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HIPPOCAMPAL VOLUME IN DIFFERENT NEUROPSYCHIATRIC COHORTS USING A SEMIAUTOMATIC, STEREOLOGICAL AND AUTOMATIC METHOD

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ABSTRACT

Introduction: In the recent literature on the course of different neuropsychiatric disorders, volumetric changes in the hippocampus, a limbic structure with anterior-posterior anatomical and functional segmentation, have been reported. We examined hippocampal volumes in subjects with Cushing's disease (CD), in female patients with recurrent Familial Pure Depressive Disease (rFPDD) and in a population with mild Traumatic Brain Injury (mTBI), with the hypothesis that cortisol damage on the hippocampus (HC) has an anterior-posterior gradient , depending on the clinical situation and on the interaction between environment and genetic. Additionally, we decided to validate FreeSurfer, an automated software program developed at the Martinos Center for Biomedical Imaging (Harvard University, Boston, USA). This aim is in line with the current research for determining the volume of brain areas, which has been geared towards the development of automated methods of volumetric analysis that are highly reproducible, accurate and potentially more efficient than the current gold standard of the manual technique. I

Methods: We used a semi-automated software program (DCM view, Padova Ricerche, Padova, Italy) for Magnetic Resonance Imaging (MRI) to measure hippocampal volumes in ten patients with CD prior to selective surgical resection of the adrenocorticotropic hormone (ACTH)-secreting pituitary micro-adenomas using a trans-sphenoidal approach and after an interval of one year following surgery. This same program was used to measure hippocampal volumes in 15 female patients with familial recurrent Major Depression (MD) and 15 healthy female subjects. We also examined hippocampal volumes using a stereological method, using Analyze software (version 10., Biomedical Imaging Resource, Mayo Foundation, Rochester, MN, USA), in a sample of subjects with mTBI within a month and a year after head injury. In the validation study, the hippocampal volumes of a sample of elderly subjects with unipolar major depression and healthy controls were estimated using a automated method. Using FreeSurfer software, a stereological method (manual) was then used to validate it. Moreover, the accuracy and precision of the automatic delineation of all images acquired were verified. Using ANCOVA with whole-brain volume and age as covariates, volumetric measurements in the two groups with the

different disorders were compared. The agreement between the volumetric measures within and between the two methods was determined by calculating Cronbach's alpha coefficient for intra- and inter-rater reliability tests.

Results: We found a significant difference in the head of the hippocampus bilaterally 12 months after selective trans-sphenoidal surgical resection of the adrenocorticotropic hormone (ACTH)-secreting pituitary micro-adenomas. The right hippocampal body and tail volumes were significantly smaller in female patients with familial depressive disorder when compared to healthy subjects. No significant differences in hippocampal volumes were found in subjects with mTBI. The test of agreement between the volumetric methods was good, whereas the verification analysis for the quality of the tracing using FreeSurfer was not found to be favourable for segmentation of the hippocampus.

Conclusions: The pre-post surgery difference found in our CD patients could contribute to increased the understanding of the pathophysiology of CD as an in vivo model for stress-related hypercortisolemic neuropsychiatric disorders. Our data provide evidence for structural lateralised hippocampal body and tail abnormalities in women with a familial history and recurrent episodes of depression. Although global reductions in hippocampal volume have already been widely reported, data on lateralised regional reductions in samples with familial recurrent depression had not been previously reported. Reductions in right posterior hippocampal volume may be a structural endophenotype for recurrent depressive disorders in women. A bigger sample of subjects and functional neuroimaging studies are necessary to form conclusions concerning changes in the brain structures of subjects with mTBI. The validation data from this study indicate that the automated method should be improved.

RIASSUNTO

Introduzione: Variazioni volumetriche dell' ippocampo, regione cerebrale del lobo limbico che presenta una differente segmentazione anatomica e funzionale lungo il proprio asse antero-posteriore, sono state riportate dalla recente letteratura nel corso di diversi disturbi neuropsichiatrici. In questo studio sono stati misurati i volumi ippocampali di soggetti con Morbo di Cushing, di donne con disturbo depressivo ricorrente familiare e di una popolazione con trauma cranico lieve, supponendo che il danno indotto dal cortisolo nell' ippocampo possa variare lungo l' asse antero-posteriore, a seconda della condizione clinica. Ciò sottolinerebbe l'importanza dell'interazione tra ambiente e genetica in questo fenomeno. Inoltre, ci siamo proposti di convalidare FreeSurfer, un software automatico sviluppato presso il Centro Martinos for Biomedical Imaging (Harvard University, Boston, USA), per determinare il volume di differenti regioni cerebrali., in linea con l'attuale ricerca orientata verso lo sviluppo di metodi automatici che avrebbero il vantaggio di elevate riproducibilità, accuratezza, e, potenzialmente, maggiore efficienza di quelli manuali, attualmente considerati il "gold standard" per la determinazione del volume delle aree cerebrali.

Metodi: Un software semi automatico (DCM view, Padova Ricerche, Padova, Italy), su immagini di Risonanza Magnetica Nucleare cerebrale, è stato utilizzato per misurare il volume dell'ippocampo in 10 pazienti con Morbo di Cushing prima e dopo 12 mesi dalla resezione chirurgica selettiva, mediante un approccio transfenoidale, del microadenoma ipofisario ormone-adrenocorticotropo secernente, in 15 pazienti donne con depressione maggiore ricorrente familiare (MD) e 15 soggetti sani di sesso femminile. Un metodo stereologico, utilizzando come programma di riferimento l'Analyze software (versione 10, Biomedical Imaging Resource, Mayo Foundation, Rochester, Minnesota), è sato usato per la determinazione del volume ippocampale, in un campione di soggetti (13) con trauma cranico lieve, entro un mese e un anno dopo il trauma. Nello studio di validazione, su un campione di soggetti anziani con

depressione unipolare maggiore e controlli sani, è stato calcolato il volume della regione cerebrale d'interesse sia mediante un metodo stereologico (manuale), sia attraverso il metodo automatico del programma in corso di validazione (FreeSurfer). Successivamente è stato eseguito un controllo sull'accuratezza e sulla precisione della delimitazione automatica di tutte le immagini acquisite. Sono state confrontate le misurazioni volumetriche nei due gruppi delle differenti coorti utilizzando ANCOVA, con il volume cerebrale totale ed età come covariate. L'accordo tra le misure volumetriche all'interno e tra i due metodi, nello studio di validazione, è stato testato usando Cronbach's alpha mediante l'analisi dell'intra-rater e della inter-rater reliability.
Risultati: Abbiamo trovato una differenza significativa nella testa dell'ippocampo bilateralmente nei soggetti con Morbo di Cushing dopo 12 mesi dalla resezione chirurgica selettiva, mediante approccio trans-sfenoidale, del microadenoma ipofisario ormone adrenocorticotropo secernente. I volumi del corpo e della coda dell' ippocampo erano significativamente minori nei pazienti di sesso femminile con disturbo depressivo familiare, rispetto ai soggetti sani. Nessuna differenza significativa nei volumi hippocampali sono stati trovati nei soggetti con trauma cranico lieve. In conclusione il metodo automatico dimostra un buon accordo con il metodo stereologico, mentre il controllo sulla qualità della delimitazione automatica ha rilevato una non precisa segmentazione della regione cerebrale d' interesse.

