yielding more information on the processes underlying perfusion distribution measurements in MDD.

Depression, Neuroimaging and Related Methodology

Background

Major Depressive Disorder (MDD) is a primary idiopathic condition (i.e. arising spontaneously, or from an unknown cause) characterized by the occurrence of depressive episodes (Unipolar Depression). The symptoms ought to be present for at least two weeks and cause significant distress in important areas of brain functioning. It has been suggested that MDD episodes are the most severe state of illness representing only the tip of the iceberg in a common, chronic and disabling disease with alternating symptom severity [1].

The lifetime prevalence of MDD has been reported to be as high as 26% [2]. MDD is now included among the ten leading disorders for global disease burden and in the next years will become one of the dominant neuro-psychiatric and social issues.

The concept of neuronal activity may refer to spiking activity or local synaptic activity, and is associated with several physiological variables such as energy and oxygen consumption, glucose utilization and regional cerebral blood flow (rCBF). In neuroimaging studies using Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) the measured signals are indirectly related to the neuronal activity reflecting, to various extents, the above mentioned variables. Increases of brain energy demands have for more than a century been believed to increase brain blood flow, but the precise nature of the relationship remains unknown even if wide experimental evidence provides support for direct coupling between glucose metabolism and neuronal activity. However cerebral blood flow is easier to measure than glucose metabolism, and measurements of blood flow have a better temporal resolution. Positive correlations exist between capillary density, metabolic rate and blood flow and interpreting the magnitude of rCBF changes as changes in synaptic and neuronal activity may be reasonable foremost during normal circumstances.

Increased SPECT tracer distribution in various regions, including frontal regions, was reported in depression studies [3]. However, reviewing functional imaging studies in depression

it was found that the decreased tracer distribution in frontal regions compared to control subjects was the most common finding [4-22]. It is yet not clear if alterations of tracer distribution reflect 'trait' phenomena that are present prior to onset of overt depression, or 'state' phenomena. It is also unknown if abnormalities of tracer distribution are pathophysiologically involved in the evolution of depression, or are an additional expression of an as yet unknown etiological factor of the disease. For all these above mentioned reasons the deeper investigation level allowed by multivariate analysis might help in clarifying in MDD the fine and hidden relationships between regions not detectable with conventional statistical analysis [23].

In a meta-study, the trend for reductions in all cortical and subcortical regions remained for remitted depressed patients [24]. Increased tracer retention in the left frontal and anterior cingulate gyrus has been reported after electro-convulsive treatment [25], and decreased retention was found in the orbitofrontal and/or anterior cingulate after treatment with repetitive transcranial stimulation [26].

In Alzheimer's Disease and Parkinson Disease, decreased radiopharmaceutical retention has been linked to regional cell loss. The decrease retention that has been reported in depression may, hypothetically, reflect the spine loss indicated by the decreases of synaptic "products" [27], the decrease of inhibitory local circuit neurons [28], and/or the reduced cortical glial cell numbers [29]. Decreased activity in a few of the functional circuits is another possibility.

Conversely, increased retention in depression may reflect increased activity in some functional units as well as other phenomena such as increased intracellular GSH levels. This phenomenon has been described in the early stages of mitochondrial disease and is considered to be due to increased oxidative stress secondary to reduced respiratory chain enzyme activity [30].

Single Photon Emission Computed Tomography (SPECT)

Modern gamma camera, operating on-line to a computer system for additional signal and image processing, image display and tomographic reconstruction, are designed with more than one camera head, typically three. Tomographic examination is a pre-requisite for rCBF SPECT, due to the complex brain anatomy with superimposed anatomo-functional structures. The resulting contrast-enhancing effect of the tomographic registration technique is of great importance since the differences between normal and pathological uptake in various brain regions may be rather small.

The studies of rCBF using SPECT are based on depicting the distribution of ^{99m}Tc - *d,l* - hexamethylpropylene amine oxime (^{99m}Tc -HMPAO), ^{99m}Tc - ethyl cysteinate dimer (^{99m}Tc -ECD) or [^{123}I]-Iodoamphetamine, which are imaged in the brain after intravenous administration. Despite that [^{123}I]-Iodoamphetamine may best represent rCBF, this tracer is rarely used due to a high cost and restricted availability. In this paper we have reviewed only studies performed with ^{99m}Tc -HMPAO.

The intracellular retention of ^{99m}Tc -HMPAO in the central nervous system is the effect of a rapid conversion from the lipophilic into the hydrophilic form at the exposure to endogenous intracellular glutathione, which is a powerful reducing agent. Trapped in the cell, ^{99m}Tc -HMPAO remains practically unchanged being its detection limited by decay of ^{99m}Tc ($t_{1/2}=6.02$ h). This specific property allows for scans performed several hours later to still depict the rCBF at the moment of administration and is of paramount importance when examining physically and mentally impaired patients allowing for the administration to be made in a quite environment and the scan postponed according to patients physical and psychological status.

