NeuroImage 45 (2009) 29–37

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Can voxel based morphometry, manual segmentation and automated segmentation equally detect hippocampal volume differences in acute depression?

Loretxu Bergouignan [a,*], Marie Chupin [b,g,i], Yvonne Czechowska [c,d,e], Serge Kinkingnéhun [f,g,h,i], Cédric Lemogne [a,j], Guillaume Le Bastard [a], Martin Lepage [c,d,e], Line Garnero [b,g,i], Olivier Colliot [b,g,i], Philippe Fossati [a]

[a] CNRS UMR 7593, Emotion Center, Department of Psychiatry, Groupe Hospitalier Pitié-Salpétrière, 47-83 Boulevard de l'Hôpital, Paris 75013 and IFR Neuroscience, France
[b] CNRS UPR 640, LENA, Cognitive Neuroscience and Brain Imaging Laboratory, 47 Boulevard de l'Hôpital, Paris 75013 and IFR Neuroscience, France
[c] Brain Imaging Group, Douglas Hospital Research Centre, Verdun, Canada
[d] Department of Psychiatry, McGill University, Montreal, PQ, Canada
[e] Department of Neurology, McGill University, Montreal, PQ, Canada
[f] INSERM U610, Paris, France
[g] IFR-49, Orsay, France
[h] e(ye)BRAIN, Paris, France
[i] UPMC Univ Paris 06, France
[j] Department of C-L Psychiatry, European Georges Pompidou Hospital, 75015 Paris, France

ARTICLE INFO

Article history:
Received 30 May 2008
Revised 5 November 2008
Accepted 10 November 2008
Available online 21 November 2008

Keywords:
MRI
Depressed patients
Hippocampus
Head
Body
Tail
VBM
DARTEL

ABSTRACT

Context: According to meta-analyses, depression is associated with a smaller hippocampus. Most magnetic resonance imaging (MRI) studies among middle aged acute depressed patients are based on manual segmentation of the hippocampus. Few studies used automated methods such as voxel-based morphometry (VBM) or automated segmentation that can overcome certain drawbacks of manual segmentation (essentially intra- and inter-rater variability and operator time consumption).
Objective: The aim of our study was to compare the sensitivity of manual segmentation, automated segmentation and VBM to detect hippocampal structural changes in middle aged acute depressed population.
Method: Twenty-one middle aged depressed inpatients and 21 matched controls were compared regarding their hippocampal structure using VBM with SPM5, manual segmentation and an automated segmentation algorithm. The VBM-ROI analysis was performed using two different normalization methods: the standard approach implemented in SPM5 and the most recent DARTEL algorithm.
Results: Using VBM-DARTEL, when corrected for multiple comparisons, significant volume differences were detected between groups in different regions and more specifically in hippocampus with ROI analyses. Whereas using standard VBM (without DARTEL), ROI analyses did not show bilateral volume between group differences.
Significant hippocampal volume reductions between patients and controls were also detected using manual segmentation (−11.6% volume reduction, $p<0.05$) and automated segmentation (−9.7% volume reduction, $p<0.05$). VBM-DARTEL and automated segmentation show equal sensitivity in detecting hippocampal differences in depressed patients, while standard VBM was unable to detect hippocampal changes. Both VBM-DARTEL and automated segmentation could be used to perform large scale volumetric studies in humans. The new automated segmentation technique could further explore and detect hippocampal subpart differences that could be very useful for clarifying physiopathology of psychiatric disorders.
© 2008 Elsevier Inc. All rights reserved.

Introduction

The hippocampus is a central component of the limbic system and has a complex set of interconnections with limbic elements involved in emotional processing (Nieuwenhuys et al., 1988). Depression is characterized by emotional impairment. Hippocampal abnormalities could then have a pathophysiologic role in depression regardless of their ultimate etiology.

Several neuroimaging studies evaluating structural changes of the hippocampus in acute depression have reported significant volume reduction in patients compared to healthy subjects (Frodl et al., 2002, 2006; Bell-McGinty et al., 2002; Saylam et al., 2006; Maller et al., 2007; Colla et al., 2007; Ballmaier et al., 2008; Vasic et al., 2008). Two

* Corresponding author. Fax: +33 153790770.
E-mail address: loretxu@alternatiba.org (L. Bergouignan).

1053-8119/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2008.11.006

meta-analyses reviewing 12 studies and over 300 patients concluded that hippocampal volume is reduced in unipolar depression (Videbech and Ravnkilde, 2004; Campbell et al., 2004). Although the patient populations were highly heterogeneous regarding age, gender distribution, age at onset of the disorder, average number of depressive episodes, and response to treatment, depression was associated with significant reduction in hippocampal volume in both hemispheres. Videbech and Ravnkilde (2004) reported a weighted average reduction of hippocampal volume of 8% on the left side and 10% on the right side.

Nevertheless, some studies did not observe any difference in hippocampal volume between acute depressed patients and controls (Mervaala et al., 2000; Vakili et al., 2000; Von Gunten et al., 2000; Rusch et al., 2001; Posener et al., 2003; Caetano et al., 2004; Hastings et al., 2004). Clinical characteristics of the studied acute depressed populations, such as first vs. multiple depressive episodes, duration of illness and presence of sexual abuse may account for the discrepancies between findings; however the divergent results could also be partly explained by methodological differences.

In the vast majority of these studies, hippocampal volumetry was performed using a manual segmentation protocol. Divergent measuring protocols could explain the divergent findings (Geuze et al., 2005). Furthermore, manual segmentation of the hippocampus is operator time consuming, requires specific anatomical expertise and may result in high intra- and inter-rater variability.

Two studies used voxel-based morphometry (VBM) with SPM (Bell-McGinty et al., 2002; Vasic et al., 2008). VBM is a fully automatic technique which allows an objective analysis of anatomical differences between groups across the whole-brain. It involves a voxel-wise comparison between two groups of subjects of the local concentration of gray matter or volume comparison using Jacobian modulation (Ashburner and Friston, 2000). VBM has been applied in different types of neuropsychiatric pathologies. Among psychiatric disorders, several studies have used VBM in patients with schizophrenia (Seidman et al., 1999; Kubicki et al., 2002; Job et al., 2002, 2003; Moorhead et al., 2004; Borgwardt et al., 2007a; Rametti et al., 2007), and all of these studies reported hippocampal volume differences between patients and healthy subjects. To the best of our knowledge, only Bell-McGinty et al. (2002) and Vasic et al. (2008) have applied VBM in acute depressed patients. Bell-McGinty et al. (2002), using SPM99, reported gray matter concentration differences in the hippocampus between patients and controls but their p-value was not corrected for multiple comparisons ($p=0.001$, uncorrected), and their research population was composed exclusively of elderly subjects. Recently, Vasic et al. (2008) interested in the relationship between gray matter (GM) abnormalities, psychopathology and cognitive impairment, studied GM concentration and volume differences between middle aged acute depressed patients and controls. Using SPM5, they have shown significant volume differences for the left hippocampus. However, as these volume differences were observed with an uncorrected voxel level height threshold of $p<0.001$, it is unclear whether, in middle aged depressed patients, VBM can detect hippocampal volume differences when using a properly corrected statistical threshold.

Also, a preprocessing step of the VBM in SPM has recently been improved with the Diffeomorphic Anatomical Registration using Exponentiated Lie algebra (DARTEL) registration method (Ashburner, 2007). This technique, being more deformable, notably improves the realignment of small inner structures (Yassa et al. 2008). To the best of our knowledge, no studies have yet used DARTEL in acute depressed patients. More generally, this very recent technique has only been used in two VBM studies (Martino et al. 2008; Stonnington et al. 2008).