Discussione e Conclusioni: Il dato di una differenza del volume della testa dell' ippocampo pre-post intervento chirurgico potrebbe contribuire a comprendere meglio la fisiopatologia del Morbo di Cushing come modello in vivo per i disturbi neuropsichiatrici legati allo stress. Il dato di una riduzione del volume del corpo e della coda ippocampale in donne con storia familiare ed episodi ricorrenti di depressione. forniscono la prova di un danno strutturale lateralizzato dell'ippocampo. Sebbene la riduzione globale delle ippocampo sia già stata ampiamente riportata, i dati sulle riduzioni regionali lateralizzate nella depressione ricorrente e familiare non erano stati precedentemente segnalati. Una riduzione posteriore del volume ippocampale di destra potrebbe essere un endofenotipo strutturale per disturbi depressivi ricorrenti nelle donne. Un campione più elevato di soggetti e studi di neuroimaging funzionale

sarebbero necessari per fare considerazioni sul cambiamento delle strutture cerebrali in soggetti con trauma cranico lieve. I risultati, circa la validazione, indicano che il metodo automatizzato dovrà essere ulteriormente migliorato.

INTRODUCTION

The hippocampus (HC) is a core region in the limbic system that has several connections to cortical areas, such as the prefrontal cortex, anterior thalamic nuclei, amygdala, basal ganglia and hypothalamus (Rosene and Van Hoesen, 1987). It is known to have multiple regulatory functions on mood, the hypothalamic-pituitary-adrenal (HPA) axis, learning and memory (McEwen, 2001). In recent years, the HC has been indicated by many groups to be the principal site for constitutive neurogenesis in the mammalian brain (Gould et al., 1998; Eriksson et al., 1998; Kempermann and Gage, 1999). Many preclinical findings have stressed that chronically elevated levels of glucocorticoids (GCs) could cause neuronal damage within the HC via the stimulation of glucocorticoid receptors in this area (McEwen, 2000). More specifically, studies in experimental animals have demonstrated that an interaction between GCs and glutamate is involved in hippocampal atrophy (Sheline, 2000) via a decrease in neuropil volume, a loss of cells (glia and neurons) and the inhibition of neurogenesis (Duvernoy 1988). In contrast, a body of research has established that the toxic effects of GCs can be suppressed and that HC atrophy may be reversed by increasing neurogenesis (Schmidt and Duman, 2007). These findings all suggest that the HC has a certain degree of plasticity (Jorge et al., 2007).

Several volumetric magnetic resonance imaging (vMRI) studies have investigated this brain structure and its involvement in the pathogenesis of altered mood and its function in many stress-related neuropsychiatric disorders, including major depression disorder (MDD) (Koolschijn PC et al., 2009; Lorenzetti et al., 2009; McKinnon et al., 2009), Cushing's disease (CD) (Starkman et al., 1999), and traumatic brain injury (TBI) (Jorge et al., 2007).

With respect to Cushing's disease, one study found significant bilateral reductions in HC volumes in 27% of patients with Cushing's syndrome as compared to a healthy group (Starkman et al., 1992). Interestingly, this

study found a positive association between reduced hippocampal volume, memory function and elevated cortisol levels. Another study found increased volumes in both the left and right HCs after pituitary microadenomectomy (Starkman et al., 1999). This increase in HC volumes was correlated with a decrease in 24-hour urinary free cortisol (UFC). In a subsequent study, these authors also showed that structural volumetric increases in HC were accompanied by functional improvements in the learning of unrelated words (Starkman et al., 2003). Similar losses of brain volumes among patients with endogenous Cushing's syndrome, as compared to normal controls, have been reported when using an indirect assessment method based on measurements of the diameter of the bicaudate and the third ventricle (Bourdeau et al., 2002).

With regard to MDD, the volumetric MRI data are not homogeneous. Indeed, the literature has reported reductions, enlargements, and even a lack of difference in hippocampal volumes between patients and controls (Lorenzetti 2009 et al). The inconsistencies between HC volumetric studies may be due to differences in the patient groups with respect to clinical and demographic variables, such as sex, current mood state, lifetime burden of illness, family history of illness, and medication side effects. For example, a recent meta-analysis examining clinical predictors in 2,000 patients with MDD found that hippocampal volumes were significantly reduced. However, this occurred only in patients with a duration of illness longer than two years or in those with recurrent depression; this was true both in children and in middle-aged adults (> 34-65) or older adults (McKinnon et al., 2009). Furthermore, it has been presumed that the heterogeneity in hippocampal volume data can be ascribed to MRI methods. MRI results can be influenced by factors including scan acquisition parameters, image quality, computer hardware employed in the tracing procedure and type of segmentation technique used (manual or automatic). Automatic segmentation techniques have generated intense interest given the wide range of clinical applications for MRI volumetry in the last years. Currently, the "gold standard" for determining the volumes of specific brain areas is the manual method; however, this method has the disadvantage of taking a significant amount of time to process and of being operator-

dependent. The current research is, therefore, geared toward the development of automated methods that would be highly reproducible, accurate and potentially more efficient than the current manual methods.