Standardization Software

In the recent past standardization software and novel statistical methodologies have been implemented by several groups in both neurodegenerative and psychiatric research in order to improve the diagnostic accuracy of functional neuroimaging.

The assessment of CBF patterns in various brain disorders by SPECT or PET have in the past mainly been carried out either by visual evaluation or by outlining the regions of interest (ROIs) in a manual or semiautomatic mode. Such methods might suffer from excessive operator's influence in the choice of the ROIs and, due to the variable shape of human brains, lack of spatial normalization, resulting in anatomical in-homogeneous brain samples among subjects.

Recently, semi-automatic approaches to assess regional CBF changes in group comparison have been developed opening up the conditions for a new approach in research and allowing an easier and earlier diagnosis. By carefully spatially standardizing each scan is possible, by means of subtractions images and/or statistical comparisons, to precisely identify regions with abnormal flow. The advantage of this techniques is the possibility to exploit the knowledge of rCBF patterns as assessed in normative or pathological scans identifying regional difference on a group to group basis.

In 3D analysis, the inclusion of the white matter makes the sample more representative for global neurodegenerative changes and volumes of interest (VOIs) can be positioned on both anatomical and functional regions improving the physiological significance of the analysis. White matter is an important part of the neuronal system and it is affected by neurodegenerative, cerebrovascular and psychiatric disorders to the same extent as grey matter. Grey matter perfusion is 2.0-3.5 times higher than white matter perfusion. When 3D analysis is performed on extensive data the resulting rCBF is then calculated by averaging, in a certain volume, a variable number of counts detected in both grey and white matter kernels.

The most currently available 3D standardization software share similar principles and can be classified into two categories: the voxel-based ones (i.e. SPM) and the ones based on neuroanatomy (i.e. CBA). We briefly describe these two different approaches.

Statistical Parametrical Mapping (SPM) [31], is the worldwide mostly used voxel-based standardization software in brain imaging for between- and within-subject group comparisons. Images are spatially standardized into a common space, and smoothed. Parametric statistical models are summed, at each voxel, using the General Linear Model to describe the variability

in the data. Hypotheses expressed in terms of the model parameters are assessed at each voxel with univariate statistics. This results in an image whose voxel values are statistics, producing t-statistical maps of significant changes in distribution and basing the output on the analyses of clusters of voxels. Such analysis should take into account the statistical threshold as well as the size of the cluster in relationship to the implemented methodology: the higher the spatial resolution of the camera the smaller the size of cluster of voxels for statistically significance.

The Computerized Brain Atlas (CBA) is a software tool originally developed by Greitz et al. [32], and applicable to any brain imaging modality. It is based on data from one cryosectioned brain in combination with information from the literature. It contains 3D surface descriptions, or volumes of interest (VOIs) of approximately 400 brain structures including the brain surface, the ventricular system, the cortical gyri and sulci, as well as the cortical cytoarchitectonic areas (Brodmann areas, BAs). The major basal ganglia and the brain stem nuclei are also included.

All image sets are spatially normalized into the stereotactic space of the atlas by using global polynomial transformation [33]. It 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 individualize the shape of the brain. CBA identifies the brain surface, the ventricular system and some central nuclei and fits to the aforementioned structures by minimizing the difference in voxel intensity. Subsequently, it deforms and stretches these structures, maximizing a simple similarity measure, to fit them to a previously defined reference SPECT scan. A major advantage of the technique is that it creates an almost fully automatic tool able to decrease the analysis time and to standardize subjects' brains providing additional anatomic information. The segmentation of the brain in VOIs reduces the number of variables to an amount that is possible, in a second step, to submit to multivariate analysis.

Statistical Approach

Univariate Analysis

In univariate analysis there is only one variable under consideration. It can be independent, as in the case of CBF data or dependent as in the case of the same subject measured at two different times. In both cases it is possible to describe the data in terms of mean and variance (the two parameters of the normal distribution). After testing the two means, possible significant differences need to be explained. The standard approach is to assume that the difference is due to an experimental effect and sources of variance are under control. However, this is not so obvious in neuro-functional studies in which many sources of variance are present. Hence, if we want to study the relationships among those sources, multivariate analysis has to be implemented.

The t-test for dependent samples is the most commonly used method to evaluate the differences in means between two groups of observation made on the same sample of subjects who were tested twice. When group of observation are made on different subjects a t-test for

independent samples is used. One-way Analysis of Variance (ANOVA) is performed when groups are three or more. In such cases nothing can be done about the variation due to individual differences since it is not possible to identify, or subtract, such differences. This is why the t-test for independent samples is always less sensitive.