In addition, one study used a semi-automatic hippocampal segmentation method (Posener et al., 2003). Automated and semi-automatic methods have been developed for systematic segmentation of the hippocampus. These methods are less operator time-consuming and generate less inter and intra-rater variability than manual segmentation (Csernansky et al., 2002; Fischl et al., 2002; Chupin et al., 2007). Using a semi-automated segmentation of the hippocampus (Csernansky et al., 2002, Posener et al. (2003)) were able to detect hippocampal changes in acute depressed patients. Chupin et al. (2007) have recently developed an automated method, called SACHA ("Segmentation Automatisée Compétitive de l'Hippocampe et de l'Amygdale"), to segment the hippocampus and the amygdala. This method has been validated by comparison with manual tracing in healthy controls and in patients with Alzheimer's disease (AD) (Chupin et al., 2007). It has also been successfully applied to detect significant hippocampal volume reduction in patients with AD and in patients with mild cognitive impairment (MCI) (Colliot et al., 2008). However, hippocampal volume reductions in AD and in MCI are more severe than in acute depressed patients, with the latter average reduction ranging at 9% according to meta-analyses (Campbell et al., 2004; Videbech et al., 2004).

The purpose of our study was to compare the sensitivity of VBM, manual segmentation and automated segmentation to detect hippocampal volume differences in depression. To achieve this goal we compared VBM with two segmentation methods (a manual segmentation method and the automated method SACHA) in the same groups of middle aged acute depressed patients and healthy controls.

Methods

Participants

Depressed inpatients fulfilling the DSM-IV criteria for a major depressive episode (unipolar depressive disorder) were recruited from the psychiatry department of the Pitié-Salpêtrière Hospital. Healthy controls with no history of psychiatric disorders were recruited from the community to match the patients for age and level of education. All participants were right-handed. Participants were screened for past or current DSM-IV axis I diagnoses by two psychiatrists (CL and GL) with the Mini International Neuropsychiatric Interview (Sheehan et al., 1998). Severity of depression was assessed using the Montgomery–Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979) and the Beck Depression Inventory (BDI) (Beck et al., 1961). Exclusion criteria were history of manic episode, psychotic features, neurological illness, medical disorders or medication likely to affect cognition, history of substance-related disorders or electroconvulsive therapy in the previous 12 months. Written informed consent was obtained for each participant. The study was approved by the Ethics Committee for Biomedical Research of the Pitié-Salpêtrière Hospital.

MRI acquisition

High-resolution three-dimensional (3D) T_1-weighted images were acquired on a 1.5-T whole-body scanner (SIGNA, GE, Milwaukee, WS, USA). The MRI parameters of the 3D magnetization-prepared rapid gradient-echo (3D-MPRAGE) (Mugler and Brookeman, 1991) sequences were as follows: TR=10.2 ms; TE=2.04 ms; TI=400 ms; FOV=256 mm, flip angle=10°. The images were acquired with an in-plane spatial resolution of 0.9375 mm and with 106 contiguous sagittal 1.5 mm thick slices for 39 participants and with 124 contiguous sagittal 1.3 mm thick slices for 3 participants. Thus, nearly isotropic three-dimensional MR data sets were obtained, making highly accurate volumetric MR measurements possible.

The three methodologies

The main goal of our study was to compare each method's (manual, automated, VBM) sensitivity to detect subtle hippocampal

volume differences between the depressed and normal control groups. We then matched the statistical designs of all three analyses as close as possible. We have included gender and age as covariates in the three statistical analyses.

VBM-DARTEL analysis

Voxel-based morphometry (Ashburner and Friston, 2000) was performed using SPM5 (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience, London, UK). The VBM pre-processing included five steps:

(1) Check for scanner artefacts and gross anatomical abnormalities for each subject. (2) Set image origin at the Anterior Commissure AC. (3) Use of Hidden Markov Random Field (HMRF) option in the segmentation part of the VBM5 toolbox to minimize the noise level of the segmentation. (4) Use DARTEL toolbox to have a high-dimensional normalization protocol. We followed John Ashburner's chapter in its standard version including the MNI space transformation (Ashburner, 2007) (see Fig. 1), but we performed the MNI space transformation with Donald McLaren's script that modifies John Ashburner's script of code for transforming DARTEL templates and images to MNI space (see Appendix A, Fig. 1). (5) Check for homogeneity across sample and use the standard version of the smoothing (i.e. 8). After this pre-processing we obtained smoothed modulated normalized data that we used for the statistical analysis.

Standard VBM analysis

For comparison on hippocampal difference sensitivity, we also repeated the ROI analysis using the more widely used standard SPM5 segmentation code (Ashburner and Friston, 2005) instead of the diffeomorphic registration algorithm. For this standard version we used HMRF segmented modulated images (obtained with step 3) and smoothed those images at 12.

Whole-brain volume comparison

The two groups (depressed patients and controls) were compared using multiple factorial comparisons with the sample as studied factor.

As the Jacobian modulation takes into account both local and global normalization, we had to correct for head-size, we thus added the Total Intracranial Volume (TIV) as covariate in the statistical analysis. TIV was calculated as the sum of GM, White Matter (WM) and Cerebrospinal Fluid (CSF) volumes, derived from SPM5 toolbox's HMRF segmentations. Thus, an analysis of covariance (ANCOVA) was computed to detect differences in gray matter volume between groups. Gender, age and TIV were included as nuisance covariates. To correct for multiple comparisons, we applied the false discovery rate (FDR) approach (Genovese et al., 2002), which controls the expected proportion of false positives among suprathreshold voxels (Benjamini and Hochberg, 2000). We used a threshold of $p<0.05$.

ROI analysis using hippocampal masks (MARINA)

Since the purpose of our study was to assess hippocampal changes, we also performed a region of interest (ROI) analysis restricted to the hippocampus. Indeed, when an a priori hypothesis is made on a specific region, an ROI analysis is more sensitive than a whole-brain comparison. We created a mask of right and left hippocampus with the MARINA software (MAsks for Region of Interest Analysis, Version