Among patients with TBI, the hippocampus is one of the most vulnerable structures (Bigler et al 1997, 2002; Tate et al. 2000; Arciniegas et al., 2001; Tomaiuolo et al., 2004; Himanen et al., 2005; Ariza et al., 2006; Jorge et al., 2006; Wilde et al., 2007; Ng et al. 2008, Levine et al. 2008). Hippocampal volume in patients with mild head injury has been found to be reduced compared to uninjured control groups and is higher than in subjects with more severe head injury, as measured by the initial Glasgow Coma Scale (GCS) (Bigler et al 1997, 2002; Tate et al. 2000; Himanen et al. 2005; Jorge et al. 2006; Levine et al. 2008). For several reasons, however vMRI studies have not yielded clear information concerning the structural changes that occur in subjects with TBI. First, there is substantial heterogeneity within the samples with respect to the severity of head injury. Second, the time between the injury and the acquisition of the scan varies widely, ranging from 1.2 months to 31.5 years. Such lengthy intervals can be a serious limitation for the study of a structure such as the hippocampus, which is sensitive to many factors, including stress and aging. Third, the retrospective nature of the studies, which involves enrolling subjects after some time after the trauma, especially in mild to moderate trauma, could lead to the observation especially patients who continue to experience problems as a result of head injury with a significant bias. Fourth, there are few vMRI studies that aim to assess changes over time to brain structures in relation to an acute event using a longitudinal design. There are two studies focusing on the brain parenchyma (McKenzie et al., 2002; Trivedi et al., 2007) and one focusing on the hippocampus (Ng et al., 2008). Mackenzie et al (2002) and Trivedi et al. (2007) have observed that the rate of decline in brain parenchyma between the first and second time points (range of 4 and 16 months post-injury) were significantly greater in patients with TBI (11 with mild TBI and 3 with moderate TBI in the first study and 11 with mild, 10 with moderate and 16 with severe TBI in the second study) when compared with healthy control subjects. In subjects with moderate to severe TBI, Ng et al. (2008) found a significant decrease in right and

left hippocampal volumes from 4.5 months to 2.5 years post-injury and a significant percent annual volume change for hippocampi when compared with published normative data.

On the basis of the above findings, the goal of our study was to investigate hippocampal volume in homogeneous samples of subjects with different neuropsychiatric disorders using a semiautomatic and stereological method and to validate FreeSurfer, an automated software program developed at the Martinos Center for Biomedical Imaging (Harvard University, Boston, USA), on a sample of elderly patients with unipolar major depression (eMDD) and healthy controls. More specifically, we sought to examine HC volume and its subregions (hippocampus head (HH), hippocampus body (HB) and hippocampus tail (HT)) in patients with CD 12 months after pituitary micro-adenoma trans-sphenoidal surgical selective resections; those with recurrent familial depressive disorder (rFPDD) and healthy subjects; and those with mild TBI, who were examined within a month of injury and again a year after head injury. A morphometric study of hippocampal subregions was recently conducted; neuroimaging and histological studies have observed that HC is a structure, along its anterior-posterior axis, heterogeneous for neuroanatomical connections, functions (Strange and Dolan, 1999), cytoarchitecture (Barbas and Blatt, 1995) and metabolite concentrations (King et al., 2008). More specifically, the posterior HC, which comprises the body and the tail, is connected to sensory cortical areas, including the parietal cortex (Moser and Moser, 1998). It plays a role in spatial learning and memory (Hampel et al., 2008) and shows a higher density of pyramidal cells, a higher concentration of metabolites, and a smaller density of granular cells (King et al., 2008). In contrast, the anterior HC or head has connections with prefrontal regions, regulates the hypothalamo-pituitary-adrenocortical axis (HPA) via negative feedback (Szesko et al., 2008) and may be associated with explicit memory (Strange et al., 2005). Therefore, the HC should not be considered a single entity; instead, all three of its anatomical segments should be studied: hippocampus head (HH), body (HB) and tail (HT) (Fanselow and Dong, 2010). We believe that cortisol-induced damage of the HC can vary in its anterior-posterior localisation depending on the

clinical situation, thus highlighting the importance of gene-environment interactions in this phenomenon.

MATERIALS AND METHODS

Study Population

CD

Eight women and two men with CD (see for criteria Albiger et al, 2006) were recruited in the Neurosurgery Unit at the University-Hospital of Padova at the time of admission for treatment. Their mean age was 38.2 years (S.D. = ± 13.1). The mean estimated duration of illness and school education was 3.5 (S.D. = ± 1.1) and 12.4 years (S.D. = ± 3.6), respectively, based on an assessment of the patient's history. All subjects were without a current or previous psychiatric illness, as determined by the structured Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheean et al., 1998). Exclusion criteria included other acute or chronic medical conditions, neurological diseases, head trauma, history of alcohol or other substance abuse and contraindications to MRI scanning. Written informed consent was obtained from all participants after a complete description of the study. All patients received an MRI shortly before the trans-sphenoidal surgical selective resection of the adrenocorticotropic hormone (ACTH)-secreting pituitary micro-adenomas and again one year after surgery to determine the volumetric measurements of the brain regions of interest (ROIs).

rFPDD

Fifteen female outpatients with unipolar recurrent depression were recruited for this study. A structured Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998) was administered to all patients. All patients met DSM-IV diagnostic criteria for current major depressive episode with a minimum score of 15 points on the 17-item Hamilton Depression Rating Scale (HDRS) and for recurrent major depression. The clinical interview revealed that all patients had at least one first-degree relative (parent, sibling or offspring) with primary depression but no family history of alcoholism, antisocial personality disorder, or mania. Thus, all patients met the criteria for FPDD as

described in the work of Winokur (Winokur, 1982). Exclusion criteria included additional Axis I co-morbid psychiatric disorders, any current clinically relevant medical condition, a history or evidence of stroke or transient ischaemic attack and alcohol/substance use, and age above 55. Late-onset depression was excluded since it presents with clinical, cognitive, biological and neuroimaging characteristics that could have affected our sample homogeneity (Ballmaier et al., 2008).

Patients' symptoms were assessed with the 17-item HDRS (Hamilton, 1960), Beck Depression Inventory (BDI) (Beck et al., 1961), and Clinical Global Impression scale-severity (CGI-S) (Guy, 1976) on the day that patients received MRI. These data were used to analyse the relationships between volumetric data and concurrent symptom state and severity. Age at onset of illness, length of illness (expressed in months) and number of previous affective episodes were recorded during the clinical assessment.

The control group consisted of fifteen healthy female controls who did not differ from the patients with respect to age ($t= 1.63$, $df= 28$, $p= 0.11$) or years of education (Mann-Whitney $Z= -0.54$, $p= 0.59$). The M.I.N.I. was also administered in the control group to rule out the presence of current or past psychiatric conditions. Further exclusion criteria for healthy controls were first-degree relatives with a psychiatric disorder, any relevant medical illnesses and a history of alcohol/substance abuse. All participants were right handed and had given written informed consent. The demographic and clinical characteristics of the study sample are described in Table 1.