Multivariate Analysis

Multivariate statistic provides a simultaneous analysis of multiple independent (i.e. gender, disease) and dependent (i.e. hemispheres, VOI) variables in order to determine the differences within them and their relationship. Such statistical approach also introduces regional analyses based on the assumption that correlated patterns exist among different brain regions and such relationships affect reciprocally the investigated variable. Variables may be correlated with each other, and their statistical dependence is often taken into account when analyzing such data. In fact, the consideration of statistical dependence and intercorrelations between variables make multivariate analysis somewhat different in approach and considerably more complex than the corresponding univariate analysis, in which there is only one variable under consideration. In the multivariate perspective each voxel is considered conjointly with explicit reference to the interactions among brain regions rendering it particularly appropriate for brain studies and providing a complementary characterization of CBF patterns.

Multivariate analysis requires the number of observations (scans) to be greater than the number of components of the multivariate observation (variables, i.e. voxels). In neuroimaging techniques (in which the raw images contain an extremely high number of voxels) the number of variables needs therefore to be reduced, by using VOIs or factorial groupings.

Multivariate analysis takes also into account the statistical inference about the response of the entire brain, without regional specificity. If interactions are present one can move from an “omnibus” effect to regional changes with the limitation of the sample size (ROI/VOI/factor).

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a data driven technique (i.e., there is no a-priori model or hypothesis) that transforms a number of (possibly) correlated variables into a (smaller) number of not-correlated factors, called principal components. PCA is totally data-led and is independent by any model or a-priori hypothesis. It does not create effects that are not present in the data, nor does lose information. The first principal component accounts for the highest percentage of the variability in the data and each of the following component accounts for a portion of the remaining variability in a descending scale. Variables are summarized in fewer dimensions while retaining most of the information. Each factor, or PC, explains a different part of the total variance of data set. This statistical approach introduces regional analyses based on the assumption that correlated patterns exist among different brain regions and such relationships affect reciprocally the rCBF or the metabolism. It sorts subject-region

interaction and it guarantees that regional coupling has been accounted for. In PCA each component is orthogonal and functionally not correlated to the remaining ones.

An advantage with a neuroanatomic atlas-VOI-based approach is that it allows for the investigation of the rCBF relationships between anatomically distributed but physiologically correlated brain regions using PCA. Applying PCA to the VOIs permits for a reduction of the number of variables through the grouping of VOIs into PCs. This latter characteristic of PCA might be of utmost importance in analyzing pathological conditions, as in the case of psychiatric disorders, in which functionally integrated pathways are involved in the disease process.

Functional Connectivity

Functional connectivity implies that pool activities of brain areas “go up and down” together and regions share a significant number of neurons whose dynamic interactions occur at the same time. Correlated areas will have correlated perfusion and neuronal activity. Functional connectivity is simply a statement about the observed correlations and characterizes distributed brain systems.

The functional role played by any component (neuron) of a connected system (brain) is largely defined by its connections. Extrinsic connections between cortical areas are not continuous but occur in patches or clusters (functional segregation, in which cells with common functional properties are thought to be grouped together).

On the other hand functional integration is mediated by the interactions between functionally segregated areas resulting in a general functional connectivity effect on the brain. Functional connectivity characterizes distributed brain systems and implies “model-free” temporal correlations between neurophysiological events: correlated areas will have correlated perfusion and neuronal activity.

The issues related to functional segregation are generally investigated by means of univariate analysis while functional integration is better analyzed by multivariate analysis.

SPM is typically predicated by functional segregation and analyses regionally specific aspects of functional organization. PCA and multivariate analysis are inspired by functional integration mediated by anatomical, functional and effective connections that form the basis for characterizing patterns of correlations and describe distributed changes in terms of systems.

Networking rCBF Gender Differences in Major Depression

Introduction

In a recent article Halbreich and Kahn reviewed the gender differences in various form of depression [34]. They reported that women as compared to men have a higher probability to suffer from MDD, have a two-fold greater risk for recurrent MDD and an higher lifetime

MDD estimates having been these variability mainly due to biological vulnerabilities and environmental provoking experiences [35]. Chronicity of depression appears to affect more women than men [36] but in a MDD study Lacerda et al. [37] found a significant reduced left lateral orbito-frontal cortex volume in males but not in female.

However, brain functional studies have reported few and sparse findings about gender differences in MDD and no conclusive results about an established rCBF pattern common to the most of MDD patients have reached a wide consensus within the neuroimaging community.

