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The Danish PET/depression project: PET findings in patients with major depression

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ABSTRACT

Background. It is hypothesized from previous positron emission tomography (PET) studies of patients with major depression that dysfunction of regions of the limbic system and the frontal lobes in close connection with the basal ganglia is involved in the pathophysiology of major depression.

Methods. By means of PET and ^{15}O labelled radioactive water we determined an index of the neuronal activity by mapping the cerebral blood flow distribution of 42 unselected in-patients suffering from moderate to severe depression and 47 healthy controls controlling for age and gender. The PET maps were co-registered to magnetic resonance images of the anatomy of the brain.

Results. The functions-of-interest analysis revealed significant gender differences in cerebral blood flow and changes in the relative distribution of the blood with increasing age. The patients had increased activity of the hippocampus and the cerebellum compared to the healthy controls when corrected for these confounders and the influence of antidepressant medication. Furthermore, data in the Danish Psychiatric Central Register showed that the patients studied were representative of the population of depressed patients admitted to the hospital during the study period.

Conclusion. Our main finding is increased blood flow to the hippocampus, even when controlling for a number of confounders. This is in accordance with a rapidly expanding literature suggesting an important role for this structure in major depression.

INTRODUCTION

Major depressive disorder is aetiologically, and most probably also pathophysiologically heterogeneous (Winokur, 1997). Furthermore, the patients often have different symptom profiles, some displaying psychomotor retardation and others suffering from symptoms of anxiety. As shown by Bench *et al.* (1993), changes in cerebral blood flow measured using PET technique is highly correlated with these clinical dimensions. A large sample size is thus required to reveal brain function in major depression; not fulfilling this requirement is one reason why consensus regarding functional brain abnormalities in this disorder has not yet been achieved (Drevets, 1999). Previously, we did an extensive literature review of PET studies of depressed patients (Videbech, 2000). The main findings were that patients with major depression have reduced blood flow and glucose metabolism in the prefrontal cortex, anterior cingulate cortex and caudate nucleus when scanned in the resting state and during stressful tests. In a large study by Bench *et al.* (1993) the prefrontal blood flow was negatively correlated with psychomotor retardation. This may be analogous to the symptoms seen in patients with focal lesion of the frontal lobes, who develop apathy and difficulties in planning and initiating behaviour.

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It is as yet unsettled whether unipolar and bipolar depressions are distinguishable on the basis of functional neuroimaging studies.

Among the limbic structures the amygdala and hippocampus attract attention because of their role in emotional stress reactions (Arborelius *et al*. 1999). Some neuroimaging studies have shown increased blood flow to the amygdala (Videbech, 2000) and atrophy of the hippocampus (Soares & Mann, 1997; Sheline *et al*. 1999) in depression, although discrepancies exist, maybe because of difficulties in delineating these structures. Few authors have thus combined PET with magnetic resonance imaging (MRI) to achieve optimal co-registration of the PET images and facilitate measurements of the activity in these small regions. Co-registration of the PET images also makes it possible to control for systematic structural differences among and between patients and controls. A matter that is becoming increasingly important, as there is growing evidence for the existence of structural abnormalities in the brains of patients with major depression (Videbech, 1997).

PET studies of depressed patients are significantly different in subject selection, control group selection, imaging protocol and the image analysis tools employed, which also could account for some of the inconsistencies in functional imaging studies (Drevets, 1998; Videbech, 2000). We therefore designed the Danish PET/depression project to counter some of these challenges. On the basis of the PET literature, the present study was designed to test the hypotheses that patients with moderate to severe major depression have: (1) decreased blood flow to the dorsolateral prefrontal cortex, anterior cingulate gyrus and basal ganglia; and (2) increased blood flow to the amygdalo-hippocampal complex and the cerebellum. We first tested whether age or gender of the patients were confounders.

METHOD

Subjects

We recruited patients between 18 and 70 years of age admitted to the Psychiatric Hospital in Aarhus between 1996 and 1999. The Structured Clinical Interview for DSM-III-R (SCID-P (Spitzer *et al*. 1990, 1992)) was administered and patients who met DSM-III-R criteria for major depression were further evaluated and excluded if there was a history of organic brain disease, drug or alcohol dependence or ongoing abuse. Subjects were screened by blood tests and urine samples for significant medical diseases or hidden abuse of psychoactive substances. Subjects were also excluded if the MRI scans showed any cerebral focal abnormality or generalized brain disease, except white matter lesions (Videbech, 1997).