Table 1. Characteristics of the study population

	Depressed patients	**Control subjects**
Number of subjects	15	15
Age (years)	43.9 (±11)	37.8 (±11)
Education (years)	14.6 (±3)	15.3 (±3)
HDRS (points)	21.8 (±5)	
BDI (points)	31.1 (±8)	
CGI-S (points)	5.3 (±1)	
Age at onset of illness (years)	30.9 (±11)	
Length of illness (months)	164 (±110)	
Number of depressive episodes	4.2 (±1)	

Continuous variables are displayed as mean values ± SD.
BDI: Beck Depression Inventory, HDRS: Hamilton Depression Rating Scale, CGI-S: Clinical Global Impression scale-severity.

TBI

The participants were recruited in the Emergence Unit at the University Hospital of Padova after medical and neurological interviews and evaluations had been conducted. The evaluations were performed by a board-certified internist (G.V.) and a neurosurgeon (M.S.), respectively. To be eligible, subjects had to be between 18 and 50 years of age, lack neuroradiological findings on a standard CT scan completed within 24 hours of head trauma and meet ACRM criteria for mild TBI (Kay et al., 1993), which is defined as a GCS score between 13 and 15 and any period of loss of consciousness for 30 minutes or less, any loss of memory of events immediately before or after the accident for less than 24 hours, any alteration in mental state at the time of the accident (e.g.,

feeling dazed, disoriented or confused), or focal neurological deficits that may or may not be transient. Exclusion criteria included the following: previous head injuries, current or previous DSM IV axis I psychiatric disorders, medical and pregnancy conditions, neurological disorders, history of alcohol or other substance abuse and any MRI contraindications. All eligible subjects were asked to return for psychiatric assessment, neuropsychological testing and blood sampling in the Psychiatric Clinic and MRI scans in the Neuroradiology Unit of the Department of Neuroscience at the University Hospital of Padova within one month of injury and approximately one year post-injury. The study was approved by the ethics committee of the University Hospital of Padova, and all participants gave written informed consent to participate after receiving a complete description of the study.

Psychiatric assessment was performed by obtaining psychiatric and medical history, performing a mental status examination and conducting a structured Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998). The 17-item Hamilton Rating Scale for Depression (HAM-D) (Halmiton, 1960), the Beck Depression Inventory (BDI) (Beck et al., 1961), the State Trait Anxiety Inventory (STAY X-Y) (Spielberger et al., 1997), the 14-item Hamilton Rating Scale for Anxiety (HAM-A) (Hamilton, 1959) and the Clinical Global Impression Scale-Severity (CGI-S) (Guy, 1966) were also used to assess the severity of depression and anxiety symptoms as well as the presence of psychiatric conditions at the two assessment times. In each subject, handedness, years of education, day of head injury, injury severity data (GCS, LOC and PTA) and cause of injury were obtained via retrospective review of acute medical chart care. Tables 2-3.

Tab. 2 Characteristic demographic, injury severity and cause of traumatic brain injury of the subject with head injury

Characteristic	**TBI group**	
Number	13	
Age (years)	25.5±5.84	
Gender (F: M)	3:10	
Handedness (R: L)	13:0	
Education (years)	14.46 ± 2.88	
Injury severity	n°	%
GCS (13-15)	13	100
LOC (< 30 min)	13	100
PTA (< 24 h)	6	46.6
Type of TBI		
Bicycle crash	2	15,38
Motorcycle crash	5	38,,46
Pedestrian car collision	3	23,08
Fall	1	7,69
Sportive	2	15,38

PTA: posttraumatic amnesia; CGS: Glasgow Clinical Score; LOC: loss of consciousness

Tab. 3 Psychometric measures (mean ± SD) at baseline and at follow up in the traumatic brain injury group.

Measures	**Baseline**	**Follow up**
HAM-D	1.92 ± 2.81	1.38 ± 2.33
BDI	3.31 ± 3.30	3.46 ± 4.20
HAM-A	4.15 ± 6.40	1.85 ± 2.30
STAY-X	32.00 ± 7.78	32.77 ± 10.69
STAY-Y	30.62 ± 5.72	32.54 ± 8.81

HAM-D: 17-item Hamilton Rating Scale for Depression; BDI: Beck Depression Inventory; HAM-A: 14-item Hamilton Rating Scale for Anxiety; STAY X and Y: State-Trait Anxiety Inventory; CGI-S: Clinical Global Impression-Severity Scale

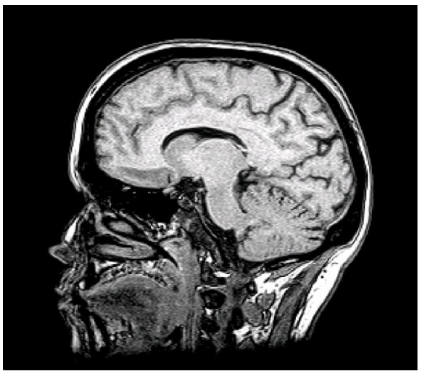
eMDD and controls

We recruited 11 patients over the age of 65 with late-onset unipolar depression, which was defined as disease onset after 60 years of age, a Geriatric Depression Scale (GDS) (Yesavage et al., 1983**)** rating greater than or equal to 15, and a lack of cognitive decline as assessed by an MMSE score >24. We also recruited 12 control subjects over 65 years of age with a GDS of less than 15, no signs of cognitive decline (MMSE ≥ 24) and without a history of psychiatric illness.

The exclusion criteria included the following: the presence of concomitant psychiatric illness, a history of alcohol or substance abuse within the last year, corticosteroid therapy within the last year, other concomitant neurological diseases, including dementia and stroke, contraindication to MRI, and presence of severe or unstable internal pathologies. Patients and control subjects were either enrolled in a hospital or were outpatients. We assessed patients for a family history of psychiatric disorders and collected a medical history to determine the presence of cardiovascular risk factors (i.e., hypertension, diabetes mellitus, hypercholesterolemia, cardiac disease, smoking).