The aim of present investigation was to contribute to the understanding of sex differences in a group of MDD patients as assessed by SPECT and multivariate analysis.

Materials and Methods

Patients and Neuropsychiatric Testing

Seventy-six subjects representing a selected group of MDD patients were included in the study. Thirty-six patients were males (mean age \pm SD 51.4 \pm 8.7 yrs) and forty females (mean age \pm SD 46.0 \pm 8.6 yrs). Thirty-six percent of males (n=13) and twenty-eight percent of females (n=11) were taking psychotropic medications at the time of the SPECT. No patient was taking any neuroleptic agent.

The remaining patients were either drug naive (n=52) or had previously been treated with antidepressant or psychotherapy without any effect. In some of them antidepressant treatment was interrupted due to side effects. Patients were mainly right-handed (88 percent for males and 90 percent for females). 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 [38-43]. 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 SPECT 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 SPECT examination without any assistance.

Neuropsychiatric tests were administered few days before SPECT. Patients were administered the Karolinska Scales of Personality (KSP), a self-report questionnaire conceived to quantify of some crucial personality or temperament dimensions representing qualities of the information processing and arousal systems of the individual [44]. The scores of the patients were compared with normative data transformed to t-scores (50 ± 10) which have been obtained from 400 subjects randomly sampled from the Stockholm population and standardized for age and sex. In patients with ongoing MDD as well as in patients who have recovered from mood symptoms, high scores have been reported on the Psychasthenia, Muscular Tension, Somatic Anxiety, and Psychic Anxiety scales, and low scores on the Socialization scale [45-46]. The self-rating 9-items version of the Montgomery Aasberg Depression Rating Scale with a 6-point response format (MADRS-S; 9), was used to assess current mood. The Structured Clinical Interview for DSM-IV (SCID), a 100-item semi-structured diagnostic interview, was

used to evaluate personality disorders and the Cognitive Failures Questionnaire (CFQ), a 25-item test to assess the frequency of everyday slips and errors, investigated the cognitive domain and the everyday memory.

The study was approved by the local ethical committee and all patients provided written informed consent.

Radiopharmaceutical and SPECT

After 30 min rest at a tranquil place with dimmed light, 1000 MBq (27.0 mCi) of ^{99m}Tc -HMPAO, (Ceretek[®], 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. SPECT brain imaging was performed using a three-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 seconds 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 made using the uniform Chang method [47]. No scatter correction was applied. Both acquisition and reconstruction were performed in 128x128 matrices with a pixel size of 2.22 x 2.22 mm².

Standardization Software

CBA was implemented for anatomo-functional standardization and for image analysis.

For evaluation and statistical analysis of the reformatted data sets, 27 VOIs, bilaterally, were selected. These regions corresponded to Brodmann Areas and numeration, in prefrontal (BA9, BA10, BA46), frontal (BA4, BA6, BA8, BA44, BA45), parietal (BA1-2-3 (SE), BA5, BA7, BA39, BA40) and temporal (BA21, BA37, BA38) cortex. Four regions, representing primary and associative auditory cortex (BA22, BA41, BA42, BA52) were merged into one single VOI (AUD). The remaining regions corresponded to cingulate (BA24, BA31, BA32) and occipital (BA17, BA18, BA19) cortex as well as putamen, nucleus caudatus, thalamus and hippocampus. In order to obtain a set of normalized relative flow data, a scaling factor was computed by averaging the brain voxels data and setting the global brain average to a pre-defined value. Before averaging the voxel data, a fixed counts/voxel threshold was selected to include in the normalization process the 30% of all brain voxels with the highest counts. The normalized value was set to 50 "uptake-units" and all rCBF values of this work were related to this value.

Statistical Analysis

After adaptation and definitions of VOIs using CBA, the ^{99m}Tc -HMPAO uptake data of all subjects were exported to a statistical package (Systat 10, 2000) for subsequent statistical analysis.

Principal Component Analysis was performed on all 76 patients and was based on all 54 VOIs (27 for each hemisphere). PCA transformed a number of correlated variables by clustering them into common factors (PCs), such that variables with higher loadings within each factor were highly correlated, but factors were uncorrelated to one another.

PCs may be treated as new variables and their values can be computed for individual cases. These values are known as factor scores, or component scores (CS), and are a linear combination of each variable included in the analysis. They should be used both to re-evaluate group differences and as predictor variables in diagnostic research. However, in the latter case, it is preferable not to use CS, but an imperfect estimate (coarse component scores, CCS) generated by summing all the VOIs with higher loading in a given factor. An advantage to using CCS is that they can more easily be computed and interpreted than CS and can also be compared between studies. The number of factors was determined by the number of eigenvalues greater than one. We considered as representative of a factor the variables with an absolute factor loadings greater than 0.5. This is an arbitrary value, but it is commonly used since it explains a moderate part of the variance of the factor. By increasing the value further, some variables should be eliminated from the calculation of CCS reducing the variance explained by these scores. Furthermore, CCS are computed only from VOIs with higher loadings on each PC and each VOI enters only one time in PC calculation. CCS were standardized to a 0-1 scale.