All patients scored 17 or more on the 17-item Hamilton Depression Rating Scale (Bech *et al*. 1986) and were rated as moderately to severely depressed. The patients were rated at the inclusion in the project, immediately before the PET scans and 7 days later. Handedness was assessed according to the Edinburgh Inventory (Oldfield, 1971). The subjects included in this study went through an extensive neuropsychological test battery. These data are presented elsewhere (Ravnkilde *et al*. 2001).

In order to perform studies of severely depressed patients and to avoid selection bias towards less distressed patients, subjects on psychotropic medication were included. Furthermore, several PET studies have substantiated that the blood flow pattern in patients would depend on their symptoms rather than the medication (Martinot *et al*. 1990; Bench *et al*. 1992, 1993; Maes *et al*. 1993; Bonne *et al*. 1999). We wanted to test this prediction in the present sample. In some of these cases the antidepressant treatment was initiated by the general practitioner shortly before admission. Like Bench *et al*. (1992, 1993) and Martinot *et al*. (1990) we considered it unethical to postpone the treatment by putting these very ill patients on a lengthy washout period. In others, due to the severity of the depression, the treatment was initiated so quickly after admission that it was not feasible to perform the PET scans first.

One of the problems in several previous PET studies was the lack of substantiation that the study sample was representative of the population of individuals with major depression (Videbech, 2000). We therefore compared the demographical data of the study subjects with all depressive patients admitted to the Psychiatric Hospital, Aarhus University Hospitals during the study period. The data was drawn from the Danish Psychiatric Central Register (Munk-Jørgensen & Mortensen, 1997).

The controls were recruited by advertisement and interviewed in the same way as the patients. None of the healthy volunteers and their first-degree relatives had any history of psychiatric disorders, and steps were taken to ensure that the controls did not have any medical disease that could possibly involve the central nervous system. Furthermore, volunteers with a history of previous or present abuse of psychotropic drugs or alcohol were excluded. The reliability of this information was ensured by blood and urine tests. None of the controls was taking any psychotropic medication. At least one control subject was allocated for each patient matched for gender, age and if possible also for length of education.

All subjects gave informed consent after thorough verbal and written information, which they had the opportunity to discuss with their next-of-kin or a nurse, who was independent of the project. The study was approved by the Regional Scientific Ethics Committee for Aarhus County and by the Danish Data Protection Agency.

PET scanning

All patients and controls underwent six measurements of cerebral blood flow distribution with radioactive water ($H_2{}^{15}O$) in a Siemens ECAT Exact HR47 positron emission tomograph. The patients and controls were scanned in random order. All scans were performed between 9 and 12 a.m. to account for diurnal variation in blood flow or symptoms.

The subjects were supine with the head fixed in a vacuum pillow to reduce head movement. Earplugs were used and the light was dimmed in the scanner room during the procedure. The subjects were asked to try to relax and look at a tread-cross on a computer monitor that was suspended in front of them, but no other instructions were given. After the resting scan five succeeding scans were made during neuropsychological activation. These results will be presented later.

Preceding the emission scans a 15 min ^{68}Ge scan in two dimensional (2D) was acquired in order to calculate the attenuation corrections. The emission scans were obtained in three dimensional (3D) mode with the collimating septa of the scanner retracted. A dose of 500 MBq (13·5 mCi)$H_2{}^{15}O$ was given as a bolus intravenous injection in the antecubital vein. One single frame of 40 s was acquired at bolus arrival to the brain. This was detected automatically at a true coincident count > 60000 counts.

MRI scanning

Magnetic resonance imaging was obtained in all subjects except one who committed suicide prior to the scan. The MR images were acquired as a T1-weighted 3D volume using a Phillips 1.5 Tesla Gyroscan (for the first 37 subjects) and a GE Signa Echospeed 1.5 T for the rest of the subjects. The same imaging protocol (SPGR, 3D, 30°, TE = 12 ms, TR = 31 ms, FOV = 280 mm) was used on both scanners providing 124 sagittal slices of 1·5 mm in thickness. These images were used to co-register the subjects' brains to the coordinate system designed by Talairach & Tournoux (1988). This spatial normalization facilitates averaging and other statistical procedures of flow data from several subjects. In order to be able to use the PET scan of the patient in whom the MRI was not obtained, his PET data was co-registered to an average T1-weighted MR brain image of 305 normal subjects.

Image processing

The PET images were reconstructed to a $128 \times 128 \times 47$ matrix with a voxel size of $0{\cdot}72 \times 0{\cdot}72 \times 3{\cdot}125$ mm using filtered back-projection with measured attenuation and scatter corrections (Watson *et al.* 1996). The volume was filtered to 12 mm isotropic Full-Width-Half-Maximum. The brain image thus consisted of 47 sections.