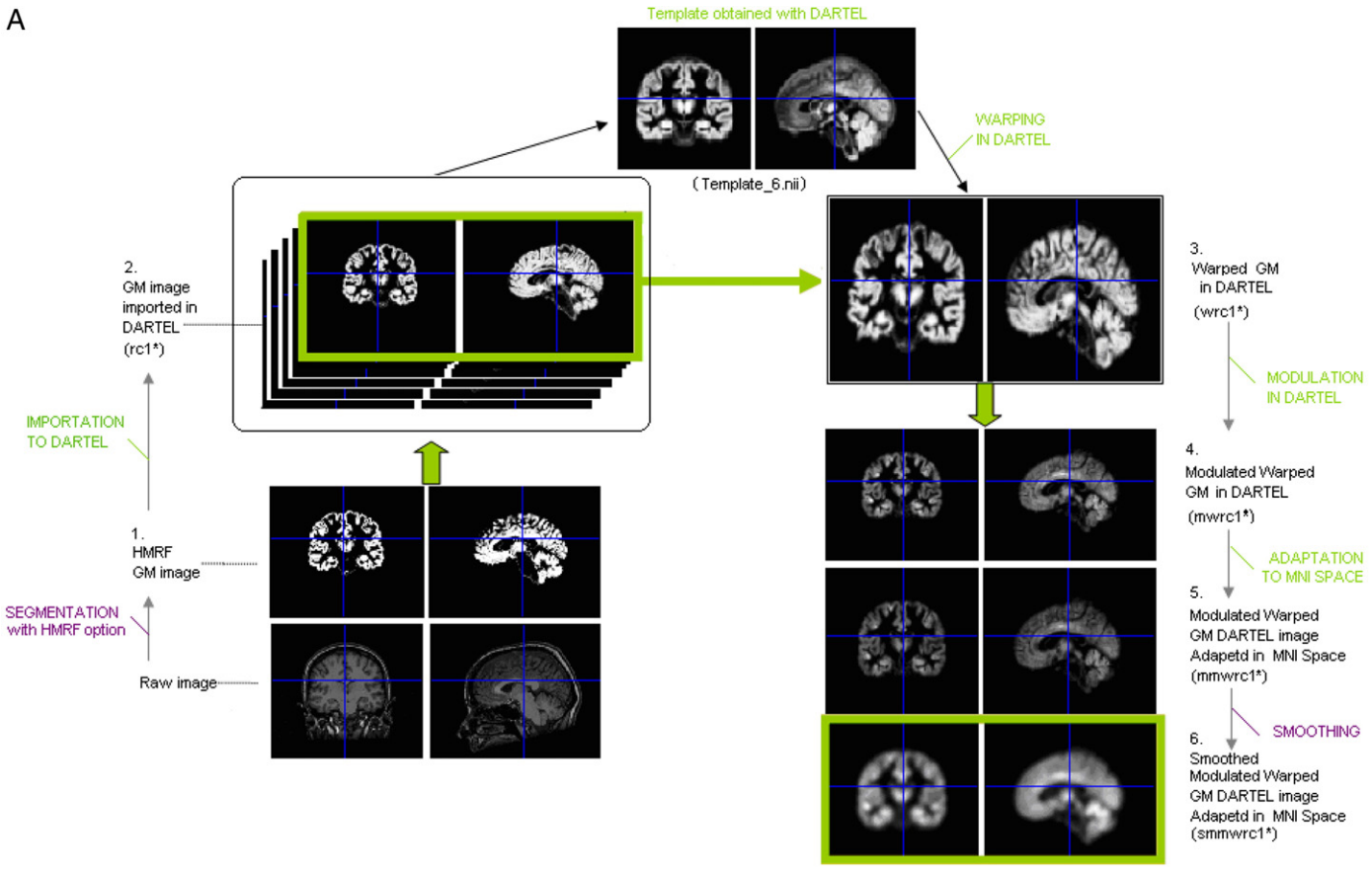


Fig. 1A. HMRF and DARTEL preprocessing in VBM 5 in a subject of our control group.

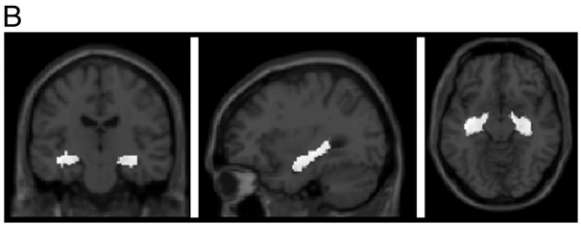


Fig. 1B. Used mask for VBM-ROI analyses.

0.6.1, B. Walter, Giessen, Germany, 2002). This mask was then inserted as the explicit mask in our VBM factorial analyses.

When doing ROI analyses in standard VBM: analysis of covariance (ANCOVA) computed on HMRF segmented modulated and smoothed images between groups with gender, age and TIV included as nuisance covariates.

Within the hippocampal mask, we set significance at a threshold of uncorrected $p<0.001$.

Manual segmentation of the hippocampus

We segmented the hippocampus according to the protocol introduced by Pruessner et al. (2000). Prior to manual hippocampal segmentation, the following preprocessing steps were performed: non-uniformity correction, linear registration to standard stereotaxic space (using the ICBM 152 template), and signal intensity normalization. Volumetric analysis was performed with the interactive software package DISPLAY developed at the Brain Imaging Center of the Montreal Neurological Institute. This program allows visualization of MR images in all orientations. The protocol includes the fimbria, alveus, dentate gyrus, cornu ammonis and excludes the subiculum. Measurements were performed by a trained rater (LB), who was blind to group membership and clinical information. The whole procedure takes about 90 min for each side. To assess intra-rater reliability, volumetric measurement of the hippocampus was carried out twice for 10 participants; the time between two successive sessions was 8–12 months. Intra-rater reliability was assessed using Pearson's intra-class correlation (Fig. 2).

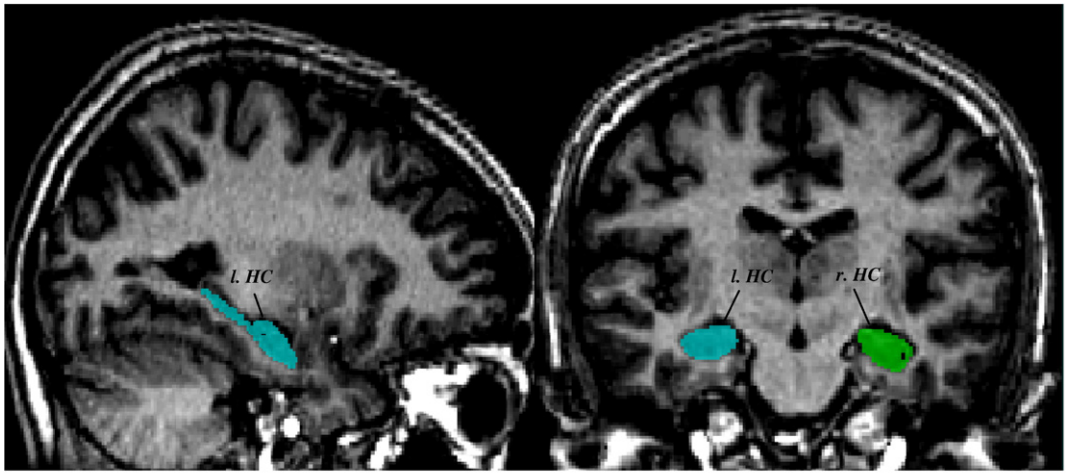
Statistical analysis

To test for differences between depressed patients and controls, a repeated measures ANCOVA (analysis of covariance) was used, with one between-subject grouping factor (patients and controls), one within-subject factor (laterality: left, right) and gender and age as covariates.