ANCOVA (age as covariate) was applied to VOIs values and then to the CCS of PCs to test rCBF differences for statistical significance, considering gender as a between subject variable. As for VOIs analysis, a third within-factor was considered, i.e. the hemisphere. Significance level for all analyses was set to $p \leq 0.05$.

Results

Demographic data of males and females are shown in Table 1. Males were older than females (t-test ($df=74$)=2.72; $p=0.008$). Therefore, all subsequent ANOVA were covaried for age. Values for the various neuropsychiatric scales are reported in Table 1. No differences were detected between males and females.

The ANCOVA performed on the 54 VOIs showed a main effect for gender ($F(1,73)=10.875$, $p=0.002$) and a significant interaction hemisphere*gender ($F(26,1898)=2.180$, $p<0.001$). We therefore performed 27 ANCOVA at single VOI level, considering only gender as factor. Gender differences were found in 10 VOIs belonging to prefrontal, temporal, parietal and occipital cortex (see Table 2). In all VOIs rCBF in females was higher than in males (44.8 vs. 44.3). Statistical analysis did not show any effect of medication on CBF.

Table 1. Demographic data relative to gender

	Male			Female		
	N	MEAN	SD	N	MEAN	SD
AGE *	36	51.4	8.7	40	46.0	8.6
MADRS	27	22.93	9.92	32	22.09	9.18
CFQ	31	57.74	18.76	32	56.75	19.04
SCIDTOTAL	20	34.75	19.27	28	37.39	17.25
KSP Somatic anxiety	22	70.36	17.3	34	65.06	10.68
KSP Psychasthenia	22	59.36	11.67	34	56.29	11.34
KSP Muscular tension	22	75.55	16.96	34	70.32	10.78
KSP Social desirability	22	49.05	9.13	34	51.03	10.21
KSP Detachment	22	48.14	14.07	34	50.06	10.21
KSP Psychasthenia	22	72.14	12.98	34	69.91	10.42
KSP Socialization	22	34.64	11.83	34	35.82	14.35
KSP Verbal aggression	22	51.32	9.13	34	47.18	9.97
KSP Irritability	22	55.55	10.45	34	55.71	11.45
KSP Suspicion	22	57.45	14.4	34	55.88	13.52
KSP Guilt	22	54.55	10.39	34	50.65	8.81
Handedness	34	88%		38	90%	
Medication free	23	63%		29	73%	

* Significant at t-test: $p < 0.01$.

Table 2. Relative mean rCBF and relative SD values for males and females. F and p values are relative to the single ANCOVA (with age as covariate) analyses in which gender was considered

VOIs	Female (n=40)		Male (n=36)		Main effect: Gender	
	Mean	SD	Mean	SD	F(1,73)	p=
AUD	44.7	1.50	43.5	1.54	11.364	0.001
BA07	46.2	1.47	45.3	1.66	4.077	0.047
BA18	41.2	1.60	40.6	1.62	5.979	0.017
BA19	41.4	1.14	40.4	1.06	22.188	0.000
BA21	41.7	1.61	40.3	1.56	15.301	0.000
BA37	43.0	1.36	41.9	1.36	10.168	0.002
BA39	41.7	1.37	40.8	1.36	10.037	0.002
BA40	43.2	1.31	42.1	1.53	9.553	0.003
BA44	45.6	1.35	44.6	1.69	4.262	0.043
SE	42.6	1.23	41.7	1.08	9.981	0.002

Mean CBF values are normalized to 50. BA=Brodman area; SE=1+2+3; AUD=22+41+42+52;

Table 3. Factorial grouping of volumes of interest (VOIs) following principal component analysis on depressed patients. F and p values are relative to gender differences