Magnetic resonance imaging study

Image acquisition



Subjects with CD, rFPDD and healthy subjects, as well as those with mild TBI, were examined using a 1.0 T MRI system (Marconi Medical Systems). Whole-brain T1-weighted three-dimensional images were acquired in the sagittal plane for volumetric measurements (time to repetition = 25 ms, time to echo = 4 ms, flip angle = 25°, field of view = 100 mm, slice thickness = 1.4 mm, matrix size = 256 × 192). A random sample of 10 subjects (from a pool of 11 elderly patients with unipolar major depression and 12 healthy controls) underwent MRI scans using a 1.5-Tesla scanner. A sequence sagittal T1-weighted magnetisation-prepared rapidly acquired gradient echo (MPRAGE) was acquired for volumetric measurements (TR = 10 msec, TE = 4 msec, TI = 300 msec, matrix size = 256 x 256, voxel resolution = 1 x 1 x 1.25 mm, slice thickness = 1.25 mm and flip angle = 8°). The sagittal images were aligned approximately parallel to the anterior-posterior commissure line. Axial proton density and T2-weighted images were obtained to enable the exclusion of structural abnormalities on the MRI scan. A board-certified neuroradiologist reviewed all scans. The procedure was well tolerated by all subjects, and no sedation was used. The imaging data were transferred from the MRI unit to a PC workstation and analysed using the different software programs.

Manual delineations of structures

Volumetric measurements of patients with CD and recurrent depression were performed on an Acer PC workstation using DCM View software

(Padova Ricerche, Italy) in which ROIs were manually outlined on consecutive coronal slices in an anterior-posterior direction and verified using axial and sagittal orientations by one rater (T.T.). Calculations of the volumes were computed automatically, excluding voxels with unwanted density (e.g., the fimbria white matter and sporadic fluid in the hippocampus complex). Hippocampal and whole-brain volumes in subjects with TBI and the random sample of subjects used to validate Freesurfer were based on stereologic estimation methods, which have been used with precision in microscopy and MR volumetry. Sampling parameters and grid size were set to yield at least 150 "hits" per measurement, a number that has previously been determined to yield reliable measurements in brain volume determination (Gundersen, 1988). From three-dimensional MRI images composed of 1.0 × 1.0× 1.0 mm voxels, coronal slices were sampled every 1.0 mm for the hippocampus and every 20 mm for the whole brain. A 3 x 3 mm_2 rigid grid of points for the hippocampus and a 20 x 20 mm_2 grid for the whole brain with a random starting position and angle of deviation the from horizontal was then superimposed on the images. Measurements were made in the coronal orientation, but all three orthogonal views were simultaneously displayed on the screen. Analysis software (version 10.0, Biomedical Imaging Resource, Mayo Foundation, Rochester, MN, USA) was used to make the measurements and to calculate the volume of brain regions. The rater (T.T.) was blind to subject identity and measured whole-brain volumes separately from left and right hippocampal volumes.

Hippocampi were traced, referencing an anatomical atlas (Durvenoy et al., 1998) and a segmentation protocol for the HC (Pruessner et al., 2000). To trace the Hippocampal Tail (HT), we considered the most anterior slice of the HT to be the first slice where the aqueduct of Sylvius was clearly seen in full profile. The right and left HT transitions did not always appear on the same slice as a result of minor differences in head position during the acquisition or due to anatomical differences. The most posterior sections of the HT were considered to be the slice at which the grey matter of the hippocampus at the level of the lateral ventricle was no longer visible. The hippocampal body (HB) was easier to outline. The first slice after the one in which the cerebral peduncles were clearly recognisable represented the front margin of the body,

while the last slice, which precedes the slice in which the aqueduct of Sylvius is well visible, represented the posterior margin of the body. The most posterior slice of the Hippocampal Head (HH) was the first slice in which the cerebral peduncles were clearly visible. Rostrally, the first slice we used was the one in which the hippocampus began to show a characteristic triangular shape from the overlying amygdala and in which the body of the caudate and the third ventricle became visible. (Figures 1 and 2) Additionally, we calculated the whole-brain volume (WBV), including both grey and white matter above the superior border of the pons and excluding the cerebellum and cerebral spinal fluid