The MR images were reconstructed in a $256 \times 256 \times 64$ matrix with a voxel size of $1{\cdot}34 \times 1{\cdot}72 \times 1{\cdot}5$ mm and co-registered to Talairach coordinate system using an automatic algorithm with a linear 12-parameter fit (Collins *et al.* 1994). Afterwards, the PET image of each subject in the resting situation was co-registered to the MR image using the MINC-TRACC algorithm (Collins *et al.* 1994), yielding a linear 6-parameter regression. This procedure failed for a few of the subjects, who instead were co-registered manually using a reference point source method (Neelin *et al.* 1993). All the co-registrations were validated visually. To reduce influence of changes in the global cerebral blood

flow, the radioactivity in every voxel was normalized to the average voxel count of all intracranial voxels.

Statistical methods

The data were analysed in two ways. The Functions-of-Interests (FOI) method was used to compare differences between average blood flow between the patients and the control subjects, as well as in a matched pairs design. To compare the mean normalized blood flow indices for patients and controls controlling for age and gender, a voxel-by-voxel multiple regression was applied with group (patient/control) and gender as categorical variables and age as a continuous variable. This was done using the DOT programme developed at the Brain Imaging Center, Montreal Neurological Institute, McGill University, Montreal, Canada. Furthermore, a term was added to test for absent interaction between group and gender (same difference between group means for the genders and vice versa), and squared age was added to test for non-linearity of the relationship between mean normalized blood flow and age. The relationship between age and mean normalized blood flow was assumed to be independent of group and gender, i.e. the same for both groups and genders. After establishment of an appropriate model for the relationship between the mean normalized blood flow and group, gender and age, the possible additional effects of medication (only patients), length of education, and handedness were considered by testing whether the effects contributed significantly to the model.

The significance of each term in the voxel-by-voxel multiple regression model was based on the usual multiple regression t statistic calculated in each voxel using voxel-specific standard deviations. A term was claimed significant in the model if at least one t statistic exceeded the appropriate threshold. To maintain an overall significance level for the whole search region (the whole brain) of approximately 5%, the thresholds were calculated according to Worsley *et al.* (1996). The threshold, t_α, maintaining an overall significance level of α for t statistics with f degrees of freedom is defined as the (approximate) solution to the equation:

$$\alpha = P(T_{\max}(f) > t_\alpha),$$

where $T_{\max}(f)$ denotes the maximal value of the theoretical field of t statistics with f degrees of freedom. However, since our tests were two-sided, i.e. both small negative and large positive t values were significant, our thresholds were defined as solutions to equations of the form:

$$\alpha = P(T_{\min}(f) < -t_\alpha \quad \text{or} \quad T_{\max}(f) > t_\alpha) = P(T^2_{\max}(f) > t^2_\alpha).$$

But, since the theoretical field of squared t statistics with f degrees of freedom is a $F(1, f)$-field (Worsley, 1994), our $T(f)$-thresholds could be calculated as $\pm$ the square-root of the corresponding $F(1, f)$-threshold. It should be noticed that these threshold are larger in absolute values than those given by $\pm$ the one-sided $T(f)$-threshold at significance level $\alpha/2$. The $F(1, f)$-thresholds could be calculated by the methods in Worsley *et al.* (1996). In these calculations, the search region was approximated by a box of volume V cc. Since the FWHM was assumed to be the same in all three directions, it was not necessary to specify the box dimensions that approximated the search region the best, because the thresholds are then identical for all box dimensions with the same volume.

Finally, the FOI methods were applied in a matched pairs design. A control was assigned to each patient according to the following criteria: same gender, nearly same age and, if possible, nearly same length of education. For every matched pair, the co-registered and normalized blood flow images were subtracted. The FOI method tested whether the mean difference in normalized blood flow was zero.