PC	VOIs with high loading on the PC	CCS VALUES				Main effect: Gender	
		Female (n=40)		Male (n=36)			
		Mean	SD	Mean	SD	F(1,73)=	p=
PC1	BA24R BA24L BA32R BA32L BA44L	0.58	0.16	0.49	0.21		
PC2	BA21L BA38R BA38L	0.61	0.22	0.54	0.22		
PC3	BA05R BA05L BA07R BA07L BA31L	0.56	0.18	0.44	0.21	4.708	0.033
PC4	BA08R BA09R BA10R BA45R BA46R	0.41	0.20	0.43	0.25		
PC5	BA17R BA17L BA18R BA18L	0.47	0.19	0.50	0.26		
PC6	BA04L BA06R BA06L BA08L	0.48	0.18	0.54	0.26		
PC7	PUTR PUTL THALL	0.46	0.20	0.49	0.28		
PC8	BA19L BA39L BA40L	0.56	0.20	0.41	0.21	13.152	0.001
PC9	BA19R BA37R BA39R	0.60	0.18	0.44	0.17	15.505	0.000
PC10	BA04R BA40R SER	0.49	0.23	0.37	0.20	5.985	0.017
PC11	CAUDR CAUDL THALR	0.63	0.21	0.60	0.17		
PC12	AUDR AUDL BA21R BA44R	0.58	0.20	0.41	0.19	12.511	0.001
PC13	BA37L HIPPR HIPPL	0.47	0.22	0.33	0.21	4.513	0.037
OVERALL MEANS		0.53	0.20	0.46	0.22		

The 13 PCs explain the 81.5% of total variance. CCS= Coarse Component Scores; CAUD= caudate nucleus; PUT= putamen; THAL= thalamus; HIPP= hippocampus; AUD= Auditory cortex (22+41+42+52); BA= Brodmann area; L= left; R= right. The PCA excluded from the final solution BA09L BA10L BA45L BA46L SEL in the left hemisphere, and BA31R in the right hemisphere. The CCS values were standardized to a 0-1 scale.

PCA, performed on the 54 VOIs, resulted in 13 factors (PCs). These orthogonal and uncorrelated factors explained the 81.5% of the total data variance. Only 6 out of 54 VOIs were excluded from the final solutions. The structure of PCA is reported in Table 3 together with ANCOVA results. Overall analysis showed both a main effect for gender ($F(1,73)=8.814$; $p=0.004$; Figure 1) and a gender*factor interaction ($F(12, 876)=3.258$, $p<0.001$).

As compared to males, females rCBF was relatively higher in 6 out of 13 PC (see Table 3 and Figure 2). Such PCs, grouped brain regions belonging to parietal-limbic cortex (PC3), parieto-temporo-occipital cortex (PCs 8 and 9), right fronto-parietal cortex (PC10), fronto-temporal cortex (PC12) and hippocampi (PC13).

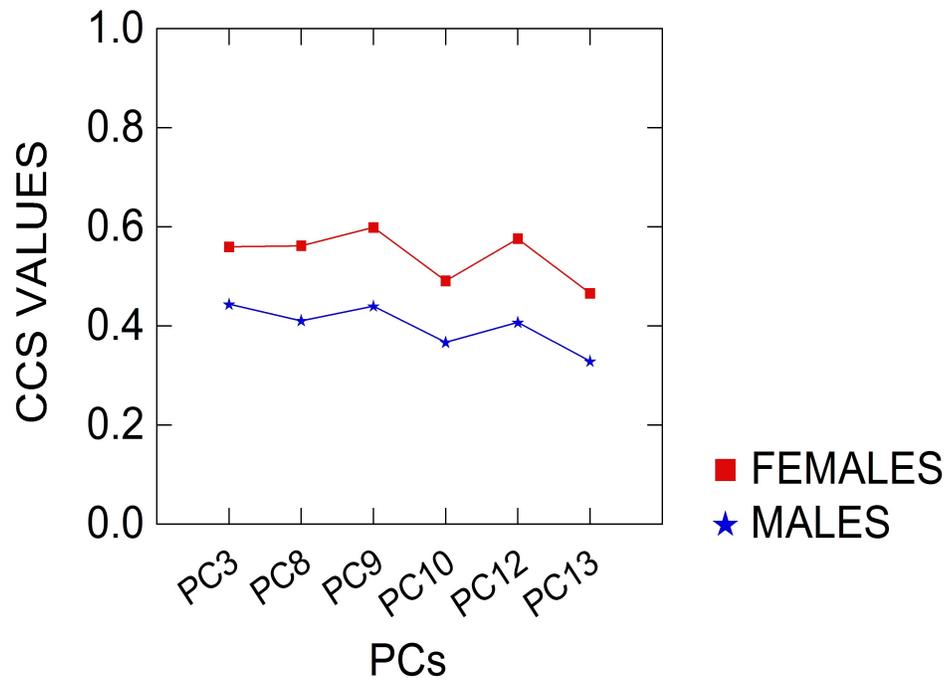


Figure 1. Overall gender effect on CCS values of significantly different PCs.