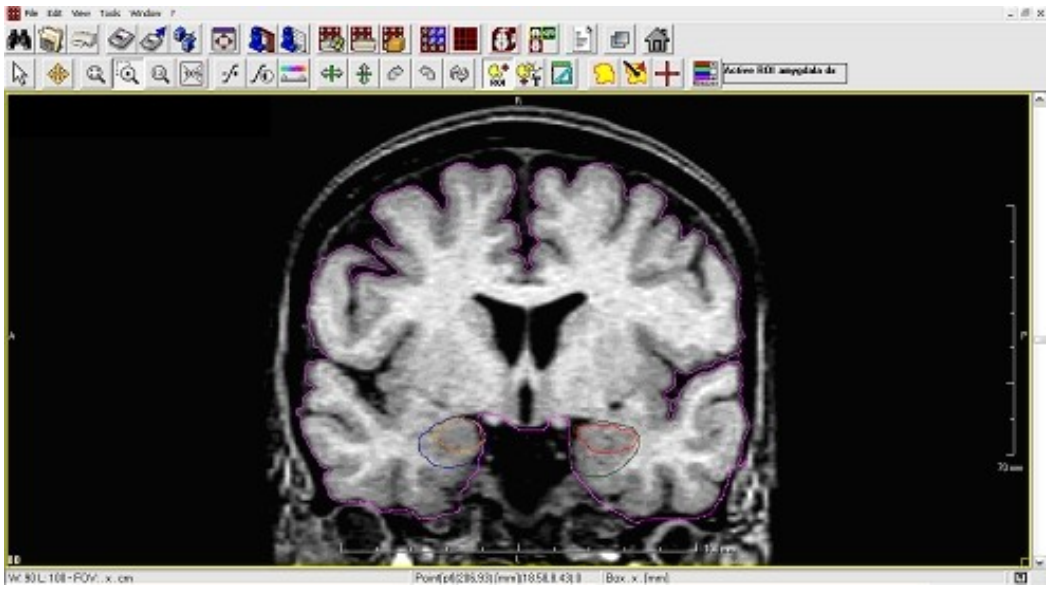


Figura 1 : Manual delineation of hippocampus and amygdala

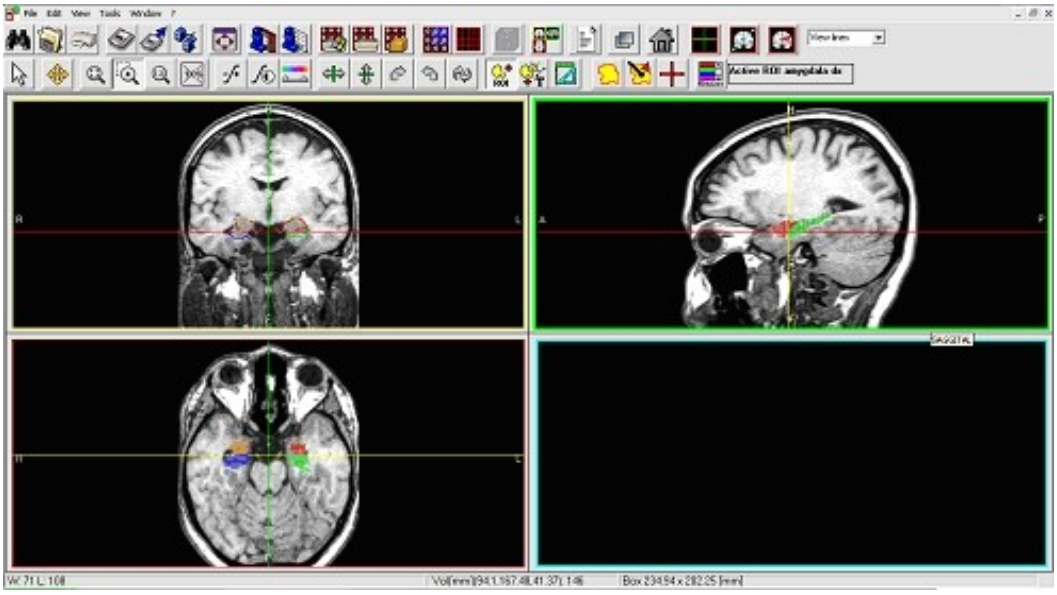


Figura 2: Hippocampus and amigdala in the three axis

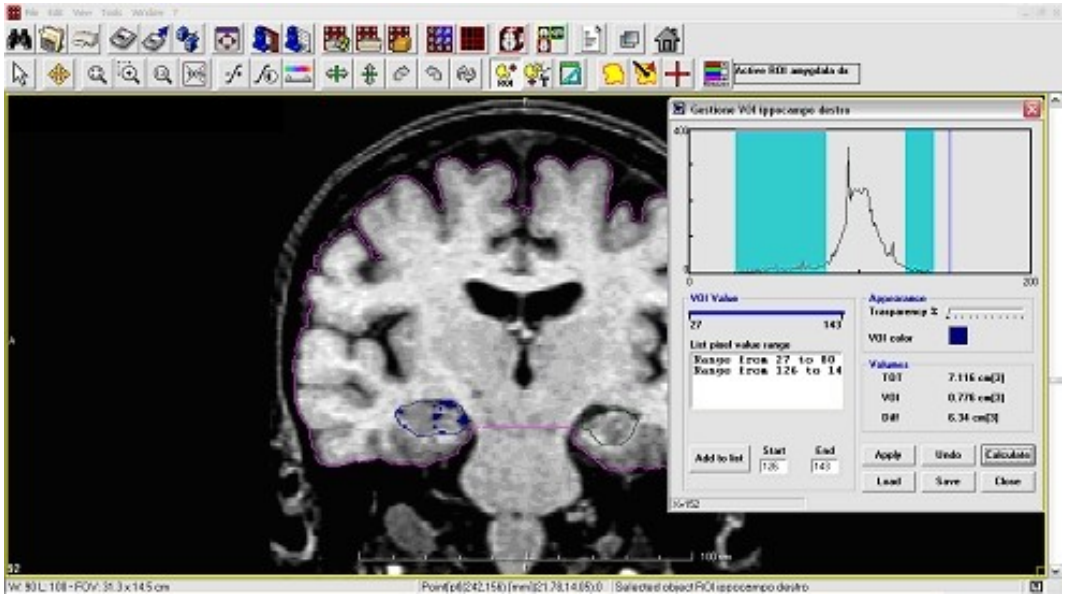


Figure 3: Calculation of the hippocampal volume

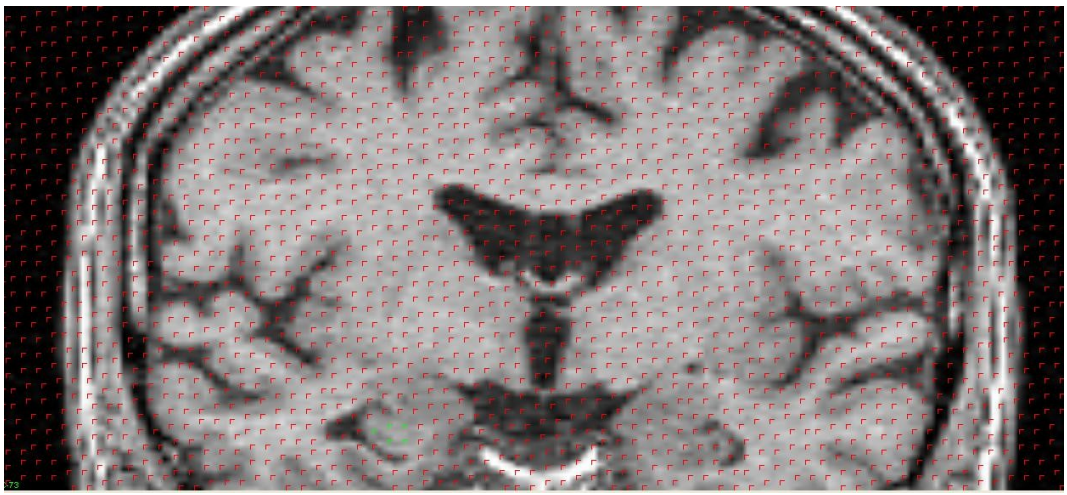


Figure 4: Anterior limit of hippocampus

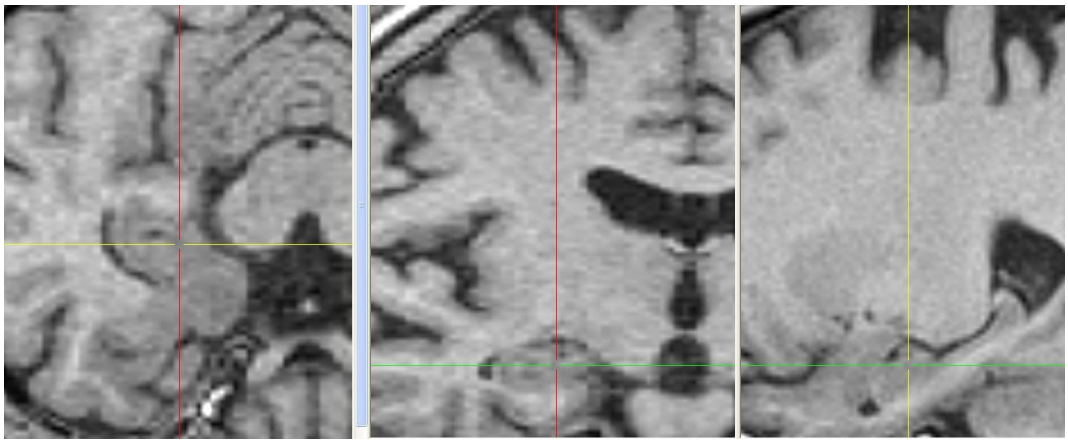


Figure 5: Hippocampus head in the three axis

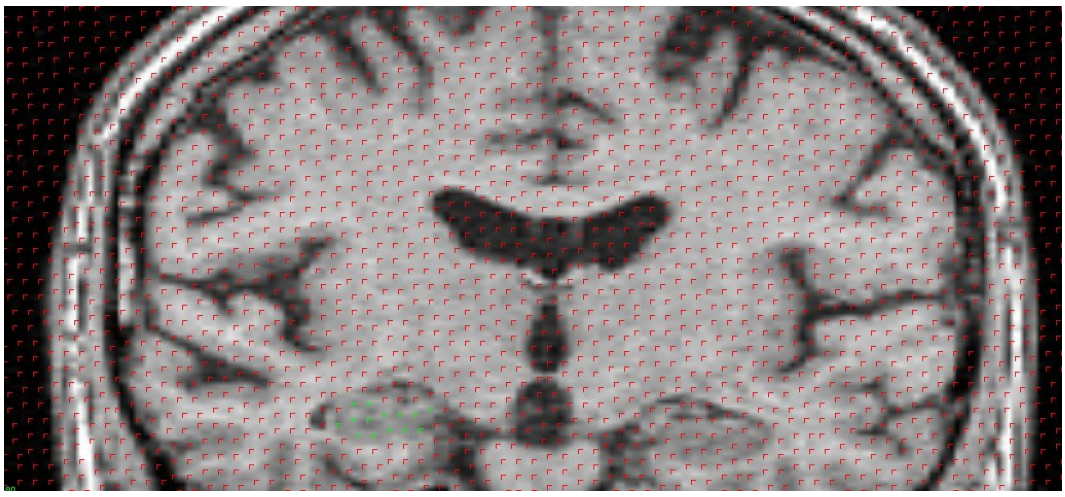


Figure 6 Posterior limit of hippocampus head

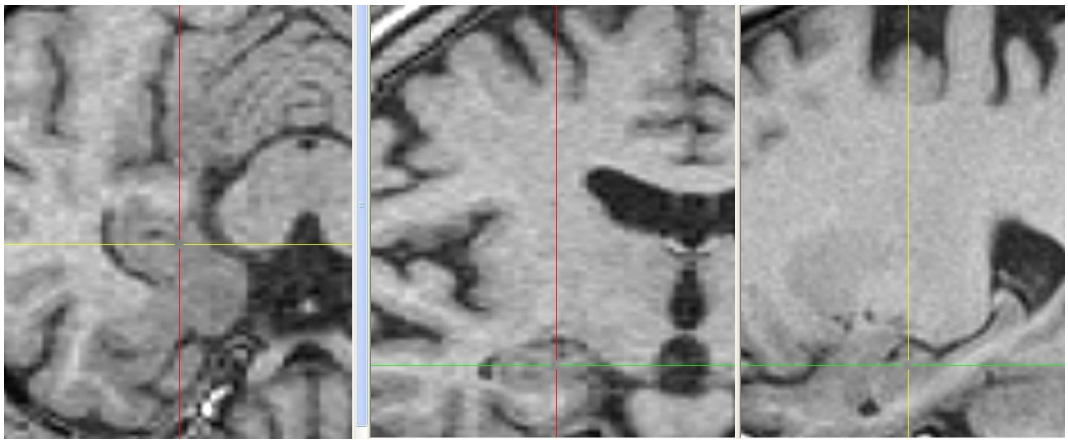


Figure 7 Posterior limit of hippocampus head

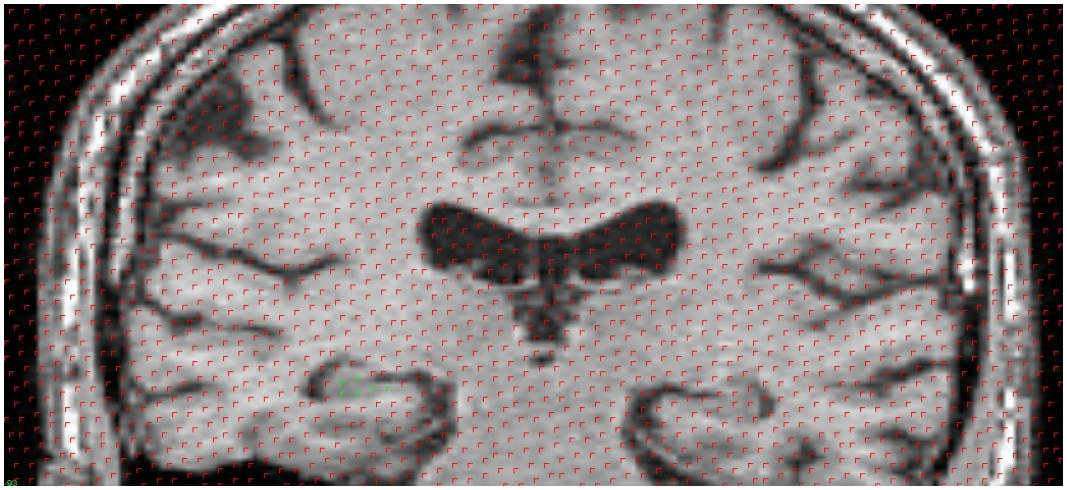


Figure 8: Posterior limit of bhipocampus body

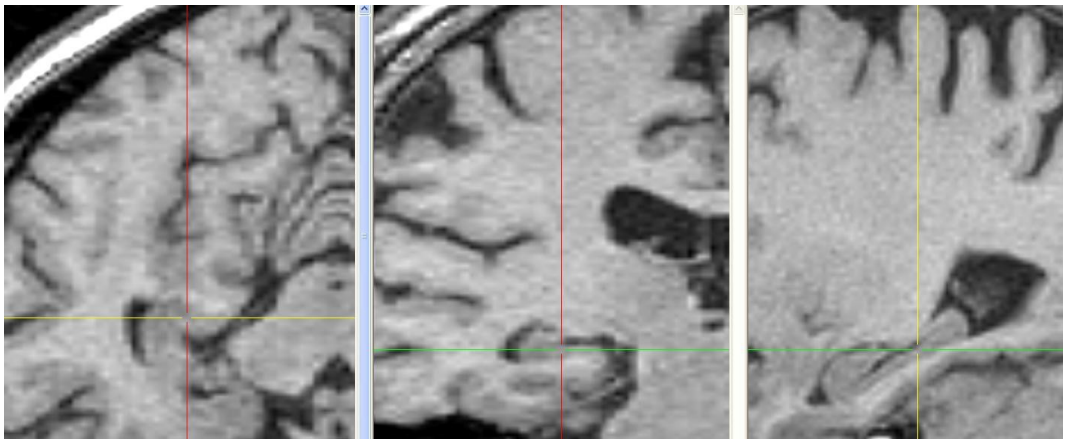


Figure 9: Hippocampus body in the three axis

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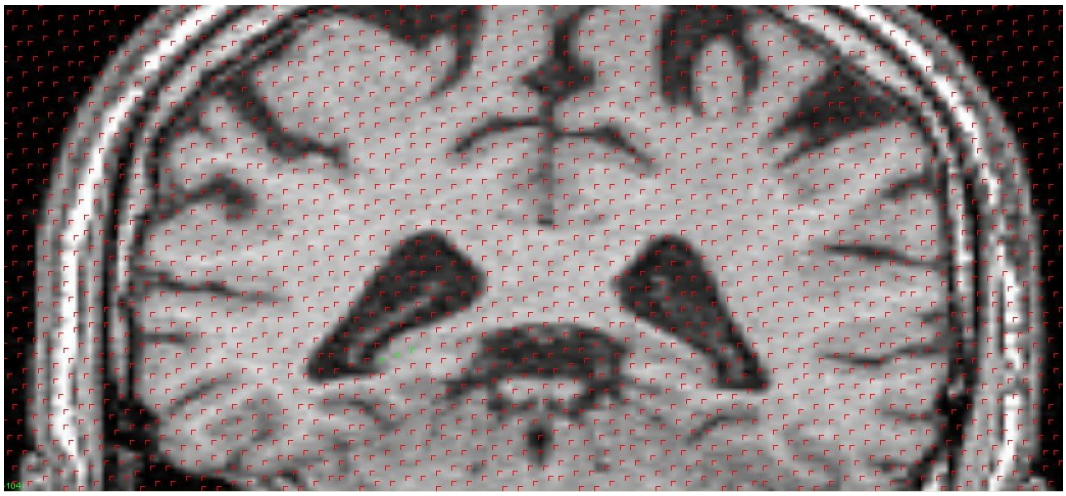

Figure10: Posterior limit of hippocampus tail

Automated volumetry

Volume measurements were made using the freely available software FreeSurfer, which was developed at the Martinos Center for Biomedical Imaging (Harvard University, Boston, USA). The detailed procedure for volumetric measurements of different brain structures has been previously described in detail by FreeSurfer's creators (Fischl et al., 2002; FreeSurfer Wiki). The visual inspection of segmentation was performed using the Analyse software.

Statistical methods