RESULTS

Forty-two patients (12 males and 30 females) and 47 controls (16 males and 31 females) entered the study (Table 1). All the patients were in-patients. The average age was the same in the two groups (42 years S.D. = 13 v. 41 years S.D. = 12). The controls were slightly higher educated than the patients (on the average one more year of education (Table 1)). In the study period between January 1996 and June 1999, 1728 patients with a main diagnosis of major depression (excluding co-morbid organic brain diseases) were admitted to the Psychiatric Hospital in Aarhus, the major psychiatric hospital in the county of Aarhus, Denmark (600000 in-

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Table 1. *Characteristics of the patients and controls*

	Patients	Controls
Number	42	47
Male/female ratio	0·4	0·5
Mean age (S.D.)	41·9 years (12·7)	41·3 years (11·5)
Years of education (S.D)	11·5 years (2·4)	12·6 years (2·4)*
Socio-economic status†		
High %	24	49
Middle %	48	26
Low %	29	26
Handedness (R/L)	39/3	44/3

* Mann-Whitney $U = 742{\cdot}5$, $z = -2{\cdot}032$, $P = 0{\cdot}04$.
† $\chi^2 = 6{\cdot}86$, df = 2, $P = 0{\cdot}03$.

Table 2. *Local maxima, regression with age corrected for influence of gender and patient/control status*

	Talairach coordinates					
Cluster localization	X	Y	Z	*N*	*t*	*P*
Brainstem	3	−26	−35	89	6·09	0·002
Left thalamus	−13	−18	6	89	5·80	0·006
Right thalamus	16	−23	3	89	5·75	0·007
Left occipital lobe white matter	−13	−81	17	89	5·74	0·007
Left medial occipitotemporal gyrus	−15	−64	−3	89	5·66	0·009
Brainstem	−7	−30	−26	89	5·50	0·017
Right hippocampal formation	29	−9	−20	89	5·46	0·02
Left occipital lobe white matter	−42	−80	−6	89	5·00	0·10
Right cerebellum	13	−62	−35	89	5·00	0·10
Left parahippocampal gyrus	−32	−28	−15	89	4·83	0·18
Right cerebellum	1	−59	−26	89	4·79	> 0·2

Blood flow increases with age in the locations mentioned.

habitants). Data from the Psychiatric Central Register show that the age and gender distribution of the present sample was the same as that of the background population with a male/female ratio of 0·5 ($\chi^2 = 0{\cdot}4$, df = 1, $P = 0{\cdot}5$) and a mean age of 43 years with nearly the same age distribution as the study sample ($\chi^2 = 1{\cdot}33$, df = 4, $P = 0{\cdot}86$).

Analysis of the MR images revealed that signs of brain atrophy and white matter lesions occurred equally frequent in the group of patients and healthy volunteers (Videbech *et al.* 2001).

According to the 17-item Hamilton depression scale, the patients were moderately to severely depressed with a mean score of 24 (range 17–36). Twenty-nine per cent of the patients had never been treated with antidepressants. One-quarter of the patients had been treated for less than 1 week, and 48 % of the patients had been treated for more than 1 week. One patient received lithium and three patients received neuroleptics as sedatives in low doses. Approximately 70 % of the patients received low single doses of benzodiazepines in the days before the PET scan, either as a sleeping aid or as a sedative when needed because of severe anxiety. On the day of the PET scan no sedatives were given.

The patients had two depressive episodes on average (range 1 to 14), and 38 % of the patients had their first episode ever. The onset of the first depression was less than 2·5 years from the present episode in 50 % of the patients. Three patients had bipolar disorders and two-thirds of the patients were endogenously depressed according to the Newcastle scale.

Functions-of-interest analysis

The interaction term was removed from the voxel-by-voxel multiple regression model, as the *t* values for interaction between group and gender were not significant. Similarly, the

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Table 3. *Local minima, regression with age corrected for influence of gender and patient/control status*

Cluster localization	Talairach coordinates X	Y	Z	*N*	*t*	*P*
Caudate nucleus	5	8	12	89	−6·65	0·0002
Left insula	−44	13	−6	89	−6·03	0·002
Left inferior frontal gyrus	−48	12	−2	89	−5·90	0·004
Right superior occipital gyrus	4	−85	45	89	−5·72	0·008
Right inferior frontal gyrus	46	12	−2	89	−5·62	0·01
Left superior temporal gyrus	−47	−1	−3	89	−5·57	0·01
Left inferior frontal gyrus	−51	12	21	89	−5·22	0·05
Right inferior frontal gyrus	51	18	24	89	−5·11	0·07
Left cingulate region	−1	18	39	89	−4·94	0·12
Left cingulate region	−1	41	24	89	−4·81	0·19
Right insula	43	−6	5	89	−4·79	> 0·2
Left middle frontal gyrus	−47	15	30	89	−4·67	> 0·2

Blood flow decreases with age in the locations mentioned.