Automated segmentation of the hippocampus using SACHA

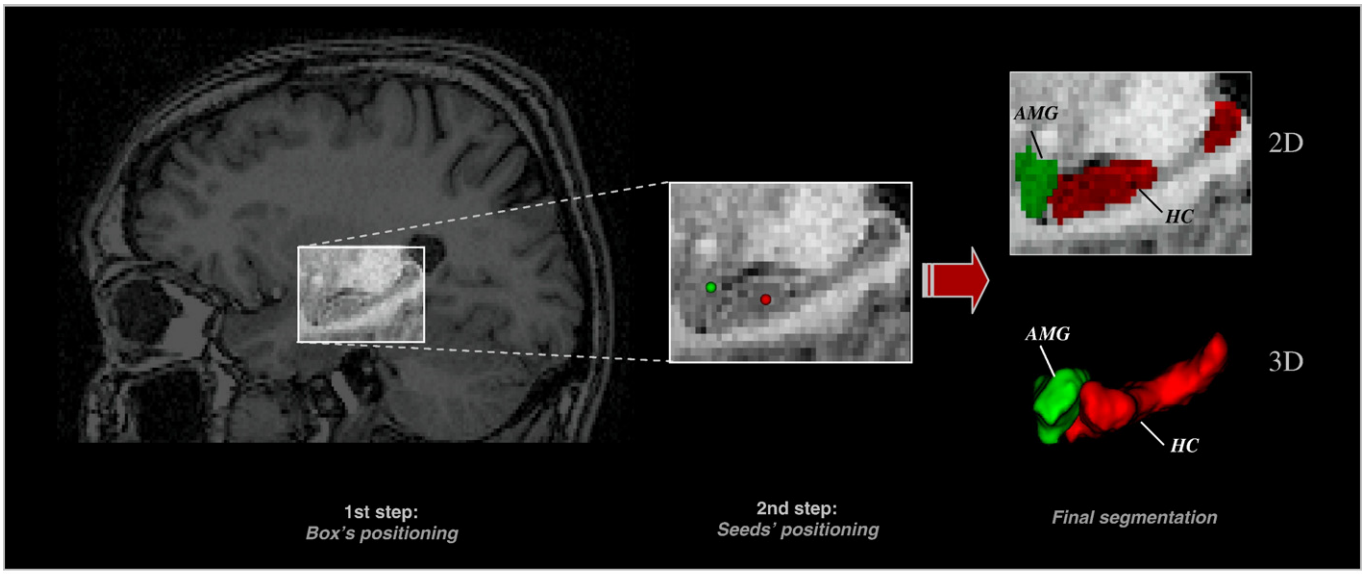
Automated segmentation was performed using the SACHA software (Chupin et al., 2007) included in the Brainvisa image analysis platform (Institut Fédératif de Recherche de Neuroimagerie, IFR 49, Saclay, France, Cointepas et al., 2001, http://www.brainvisa.info/). SACHA extracts the hippocampus from a native scan according to the protocol described in Chupin et al. (2007), Appendix C. As in the manual protocol, the fimbria, alveus, dentate gyrus, cornu ammonis are included and the subiculum excluded.

This software relies on region deformation, introducing a competition between the hippocampus and the amygdala. It gives the volume of both regions; here we only took hippocampal volumes into account. The method requires the following initialization from the operator. First, a bounding box is manually defined around the amygdalo–hippocampal complex (Fig. 3, 1st step) by selecting six slices that correspond to the limits of the hippocampus and the amygdala. The dimension of the bounding box is typically around 30×50×20 voxels. Then, two seeds are placed, one in the hippocampus and one in the amygdala (Fig. 3, 2nd step). These seeds constitute the starting points of the deformation process. They are positioned close to the centre of the amygdala and the center of the head of the hippocampus. Lastly, starting from these two seeds, the algorithm automatically aggregates voxels and converges to the segmentation of the two structures (Fig. 3, 'Final segmentation'). Additionally, two parameters of the algorithm can be adjusted: a radiometric parameter and a geometric parameter. 1) The radiometric parameter controls the ratio between the intensity characteristics (mean and Standard Deviation (SD)) of the hippocampus and the amygdala and those of the gray matter. The ratio between the mean and SD of the intensity of the amygdala and the



l. HC= left Hippocampus; *r. HC*= right Hippocampus

Fig. 2. Left hippocampus segmented with Pruessner's manual segmentation protocol in a subject of our patient group: *l. HC*=left hippocampus; *r. HC*=right hippocampus.



HC= Hippocampus; *AMG*= Amygdala

Fig. 3. Right hippocampus segmented with SACHA software in a subject of our control group: *HC*=hippocampus; *AMG*=amygdala.

hippocampus and those of gray matter may depend on image contrast. For this reason, two preset values controlling these intensity ratios can be chosen depending on the visual contrast of the image. 2) The geometric parameter controls the degree of influence of an anisotropic regularisation strategy taking into account the particular shape in the tail of the hippocampus. Namely, growth in the tail will be influenced by a parameter called tail's anisotropy. These parameters can be adjusted when the hippocampus is atrophied. The default radiometric parameter is 1 and the tail's anisotropy is at 1. The whole procedure takes about 10 min for each side. Measurements were performed by a trained rater (LB) who was blind to group membership and clinical information.

The SACHA algorithm has been validated only on native data and not on normalized data. We used this standard version and obtained non-normalized hippocampal segmentations. We thus had to take into account brain inter-subject variability during the statistical analyses.

Statistical analysis

We took TIV into account in the statistical analyses. ANCOVA was used to test for differences between depressed patients and controls, with one two-level-between-subject factor (group: patients, controls), one within-subject factor (laterality: left, right) and gender, age and TIV as covariates.

Table 1
Clinical and demographic characteristics of participants

	Patients				Controls					
	Mean	s.d.	Min	Max	Mean	s.d.	Min	Max	test *t*	
Age	33.16	9.58	20	49	28.21	5.50	21	38	$t=1.82$	$p>0.05$
Scholar level	13.05	2.50	9	17	14.14	2.06	9	17	$t=-1.51$	
Number of MDE	2.18	1.13	1	5						
Duration of illness (years)	8.45	9.03	0.33	27						
Age on the first MDE	23.8	8.65	13	46						
Duration of the last MDE (month)	4.56	3.22	1	12						
Number of hospitalizations	1.53	1.06	1	5						
MADRS	28.71	6.82	18	40						
BDI	19.36	4.67	13	27						

MDE = Major Depression Episode. MADRS = Montgomery and Asberg Depression Rating Scale. BDI = Beck Depression Inventory.

We added an ANCOVA with one two-level-between-subject factor (group: patients, controls), one within-subject factor (laterality: left, right) and gender, age and Total Cerebral Volume (TCV) as covariates. The TCV was calculated as the sum of GM and WM. We added this analyse to ensure that the segmentation of the CSF did not influence the results.

Results

Participants' description

Twenty-one unipolar depressed inpatients (17 women, 4 men) and 21 healthy controls (14 women, 7 men) were recruited. All controls had no history of psychiatric disorder. Clinical and demographic characteristics of the participants are displayed in Table 1.

All patients were taking antidepressants and were tested within the first week of receiving their treatment. Sedative drugs were not allowed on the experiment day. One male depressed inpatient was excluded from the study population due to an abnormal enlargement of the lateral ventricles revealed by the MRI scanning. This patient was hospitalized due to suicidal thoughts and subsequently experienced atypical memory symptoms.

VBM results

Whole-brain analysis with DARTEL-VBM

No scanner artefacts or gross anatomical abnormalities were found. After the segmentation and the normalization, no outlier was found when checking for homogeneity. No subject was excluded from the analyses.

Table 2
Regions of GM volume difference in VBM5 with FDR correction

Region	Side	*x*	*y*	*z*	*Z*-score (*t*-value)
Cingulate gyrus (BA 24)	Right	7	−3	46	5.77 (7.68)
Middle temporal gyrus	Right	57	−6	−21	4.56 (5.43)
Posterior lobe	Right	19	−71	−15	4.32 (5.05)
Superior parietal lobule	Right	28	−57	50	4.24 (4.94)
Parahippocampal gyrus	Right	41	−32	−11	4.21 (4.89)
Inferior semi-lunar lobule	Left	−33	−65	36	4.13 (4.78)

BA=Brodmann Area.