All statistical tests were performed using SPSS version 16.0 for Windows (SPSS, Illinois, Chicago, USA). Group differences between the demographic variables of age and educational level were analysed using a t-test for independent samples and the Mann-Whitney U-test, respectively. Descriptive statistics were used to assess the psychiatric clinical characteristics of all participants in the study. The normal distribution of morphometric data was tested using the Kolmogorov-Smirnov test. Analysis of covariance (ANCOVA) was used to determine differences in volume. Using age and whole-brain volume as covariates, we compared the volumes of the brain structures of interest between the groups. The agreement between the volumetric measures within and between the two methods (stereological and automatic) was determined by calculating Cronbach's alpha coefficient for intra- and inter-rater reliability tests. The level of significance was established at $p<0.05$.

RESULTS

Quantitative evaluation in CD

A significant increase in HH volume was found (Right HH: F (1.14)= 5.83, p=0.03; Left HH: F (1.14)= 4.87, p=0.04) after trans-sphenoidal surgery for the microadenectomy, as compared to the volume prior to surgery. No significant differences were observed for other hippocampal volumes and WBV (p>0.05). (Table 4)

Table 4. Hippocampal and Whole Brain Volumes data of Patients with Cushing's Disease compared after 1 year

cm^3	Patients with Cushing's Disease		ANCOVA	
	Pre-Treatment	Post-Treatment	F	P
Right Hippocampus Volume,	3.56 ± 0.91	3.85 ± 0.48	0.80ª	0.39ª