Table 4. *Local maxima, regression with patient/control status corrected for influence of age and gender*

Cluster localization	Talairach coordinates X	Y	Z	*N*	*t*	*P*
Right hippocampus	31	−11	−17	89	5·98	0·0025
Left cerebellum	−12	−57	−17	89	5·25	0·04
Left lateral occipitotemporal gyrus	−28	−45	−21	89	5·15	0·06
Left medial occipitotemporal gyrus	−13	−44	−8	89	4·83	0·17
Right putamen	25	10	−11	89	4·60	> 0·19

squared age could be removed from the model, indicating linearity in the relationship between age and mean normalized blood flow. However, there were several significant t values for the effect of group, gender and age.

A highly significant fraction of blood went to the parietal lobes ($t = 5{\cdot}67$, $P < 0{\cdot}009$) and a nearly statistically significant fraction to the temporal ($t = 5{\cdot}10$, $P < 0{\cdot}07$) lobes of women compared with men. Furthermore, the share of blood flow to the brainstem, thalamus, left occipital lobe white matter, left medial occipito-temporal gyrus and right hippocampal formation, increased proportionally with age when correcting for the influence of gender (Table 2). Concurrently, the flow to caudate nucleus, left insula, inferior frontal gyri bilaterally, right superior occipital, and left superior temporal gyri decreased with age (Table 3).

Controlled for the effects of gender and age, a comparison of the 42 patients and the 47 healthy subjects revealed a highly significant increase in blood flow to the right hippocampus of the patients ($P = 0{\cdot}0025$, Table 4 and Fig. 1). The left cerebellum of the patients also had an increased flow ($P = 0{\cdot}04$, Fig. 2). No other significant increase or decrease of blood flow was found, but an increase of flow to the left lateral occipito-temporal gyrus of the patients came very close to statistical significance ($P = 0{\cdot}06$). As mentioned earlier, all these P values are corrected for multiple comparisons. Correction for differences of length of education or index of lateralization measured on the Edinburgh Inventory (Oldfield, 1971) did not alter these findings.

Correspondingly, antidepressant treatment did not contribute significantly to the model. As the patients on antidepressant medication had received the drugs over various period of time (from 1 day to several months), we correlated the number of days the patients received the antidepressant to the changes in blood flow, controlling for age and gender. The highest t

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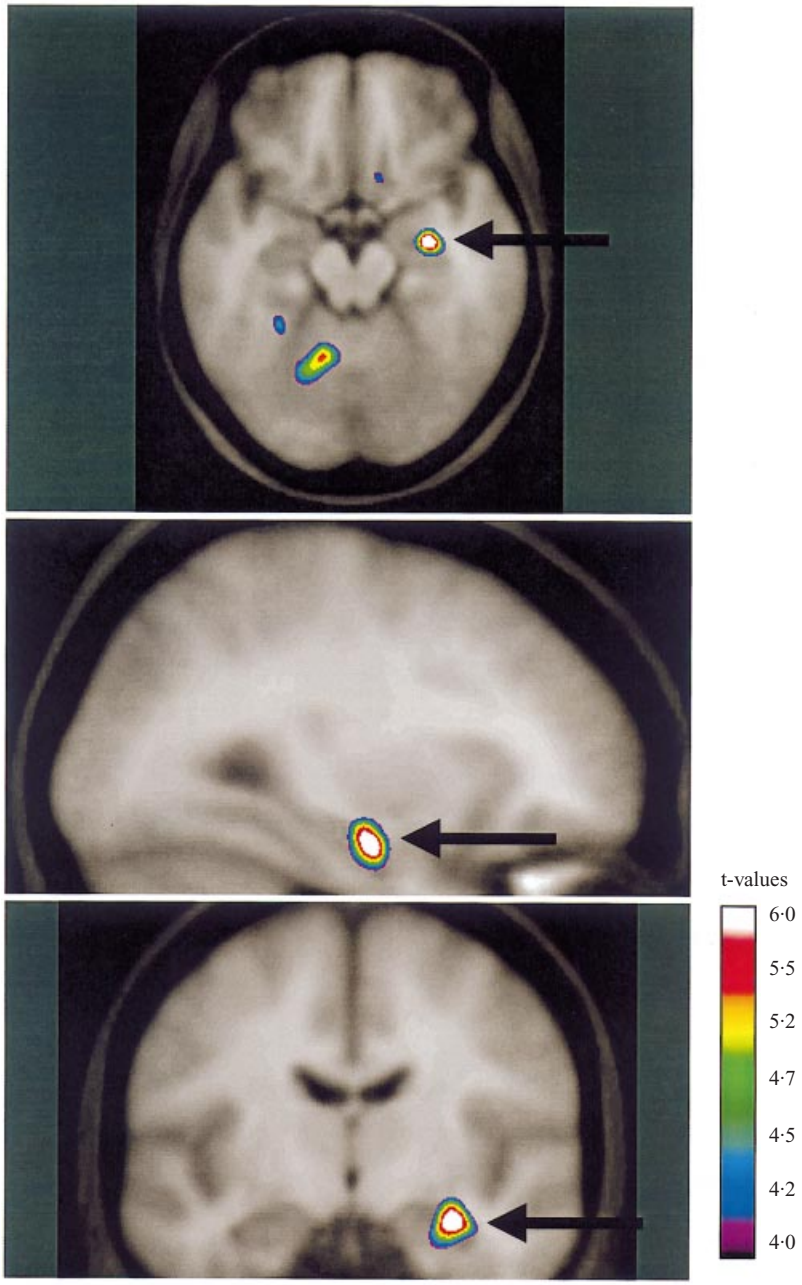