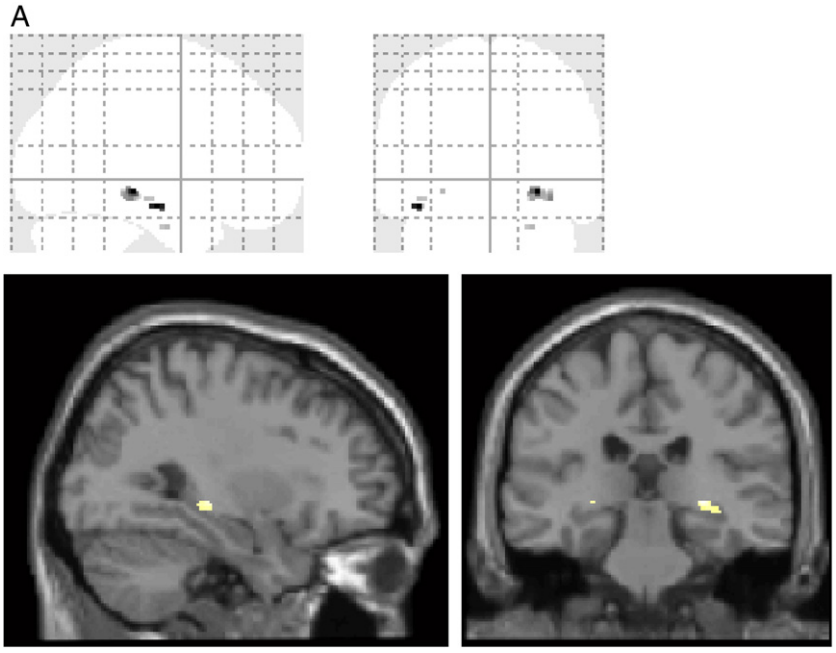


Fig. 4A. Hippocampal volume difference observed in VBM5, including DARTEL preprocessing, in MARINA hippocampus restricted ROI analyses with $p<0.001$ uncorrected threshold.

As some artefacts appeared outside the brains, which could not be due to real differences, we have added a mask, namely a canonical brain image. The whole-brain VBM analysis showed significant volume differences in different regions including the parahippocampal gyrus, but not hippocampus (see Table 2), using a threshold of $p<0.05$ and the FDR method.

ROI analysis

VBM-DARTEL. When doing ROI analyses with the SPM5 VBM-DARTEL procedure: analysis of covariance (ANCOVA) with a threshold of $p<0.001$ uncorrected showed significant bilateral hippocampal differences (see Fig. 4A).

Standard-VBM

When doing ROI analyses with the standard SPM5 VBM procedure: analysis of covariance (ANCOVA) with a threshold of $p<0.001$ uncorrected showed slight significant differences restricted to the left hippocampal region. However, the small region that appears is outside the hippocampus, and it is therefore difficult to conclude that this difference is related to hippocampal changes (see Fig. 4B).

Manual hippocampal volumetry

The intra-rater reliability (intra-class correlation) was 0.89 for the left hippocampus and 0.82 for the right hippocampus.

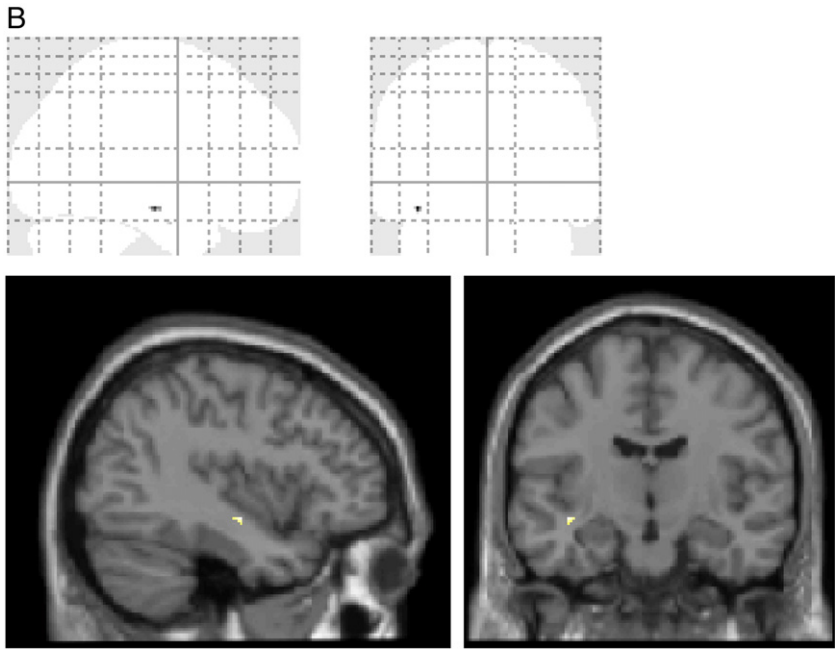


Fig. 4B. Hippocampal volume difference observed in VBM5, without DARTEL preprocessing, in MARINA hippocampus restricted ROI analyses with $p<0.001$ uncorrected threshold.

Mean left hippocampal volumes were 3.95 cm^3 ± 0.75 (range: 2.25–5.41) for depressed patients and 4.42 cm^3 ± 1.04 (range: 2.36–6.93) for control participants. Right hippocampal volumes were 3.93 cm^3 ± 0.80 (range: 2.32–5.70) for depressed patients and 4.50 cm^3 ± 1.06 (range: 2.47–7.16) for control participants. The ANCOVA with hippocampal volume as the dependent variable and gender and age as covariates revealed no side effect (F=0.514, df=38, p=0.478) or group×side interaction (F=0.031, df=38, p=0.861). However a significant group effect was revealed (F=4.718, df=38, p<0.05). The mean hippocampal volume reduction in patients compared to controls was 11.6% (10.6% on the left and 12.6% on the right). Detailed results are presented in Table 3.

Automated hippocampal volumetry

Seventy-four (74/84=88%) hippocampi were segmented with the default parameters. Ten segmentations needed parameter adjustments: for radiometric parameters, five were set at 0 (bad contrast); for tail's geometric parameter two had the tail's anisotropy set at 0, six at 2 and one at 3.

Left hippocampal volumes were 2.73 cm^3 ± 0.35 (range: 2.23–3.27) for depressed patients and 2.95 cm^3 ± 0.31 (range: 2.45–3.47) for control participants. Right hippocampal volumes were 2.71 cm^3 ± 0.47 (range: 1.38–3.40) for depressed patients and 3.10 cm^3 ± 0.33 (range: 2.63–3.73) for control participants. Detailed results are presented in Table 4.

The ANCOVA with the hippocampal volume (cm^3) as the dependant variable and the TIV, gender and age as covariates revealed no significant side effect (F=0.842, df=37, p=0.365) or group×side interaction (F<0.001, df=37, p=0.992). However a significant group effect was revealed (F=6.504, df=37, p<0.05). The ANCOVA with the hippocampal volume (cm^3) as the dependant variable and the TCV, gender and age as covariates revealed similar results [no significant side effect (F=1.467, df=37, p=0.233), or group×side interaction (F=0.022, df=37, p=0.884), but a significant group effect (F=4.234, df=37, p<0.05).

The mean hippocampal volume reduction in patients compared to controls was 9.7% (7.8% on the left and 11.6% on the right). T test on TIV values showed no significant difference between group (t=0.836, df=40, p=0.408).

Supplementary analyses

When analysing our data with the standard normalization of SPM5 (i.e. without DARTEL normalization) VBM analyses were unable to reveal bilateral hippocampal changes. This could be due to the fact that, in patients with depression, volume loss could be mostly found in the posterior subparts of the hippocampus, the hippocampal anterior part being preserved (Maller et al., 2007) and that this posterior part needs a good realignment. This may account for the discrepancies between VBM without DARTEL and with DARTEL. Since the posterior part of the hippocampus is a thin elongated structure, it might not be accurate for VBM analyses without DARTEL realignment. To test this hypothesis, we used the SACHA software and divided each automated hippocampal labels into head, body and tail. We used ANCOVA with one two-level-between-subject factor (group: patients, controls), two within-subject factors [1) subpart: head, body, tail; 2) laterality: left, right], and sex and TIV as covariates. This revealed a significant group by subpart effect (p=0.018). We then performed distinct ANCOVAs for each subpart of the hippocampus to determine which one triggered this effect. There was no significant effect on the head (p=0.299). There was a significant group effect on the body (p=0.003) and a tendency of group effect on the tail (p=0.093). Detailed results are presented in Table 5.