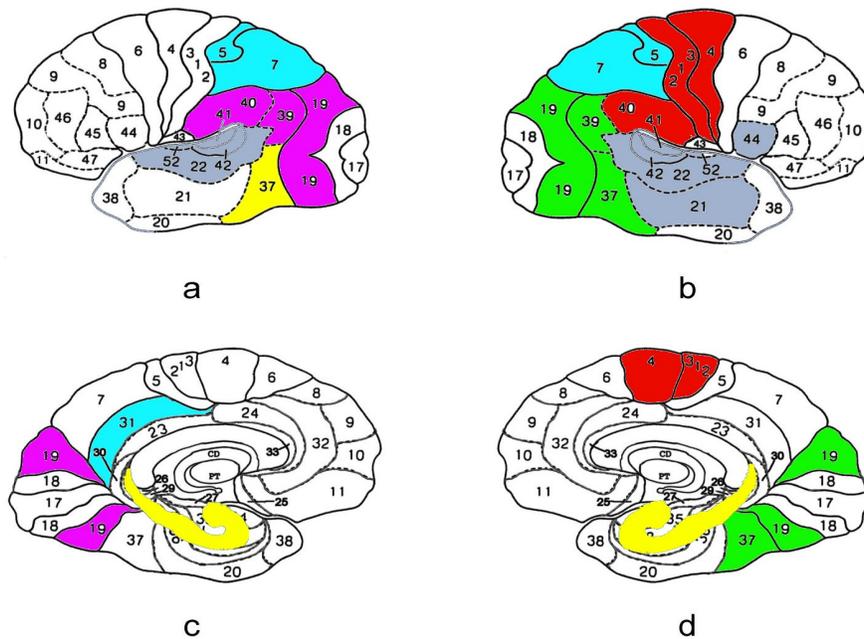


Figure 2. Representation of lateral and medial aspects of hemispheres depicting the 6 PCs for which there was a significant gender effect in 76 MDD patients. a = Left lateral aspect; b = Right lateral aspect; c = Left medial aspect; d = Right medial aspect. VOIs grouped into each PC are depicted with the same color.

At the end we conducted age-related regression analyses for both males and females and for each of the significant PC. Results showed a significant effect of age in females for PC13 ($F(1,38)=6.98$, $p=0.01$, Figure 3) but not in males. Linear regression was: $PC13=0.93-0.01*AGE$.

Conclusion

Previous investigations on rCBF in depressed patients have often been conflicting, showing either increased or decreased rCBF tracer distribution in various regions. These regions and/or clusters of voxels have almost always been analyzed 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 [23]. It has been argued in favor of methods able to determine possible networking within brain regions [48] considering brain regions not as single units (univariate analysis) but taking into account their mutual relationships (factor analysis). Functional connectivity in major depression has recently been explored by investigating pairs of structures belonging to the limbic system and connected by a simple bivariate linear mode [49]. By implementing a multivariate model we were able to correlate cortical and subcortical interactions at a higher level.

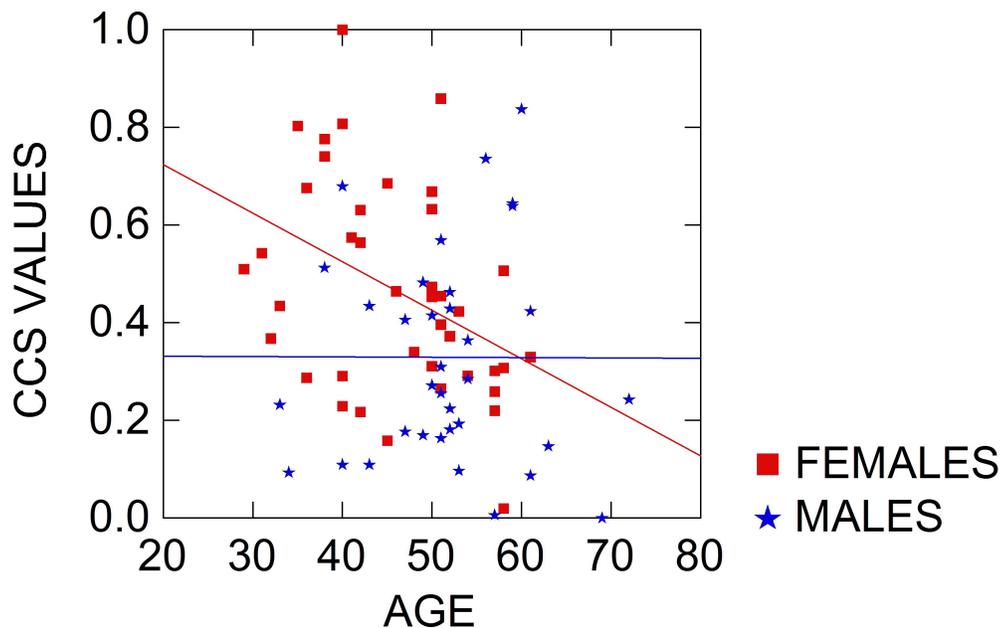


Figure 3. Regression analysis depicting the variations of PC13 CCS with age.