FIG. 1. Blood flow in right hippocampus: corrected for age and gender effect.

value of a local maximum was 4·52 in the right frontal lobe white matter, which corresponds to a *P* value much larger than 0·2 (the threshold being 5·8 with 38 degrees of freedom). The lowest *t* value of a local minimum was −5·18 in the right superior frontal gyrus, which also correspond to a *P* value $> 0{\cdot}2$. Selective serotonin reuptake inhibitors or tricyclic antidepressant treatment alone revealed no significance. Furthermore, following the analysis

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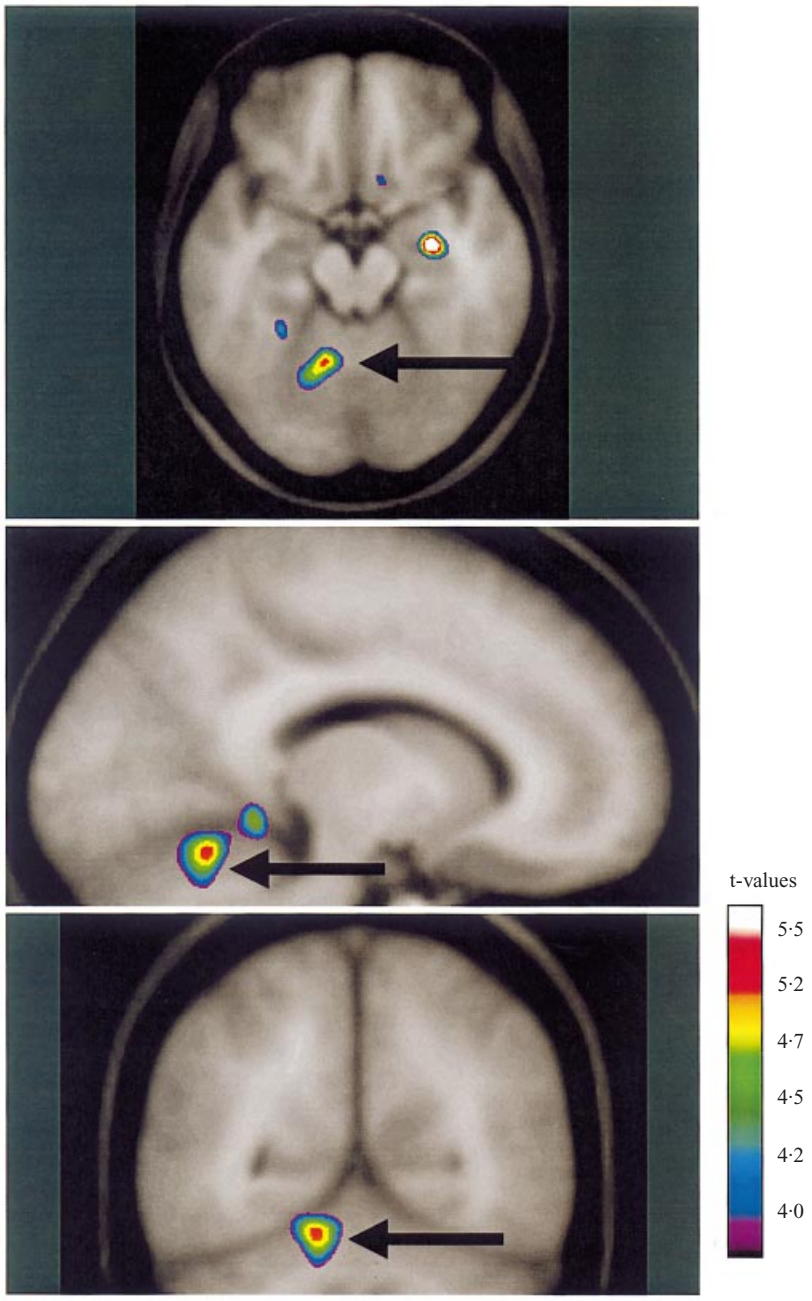

FIG. 2. Blood flow in left cerebellum: corrected for age and gender effect.

suggested by Bench *et al*. (1992) a comparison between unmedicated patients and patients in any antidepressant treatment showed no significant local maxima or minima.