Table 3
Hippocampal volumes (cm^3) using manual segmentation

	Left Hc manual segmentation				Right Hc manual segmentation			
	Mean	s.d.	Min	Max	Mean	s.d.	Min	Max
Patients	3.95	0.75	2.25	5.41	3.93	0.80	2.32	5.70
Controls	4.42	1.04	2.36	6.93	4.50	1.06	2.47	7.16

Table 4
Hippocampal volumes (cm^3) using automated segmentation

	Left Hc automated segmentation				Right Hc automated segmentation			
	Mean	s.d.	Min	Max	Mean	s.d.	Min	Max
Patients	2.73	0.35	2.23	3.27	2.71	0.47	1.38	3.40
Controls	2.95	0.31	2.45	3.47	3.10	0.33	2.63	3.73

Discussion

ROI-based VBM with DARTEL normalization (but not with standard normalization) as well as manual and automated segmentation of the hippocampus were able to detect significant bilateral hippocampal volume reduction in acute depressed patients compared to controls. There were comparable degrees of hippocampal reduction with manual segmentation and automated segmentation.

This significant hippocampal volume reduction in acute depressed patients is consistent with the two meta-analyses of Videbech and Ravnkilde (2004) and Campbell et al. (2004).

Thus, according to our results the three methods (ROI-based VBM-DARTEL, manual and automated segmentation) are sensitive enough to detect hippocampal volume differences in acute depression. As specified in the introduction, only two studies using VBM (the standard version) have been published in acute depressed patients. The study of Bell-McGinty et al. did not find any significant gray matter differences in the hippocampal region with corrected p values but found some with an uncorrected p value at p=0.001 (Bell-McGinty et al., 2002) and Vasic et al. (2008) have recently found positive results in middle aged acute depressed patients (Vasic et al., 2008). A study in chronic patients found positive results with VBM (Shah et al., 1998). Some other works might have been done with standard-VBM in acute depression, but these may have not been published due to negative results.

In our study, the results of whole brain analysis with VBM-DARTEL were close to the results of Vasic et al. (2008). In our study, when using a FDR corrected threshold of p<0.05, there were volume differences on middle temporal gyrus, parahippocampal gyrus, superior parietal lobule, and cingulate gyrus.

VBM is a fully automatic technique which allows an objective analysis of anatomical differences between groups across the whole-brain. Its main advantage is to simultaneously analyse many different brain regions. With its diffeomorphic image registration algorithm (DARTEL), it has certainly improved the realignment. Even if it is not yet totally user-friendly and needs to run during few days to get the template, DARTEL is a real improvement over the standard approach specially for the medial temporal lobe (Yassa et al. 2008), including, as we can see with our results, an improvement for the hippocampus analyses.

Using a subdivision of the automated segmentation into hippocampal head, body and tail, we observed that our depressed patients had no significant volume reduction in the head of the hippocampus but showed a significant difference in the body and a trend in the tail. This is consistent with the results of Maller et al. (2007), who found a more pronounced difference in the posterior part of the hippocampus. On the contrary, in schizophrenia, atrophy seems to mainly affect the hippocampal head (Csernansky et al., 2002). Several studies using the standard version of VBM with SPM have shown hippocampal gray matter differences when comparing schizophrenic patients with healthy controls (Kubicki et al., 2002; Job et al., 2002, 2003; Moorhead

Table 5
Hippocampal head, body and tail volumes (cm^3) using automated segmentation

	Left hippocampus						Right hippocampus					
	Head		Body		Tail		Head		Body		Tail	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Patients	1.46	0.33	0.75	0.09	0.52	0.21	1.67	0.62	0.72	0.11	0.42	0.29
Controls	1.52	0.24	0.83	0.13	0.62	0.17	1.68	0.35	0.85	0.15	0.56	0.18

et al., 2004; Borgwardt et al., 2007b; Rametti et al., 2007). These different spatial patterns of hippocampal atrophy between depression and schizophrenia might have influence VBM standard version's sensitivity. With the subdivision in head-body-tail with the automated segmentation, and in the VBM-DARTEL ROI analyses, we can observe that the difference is larger in the posterior part of the hippocampus in our population (see Fig. 4).

Manual and automated segmentation resulted in comparable volume reductions (9.7% vs. 11.6%). However, in both populations, manual volumes were larger than non TIV corrected automated volumes (mean = 4.20 cm^3 for manual segmentation, mean = 2.87 cm^3 for automated segmentation). Considering that the manual and the automated hippocampus segmentation protocols are highly similar, this difference is likely to be due to the normalization in the MNI stereotactic space that was performed prior to manual segmentation with the linear ICBM 152 template (Lancaster et al., 2007).

Our study has some limitations: we did not perform any direct comparison between the methods. The automated segmentation method was done on native scans whereas manual segmentations and VBM after normalization to a standard space (as specified on the methodology, with the modulation we also needed to take into account global differences during statistical analyses with the VBM). One would expect that the sharper edges of the native scans would be as beneficial to manual segmentation as they are to automated segmentation. However, the use of normalization may be beneficial to the manual segmentation because it improves reproducibility and Pruessner's manual segmentation has not been adapted for a segmentation on native MRI images. Whatsoever repeating the manual segmentation on the native scans to have a direct comparison of automated and manual segmentations would in turn not allow for direct comparison to the VBM-DARTEL method that includes spatial normalization as part of the preprocessing. Beyond this limitation, our results confirm the hippocampal difference between patients and controls and highlight the posterior aspect of this difference.

Episodic memory retrieval is critically dependent upon hippocampal integrity (Sapolsky et al., 1990). According to the HIPER model (HIPpocampal Encoding/Retrieval model), activations in the hippocampal region associated with episodic memory encoding are located in the rostral part of the region (i.e. head), whereas activations associated with episodic memory retrieval are located in the caudal part (i.e. body and tail) (Lepage et al., 1998). Episodic memory retrieval is impaired in depressed patients not only in acute state (Lemogne et al., 2006), but also in euthymic state (Bergouignan et al., 2008). The specific anatomical abnormality found in the posterior hippocampus in our study in acute depression may be associated with specific retrieval deficits in depression.

Structural changes in the hippocampus could be due to remodeling of key cellular elements, involving retraction of dendrites, decreased neurogenesis in the dentate gyrus, and loss of glial cells (Cameron et al., 1998; Magarinos et al., 1999; Malberg et al., 2000; McEwen, 1999; Rajkowska, 2000; Rogatsky et al., 1997). According to post mortem clinical studies and animal model studies, most antidepressants stimulate adult hippocampal neurogenesis (Malberg et al., 2000; Perera et al., 2000; Madsen et al., 2000; Van Praag et al., 1999; Duman, 2004). Hippocampal changes could be associated with acute depressed patient's responsiveness to antidepressants. Large scale longitudinal studies evaluating the link between hippocampal atrophy and medication responsiveness are necessary to test this hypothesis.

To summarize, our results demonstrate that automated segmentation and VBM with DARTEL can constitute a viable alternative to manual segmentation to detect hippocampal atrophy in acute depressed patients. The two automated techniques can be used to perform large scale volumetric studies in humans. The new automated segmentation technique could further explore and detect hippocampal subpart differences that could be very useful for clarifying physiopathology and providing further light on the clinical implications of these structural brain abnormalities.

Acknowledgments

We are particularly grateful to the reviewers for their requirement in the VBM part of the paper that has made us notably improve the manuscript. We are also grateful to Lisa Buchy and thank her for the helpful comments and corrections on English language regarding the manuscript.

L.B. is supported by funding from the Institut National du Cancer (The Cancer National Institute).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2008.11.006.