Compared with male MDD patients, we found a significantly higher rCBF in female patients in large regions (as defined by PCA) in frontal, temporal, parietal and occipital cortex. In our study regions showing significant group differences were larger when changes were analyzed by PCs as compared to single VOI analysis (see Tables 2 and 3) and gender differences in the hippocampi were highlighted by PCA only. In PCA, VOIs were considered as independent variables and grouped into factors either containing or not containing the homologous contralateral Brodmann areas. This is an interesting aspect of the analysis with important neurophysiological implications in the lateralization of most of the VOIs showing statistically significant difference between genders. This effect was not present at single VOI analysis in which an hemispheric effect was not present and might have an impact on the different contribution of right and left hemisphere to emotional feelings.

The VOIs shown by single analysis to differ significantly between genders were mostly grouped by multivariate analysis in PCs containing adjacent and functionally correlated BAs (PCs 8, 9, 10, 12), reinforcing the biological significance of the analysis.

Medial prefrontal cortex (including anterior cingulate cortex bilaterally, PC1) and right dorsolateral prefrontal cortex (PC4), regions previously reported to be implicated in MDD, did not show any difference speaking against specific gender related rCBF changes in major depression. However in our specific group of patient the finding of a statistically significant increased rCBF tracer distribution in six PCs in females as compared to male deserves attention.

The majority of patients participating in the present study have been previously investigated by the same methodology [50] and a significantly higher ^{99m}Tc -HMPAO distribution was found in several cortical regions in the whole group of patients (males and females as taken together). They suffered long-standing depression with concomitant physical symptoms and represent a subpopulation of depressed patients with chronic physical problems rather than depressed patients in the general population. The vast majority of patients exhibited flight of ideas and difficulties in keeping to the topic of conversation, suggesting increased thought processes. Hence during the SPECT 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.

The finding of increased ^{99m}Tc -HMPAO distribution in MDD confirmed previous studies in which relative hypermetabolism in distinct regions of the brain was reported [51-56]. However, the most of the functional neuroimaging studies about major depression described reduced rCBF in medial prefrontal cortex and central structures. Such 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 SPECT (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 MDD, females have a significantly higher relative CBF as compared to men, as previously reported [57-62], possibly due to the higher relative percentage of grey matter in women than in men [63]. Although the performed neuropsychiatric test did not show any difference in the disease severity between males and females, the increased flow distribution in this latter group might represent the neurobiological substrate of a trend towards a more accentuated brain involvement in the disorder. This has been extensively described by epidemiological studies reporting findings of higher lifetime prevalence of MDD for women than men [64, 65] or by the higher prevalence of comorbidity disorders in women [64].

The rCBF changes between genders were found predominantly in the right temporo-parietal cortex and such findings are in accordance with the differential role of the right hemisphere in negative emotions [66, 67] and with the different lateralization between genders, being women more bilaterally organized. It has also been hypothesized a gender difference between anterior and posterior area of the brain, particularly in those processing language. However, neuroimaging studies to date have only provided support for differences in the anterior language areas and Kansaku et al [68] have shown some differences between men and women in posterior temporal lobes. Our results seems to support a higher rCBF distribution in both the whole right hemisphere and in temporo-parietal cortex in women. Moreover PCA clearly separated the angular and the supramarginal gyri of the left hemisphere (PC8) from those of the right hemisphere (PC9 and PC10) showing significant perfusion differences between males and females in these regions.

A further important difference between gender is in PC13 including hippocampi and the left posterior temporal cortex. A significant age effect was found in female with a rCBF

reduction in adulthood (Fig 3) but not in males. This finding suggests, as previously reported, that ageing had no effect on males rCBF but had a significant effect on females rCBF [62]. Moreover hippocampus is a structure in which differences have been found in both MDD and gender. For example Murphy et al. [69] showed a greater age related volumetric decrease in frontal and temporal lobes in males, and in hippocampus and parietal lobes in females. Additionally, some authors found hyperactivity of amygdala, hippocampus and parts of the temporal lobes in the depressed state [70]. Patients with depression have been found to have volume reductions or other abnormalities in the prefrontal cortex and hippocampus, areas connected to the regulation of mood [71, 72].

In conclusion, our study showed in a group of 76 MDD patients regional cerebral blood flow differences related to gender but not to the disease. PCA networked brain regions increasing the depth and the significance of the analysis and contributing to better clarify the processes underlying perfusion distribution measurements.

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