No statistically significant correlations were found between any region and the Hamilton or the Newcastle score among patients controlled for age and gender. Furthermore, the rCBF was

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not found to correlate to the number of episodes or to the length of the period since their first depression.

Matched pairs design

The average of the differences between the images of the matched pairs of patients and controls was calculated. This average and the corresponding *t* images showed the same local maxima and minima as the Functions-of-Interest analysis, when the two groups controlled for age and gender were compared.

DISCUSSION

Functions-of-Interest analysis

The patients had highly significant increased flow to the right hippocampus and the left cerebellum when controlling for the confounders age, gender and medication. The hippocampus is located adjacent to the amygdala, and is thus difficult to distinguish from this structure, especially if co-registration with MRI is not used. Therefore, both regions will be considered in the following analysis. Previous studies, which did not use co-registration, revealed increased activity in the amygdala or amygdalo-hippocampal complex in depression (Drevets *et al.* 1992; Abercrombie *et al.* 1998; Ketter *et al.* 2001). In one study, the increased blood flow persisted after treatment (Drevets *et al.* 1992), and in other studies the increased activity of the amygdalohippocampal complex predicted responsiveness to total sleep deprivation (Wu *et al.* 1992; Ebert *et al.* 1994). After significant improvement of the patients' mood in one study, the amygdala, activity declined but remained elevated compared to normal controls and depressed non-responders (Wu *et al.* 1992). Elevated baseline metabolism of the amygdala predicate relapse of depression after acute tryptophan depletion in patients successfully treated for major depression (Bremner *et al.* 1997). Although hemispheric lateralization has been suggested in depression (Cutting, 1992), we have no good explanation why the increased activity in the hippocampus in the present study was highest on the right side. A corresponding increase in blood flow was, however, also found on the left side, but this was not statistically significant. A satisfactory explanation of this difference awaits further development of our theoretical understanding of brain function in depression.

The left cerebellum had an increased share of the blood flow, as also reported by others (Bench *et al.* 1992; Ketter *et al.* 2001). Several studies showed that the cerebellum is involved in language and cognitive processing (Leiner *et al.* 1991). Interestingly, recent studies suggest that both the cerebellum and amygdala are involved in the mediation of unpleasant emotions (Paradiso *et al.* 1999) and that the cerebellum is active in recognition of emotion in spoken words (Imaizumi *et al.* 1997).

No significant changes were found in dorsolateral prefrontal cortex, cingulate gyrus, or basal ganglia. This is in contrast to earlier observations of decreased glucose metabolism in the frontal lobe compared with the ipsilateral hemisphere (Baxter, Jr. *et al.* 1989) and reduced blood flow to the dorsolateral prefrontal cortex and anterior cingulate in unipolar patients (Bench *et al.* 1993). The discrepancies between the present study and earlier reports could be caused by differences in age and gender distributions between the patient samples. Furthermore, differences in the symptom profiles could affect the measurements of the blood flow, as indicated by Bench *et al.* (1993) and Videbech (2000), but using the FOI-method we were not able to replicate this. In two smaller studies (Martinot *et al.* 1990; Biver *et al.* 1994), depressed patients had decreased prefrontal glucose metabolism, but the control group had a lower average age than the group of patients, which may explain their higher metabolism of the frontal lobes (Videbech, 2000).

To our knowledge, this study is the largest PET investigation of depressive patients so far. The size of the study population, and the representativeness of the sample of in-patients of the population of depressed patients admitted to this hospital during the study period, allow us to generalize the results. Furthermore, MRIs were not only used for stereotactic normalization but also to exclude any confounding effect of cerebral atrophy or white matter lesions. No other PET study of depression have controlled for these important confounders.

Our analyses demonstrate the importance of having a carefully selected and sufficiently large group of controls as we found significant differences between men and women, and age

dependent changes in the relative distribution of the blood flow to the regions of the brain. When interpreting studies of depressed patients it is thus especially important to take into account the finding of an inverse relationship between age and blood flow to the frontal lobes.