References

Ashburner, J., 2007t. A fast diffeomorphic image registration algorithm. NeuroImage 15 38 (1), 95–113 Oct.
Ashburner, J., Friston, K.J., 2000. Voxel-based morphometry—the methods. NeuroImage 11, 805–821.
Ashburner, J., Friston, K.J., 2005. Unified segmentation. NeuroImage 26, 839–851 2005.
Ballmaier, M., Narr, K.L., Toga, A.W., Elderkin-Thompson, V., Thompson, P.M., Hamilton, L., Haroon, E., Pham, D., Heinz, A., Kumar, A., 2008. Hippocampal morphology and distinguishing late-onset from early-onset elderly depression. Am. J. Psychiatry 165 (2), 229–237.
Beck, A.T., Ward, C.H., Mendelson, M., Mock, J., Erbauch, J., 1961. An inventory for measuring depression. Arch. Gen. Psychiatry 4, 561–571.
Bell-McGinty, S., Butters, M.A., Meltzer, C.C., Greer, P.J., Reynolds III, C.F., Becker, J.T., 2002. Brain morphometric abnormalities in geriatric depression: long-term neurobiological effects of illness duration. Am. J. Psychiatry 159 (8), 1424–1427.
Benjamini, Y., Hochberg, Y., 2000. On the adaptive control of the false discovery rate in multiple testing with independent statistics. J. Educ. Behav. Stat. 25 (1), 60–83.
Bergouignan, L., Lemogne, C., Foucher, A., Longin, E., Vistoli, D., Allilaire, J.F., Fossati, P., 2008. Field perspective deficit for positive memories characterizes autobiographical memory in euthymic depressed patients. Behav. Res. Ther. 46 (3), 322–333.
Borgwardt, S.J., Riecher-Rössler, A., Dazzan, P., Chitnis, X., Aston, J., Drewe, M., Gschwandtner, U., Haller, S., Pflüger, M., Rechsteiner, E., D'Souza, M., Stieglitz, R.D., Radü, E.W., McGuire, P.K., 2007a. Regional gray matter volume abnormalities in the at risk mental state. Biol. Psychiatry 61 (10), 1148–1156 15.
Borgwardt, S.J., McGuire, P.K., Aston, J., Berger, G., Dazzan, P., Gschwandtner, U., Pflüger, M., D'Souza, M., Radue, E.W., Riecher-Rössler, A., 2007b. Structural brain abnormalities in individuals with an at-risk mental state who later develop psychosis. Br. J. Psychiatr. (Suppl.) 51, 69–75.
Caetano, S.C., Hatch, J.P., Brambilla, P., Sassi, R.B., Nicoletti, M., Mallinger, A.G., Frank, E., Kupfer, D.J., Keshavan, M.S., Soares, J.C., 2004. Anatomical MRI study of hippocampus and amygdala in patients with current and remitted major depression. Psychiatry Res. 132 (2), 141–147 15.
Cameron, H.A., Tanapat, P., Gould, E., 1998. Adrenal steroids and N-methyl-D-aspartate receptor activation regulate neurogenesis in the dentate gyrus of adult rats through a common pathway. Neuroscience 82 (2), 349–354.

Campbell, S., Marriott, M., Nahmias, C., MacQueen, G.M., 2004. Lower hippocampal volume in patients suffering from depression: a meta-analysis. Am. J. Psychiatry 161 (4), 598–607.

Chupin, M., Mukuna-Bantumbakulu, A.R., Hasboun, D., Bardinet, E., Baillet, S., Kinkingnéhun, S., Lemieux, L., Dubois, B., Garnero, L., 2007. Anatomically constrained region deformation for the automated segmentation of the hippocampus and the amygdala: method and validation on controls and patients with Alzheimer's disease. NeuroImage 34 (3), 996–1019 1.

Colla, M., Kronenberg, G., Deuschle, M., Meichel, K., Hagen, T., Bohrer, M., Heuser, I., 2007. Hippocampal volume reduction and HPA-system activity in major depression. J. Psychiatr. Res. 41 (7), 553–560.

Colliot, O., Chételat, G., Chupin, M., Desgranges, B., Magnin, B., Benali, H., Dubois, B., Garnero, L., Eustache, F., Lehéricy, S., 2008. Discrimination between Alzheimer disease, mild cognitive impairment and normal aging by using automated segmentation of the hippocampus. Radiology 248 (1), 194–201.

Csernansky, J.G., Wang, L., Jones, D., Rastogi-Cruz, D., Posener, J.A., Heydebrand, G., Miller, J.P., Miller, M.I., 2002. Hippocampal deformities in schizophrenia characterized by high dimensional brain mapping. Am. J. Psychiatry 159 (12), 2000–2006.

Duman, R.S., 2004. Depression: a case of neuronal life and death? Biol. Psychiatry 1 56 (3), 140–145 Review.

Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., van der Kouwe, A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., Dale, A.M., 2002. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33 (3), 341–355 31.

Frodl, T., Meisenzahl, E.M., Zetzsche, T., Born, C., Groll, C., Jäger, M., Leinsinger, G., Bottlender, R., Hahn, K., Möller, H.J., 2002. Hippocampal changes in patients with a first episode of major depression. Am. J. Psychiatry 159 (7), 1112–1118.

Frodl, T., Schaub, A., Banac, S., Charypar, M., Jäger, M., Kümmler, P., Bottlender, R., Zetzsche, T., Born, C., Leinsinger, G., Reiser, M., Möller, H.J., Meisenzahl, E.M., 2006. Reduced hippocampal volume correlates with executive dysfunctioning in major depression. J. Psychiatry Neurosci. 31 (5), 316–323.

Genovese, C.R., Lazar, N.A., Nichols, T., 2002. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. 15(4):870–8.

Geuze, E., Vermetten, E., Bremner, J.D., 2005. MR-based in vivo hippocampal volumetrics: review of methodologies currently employed. Mol. Psychiatry 10 (2), 147–159.

Hastings, R.S., Parsey, R.V., Oquendo, M.A., Arango, V., Mann, J.J., 2004. Volumetric analysis of the prefrontal cortex, amygdala, and hippocampus in major depression. Neuropsychopharmacology 29 (5), 952–959.

Job, D.E., Whalley, H.C., McConnell, S., Glabus, M., Johnstone, E.C., Lawrie, S.M., 2002. Structural gray matter differences between first-episode schizophrenics and normal controls using voxel-based morphometry. NeuroImage 17 (2), 880–889.

Job, D.E., Whalley, H.C., McConnell, S., Glabus, M., Johnstone, E.C., Lawrie, S.M., 2003. Voxel-based morphometry of gray matter densities in subjects at high risk of schizophrenia. Schizophr. Res. 64 (1), 1–13 1.

Kubicki, M., Shenton, M.E., Salisbury, D.F., Hirayasu, Y., Kasai, K., Kikinis, R., Jolesz, F.A., McCarley, R.W., 2002. Voxel-based morphometric analysis of gray matter in first episode schizophrenia. NeuroImage 17 (4), 1711–1719.

Lancaster, J.L., Tordesillas-Gutiérrez, D., Martinez, M., Salinas, F., Evans, A., Zilles, K., Mazziotta, J.C., Fox, P.T., 2007. Bias between MNI and Talairach coordinates analyzed using the ICBM-152 brain template. Hum. Brain Mapp. 28 (11), 1194–1205.

Lemogne, C., Piolino, P., Friszer, S., Claret, A., Girault, N., Jouvent, R., Allilaire, J.F., Fossati, P., 2006. Episodic autobiographical memory in depression: specificity, autonoetic consciousness, and self-perspective. Conscious. Cogn. 15 (2), 258–268.