As in several previous large studies (Martinot *et al*. 1990; Bench *et al*. 1992, 1993) a limitation in our study was that some of the patients were scanned while taking antidepressant medication. We did, however, consider it unethical to withdraw medication from these very ill patients, as previously described. Alternatively, we would have been unable to investigate these severely depressed patients. We thus assumed, like Bench *et al*. (1992) and other authors (Martinot *et al*. 1990; Bench *et al*. 1993; Meas *et al*. 1993), that any effect of antidepressant medication would not confound changes due to psychopathology. This view is substantiated by our finding that antidepressant medication did not contribute significantly to our statistical model as well as the absence of significant differences between medicated and unmedicated patients in the present material. Because of the risk of a type II error, we cannot, however, reject the possibility that medication actually effects the cerebral blood flow, but this effect cannot explain the observed differences of flow between our depressed patients and the healthy volunteers. This is in accordance with several previous studies in depressives (Bench *et al*. 1993; Maes *et al*. 1993) and a SPECT study of 15 normal volunteers scanned after 2 weeks of placebo and rescanned after 6 weeks administration of fluoxetine (Bonne *et al*. 1999). One SPECT study has suggested hypoperfusion to the motor and parietal cortex in patients treated with benzodiazepines (Maes *et al*. 1993), however, this does not influence our conclusions, because the only possible outcome of this effect would be neglect of areas of high perfusion in the present analysis.

Hippocampal hyperperfusion

As mentioned several studies of patients with major depression have pointed to hippocampal pathology in this disorder. A recent study has shown hippocampal atrophy in patients with major depression (Bremner *et al*. 2000), and one study even suggests that the repeated stress during recurrent depressive episodes may result in cumulative loss of hippocampal volume (Sheline *et al*. 1999). The hippocampus is extensively connected to the serotonergic and noradrenergic systems (Dinan, 1995; Lopez *et al*. 1998; McQuade & Young, 2000) and has one of the highest concentrations of glucocorticoid receptors in the brain (Dinan, 1995; De Kloet *et al*. 1998). Normally, increased level of glucocorticoids alters the electrical properties of the cell membranes and stimulates the cytosolic MR and GR receptors in hippocampus. The overall effect is inhibition of cortisol secretion by a negative feedback via GABAergic neurons to the hypothalamus (Young & Vazquez 1996; Kaufman *et al*. 2000). This negative feedback mechanism, which is modulated by serotonergic neurotransmission to the amygdala and hippocampus (Dinan, 1995), is apparently deficient in major depression, since it is a robust finding that the majority of all depressive patients have a pathologically increased level of glucocorticoids in the blood (Wolkowitz & Reus, 1999; McQuade & Young, 2000), which cannot be suppressed by dexamethasone (Arana *et al*. 1985).

Several authors have thus hypothesized that at least in some types of depression stressful life events starts a vicious circle by increasing the cortisol level, which gradually overstimulates the hippocampal cells leading to their death and further decreases its inhibitory regulation of the HPA-axis (Holsboer, 1988; Duman *et al*. 1997; McEwen, 2000; Sapolsky, 2000). After several untreated episodes this could lead to atrophy of the hippocampus (Sheline, 2000). Accordingly, persistent hyperactivity of the HPA-axis is a predictor of poor prognosis of depression (Ribeiro *et al*. 1993; Zobel *et al*. 1999). A corresponding mechanism is known in Cushing's disease, which in 50–70 % of cases is complicated by depression and often hippocampal atrophy (Sonino *et al*. 1998; Lupien *et al*. 1999; Starkman *et al*. 1999). As mentioned elsewhere (Ravnkilde *et al*. 2001), our patients had severe disturbances in their short-term and working memory compared to the matched controls. Other studies of depressed patients have shown concordant results (Kessing, 1998; Brown *et al*. 1999; Rossi *et al*. 2000). These functions are often attributed to the hippocampus (De Kloet *et al*. 1999) and could thus be connected to the demonstrated hyperperfusion in this structure.

Thus, these results stress the importance of

combining direct measurement of HPA-axis activity with neuroimaging and neuropsychological testing in future research.

Conclusions

Hippocampal pathology has in several studies of neuroendocrine and structural brain abnormalities been linked to major depression. The present PET study, which is the largest of its kind, furthermore adds evidence along these lines from functional neuroimaging. The present analysis thus shows a highly significant increased rCBF to the right hippocampus in a representative sample of patients with severe depression, even when controlling for age, gender, structural brain abnormalities and several other factors.

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