Lepage, M., Habib, R., Tulving, E., 1998. Hippocampal PET activations of memory encoding and retrieval: the HIPER model. Hippocampus 8 (4), 313–322.

Madsen, T.M., Greisen, M.H., Nielsen, S.M., Bolwig, T.G., Mikkelsen, J.D., 2000. Electroconvulsive stimuli enhance both neuropeptide Y receptor Y1 and Y2 messenger RNA expression and levels of binding in the rat hippocampus. Neuroscience 98 (1), 33–39.

Magariños, A.M., Deslandes, A., McEwen, B.S., 1999. Effects of antidepressants and benzodiazepine treatments on the dendritic structure of CA3 pyramidal neurons after chronic stress. Eur. J. Pharmacol. 371 (2–3), 113–122 29.

Malberg, J.E., Eisch, A.J., Nestler, E.J., Duman, R.S., 2000. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J. Neurosci. 20 (24), 9104–9110 15.

Maller, J.J., Daskalakis, Z.J., Fitzgerald, P.B., 2007. Hippocampal volumetrics in depression: the importance of the posterior tail. Hippocampus 17 (11), 1023–1027.

Martino, D., Draganski, B., Cavanna, A., Church, A., Defazio, G., Robertson, M.M., Frackowiak, R.S., Giovannoni, G., Critchley, H.D., 2008. Anti-basal ganglia antibodies and Tourette's syndrome: a voxel-based morphometry and diffusion tensor imaging study in an adult population. J. Neurol. Neurosurg. Psychiatry 79 (7), 820–822.

McEwen, B.S., Tanapat, P., Weiland, N.G., 1999. Inhibition of dendritic spine induction on hippocampal CA1 pyramidal neurons by a nonsteroidal estrogen antagonist in female rats. Endocrinology 140 (3), 1044–1047.

Mervaala, E., Föhr, J., Könönen, M., Valkonen-Korhonen, M., Vainio, P., Partanen, K., Partanen, J., Tiihonen, J., Viinamäki, H., Karjalainen, A.K., Lehtonen, J., 2000. Quantitative MRI of the hippocampus and amygdala in severe depression. Psychol. Med. 30 (1), 117–125.

Montgomery, S.A., Asberg, M., 1979. A new depression scale designed to be sensitive to change. Br. J. Psychiatry 134, 382–389.

Moorhead, T.W., Job, D.E., Whalley, H.C., Sanderson, T.L., Johnstone, E.C., Lawrie, S.M., 2004. Voxel-based morphometry of comorbid schizophrenia and learning disability: analyses in normalized and native spaces using parametric and nonparametric statistical methods. NeuroImage 22 (1), 188–202.

Mugler III, J.P., Brookeman, J.R., 1991. Rapid three-dimensional T1-weighted MR imaging with the MP-RAGE sequence. J. Magn. Reson. Imaging 1 (5), 561–567.

Nieuwenhuys, R., Voogd, J., van Huijzen, C., 1988. The Human Central Nervous System: a Synopsis and Atlas3rd revised ed. Springer-Verlag, Berlin.

Perera, T.D., Lisanby, S.H., 2000. Neurogenesis and depression. J. Psychiatr. Pract. 6 (6), 322–333 Nov.

Posener, J.A., Wang, L., Price, J.L., Gado, M.H., Province, M.A., Miller, M.I., Babb, C.M., Csernansky, J.G., 2003. High-dimensional mapping of the hippocampus in depression. Am. J. Psychiatry 160 (1), 83–89.

Pruessner, J.C., Li, L.M., Serles, W., Pruessner, M., Collins, D.L., Kabani, N., Lupien, S., Evans, A.C., 2000. Volumetry of hippocampus and amygdala with high-resolution MRI and three-dimensional analysis software: minimizing the discrepancies between laboratories. Cereb. Cortex 10 (4), 433–442.

Rajkowska, G., 2000. Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. Biol. Psychiatry 48 (8), 766–777 15.

Rametti, G., Segarra, N., Junqué, C., Bargalló, N., Caldú, X., Ibarretxe, N., Bernardo, M., 2007. Left posterior hippocampal density reduction using VBM and stereological MRI procedures in schizophrenia. Schizophr. Res. 96 (1–3), 62–71.

Rogatsky, I., Trowbridge, J.M., Garabedian, M.J., 1997. Glucocorticoid receptor-mediated cell cycle arrest is achieved through distinct cell-specific transcriptional regulatory mechanisms. Mol. Cell. Biol. 17 (6), 3181–3193.

Rusch, B.D., Abercrombie, H.C., Oakes, T.R., Schaefer, S.M., Davidson, R.J., 2001. Hippocampal morphometry in depressed patients and control subjects: relations to anxiety symptoms. Biol. Psychiatry 50 (12), 960–964 15.

Sapolsky, R.M., Uno, H., Rebert, C.S., Finch, C.E., 1990. Hippocampal damage associated with prolonged glucocorticoid exposure in primates. J. Neurosci. 10 (9), 2897–2902.

Saylam, C., Uçerler, H., Kitiş, O., Ozand, E., Gönül, A.S., 2006. Reduced hippocampal volume in drug-free depressed patients. Surg. Radiol. Anat. 28 (1), 82–87.

Seidman, L.J., Faraone, S.V., Goldstein, J.M., Goodman, J.M., Kremen, W.S., Toomey, R., Tourville, J., Kennedy, D., Makris, N., Caviness, V.S., Tsuang, M.T., 1999. Thalamic and amygdala-hippocampal volume reductions in first-degree relatives of patients with schizophrenia: an MRI-based morphometric analysis. Biol. Psychiatry 46 (7), 941–954 1.

Shah, P.J., Ebmeier, K.P., Glabus, M.F., Goodwin, G.M., 1998. Cortical gray matter reductions associated with treatment-resistant chronic unipolar depression. Controlled magnetic resonance imaging study. Br. J. Psychiatry 172, 527–532.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59 (Suppl. 20), 22–33 quiz 34–57.

Stonnington, C.M., Tan, G., Klöppel, S., Chu, C., Draganski, B., Jack Jr, C.R., Chen, K., Ashburner, J., Frackowiak, R.S., 2008. Interpreting scan data acquired from multiple scanners: a study with Alzheimer's disease. NeuroImage 39 (3), 1180–1185 Feb 1.

Vakili, K., Pillay, S.S., Lafer, B., Fava, M., Renshaw, P.F., Bonello-Cintron, C.M., Yurgelun-Todd, D.A., 2000. Hippocampal volume in primary unipolar major depression: a magnetic resonance imaging study. Biol. Psychiatry 47 (12), 1087–1090 15.

Van Praag, H., Kempermann, G., Gage, F.H., 1999. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat. Neurosci. 2 (3), 266–270.

Vasic, N., Walter, H., Höse, A., Wolf, R.C., 2008. Gray matter reduction associated with psychopathology and cognitive dysfunction in unipolar depression: a voxel-based morphometry study. J. Affect. Disord. 109 (1–2), 107–116.

Videbech, P., Ravnkilde, B., 2004. Hippocampal volume and depression: a meta-analysis of MRI studies. Am. J. Psychiatry 161 (11), 1957–1966.

Von Gunten, A., Fox, N.C., Cipolotti, L., Ron, M.A., 2000. A volumetric study of hippocampus and amygdala in depressed patients with subjective memory problems. J. Neuropsychiatry Clin. Neurosci. 12 (4), 493–498.

Yassa, M.A., Stark, C.E., 2009. A quantitative evaluation of cross-participant registration techniques for MRI studies of the medial temporal lobe. NeuroImage 44 (2), 319–327.