Hippocampus Head (HH)	0.77 ± 0.36	1.27 ± 0.53	5.83ª	0.03ª
Hippocampus Body (HB)	1.72 ± 0.25	1.78 ± 0.32	0.43ª	0.52ª
Hippocampus Tail (HT)	0.90 ± 0.37	0.87 ± 0.46	0.39ª	0.54ª
Left Hippocampus Volume	3.63 ± 0.90	4.22 ± 0.89	2.31ª	0.15ª
Hippocampus Head (HH)	0.71 ± 0.39	1.26 ± 0.59	4.87ª	0.04ª
Hippocampus Body (HB)	1.82 ± 0.35	1.84 ± 0.32	0.02ª	0.90ª
Hippocampus Tail (HT)	1.10 ± 0.47	1.00 ± 0.36	0.48ª	0.50ª
Whole Brain Volume (WBV),	932.93±88.30	935.43±91.20	0.01º	0.96º

Volumes are given as mean ± SD; level of significance p<0.05;
ª age and WBV as covariates; d.f. = 1.14;
º age as covariates; d.f. = 1.15.

Morphometric data in depressed patients

Whole-Brain Volume (WBV) was similar in patients and controls, and depressed patients displayed only a trend towards significance for right hippocampal volume; there was no significant difference for left hippocampal volume.

The segmented hippocampal body (HB) and tail (HT) revealed marked differences between patients and control subjects for HB ($p \leq 0.03$) and HT ($p \leq 0.005$) only on the right side. Factorial analysis was performed to determine the number of independent brain structures to correct for multiple comparisons. Five factors were found (80% of variance explained), yielding a corrected $p < 0.01$ (0.05/5). (Table 5)

Table 5 Volume of the hippocampus in depressed patients and controls compared using an analysis of covariance (ANCOVA) with age and whole brain volume as covariates

Volumes	Depressed patients (n=15)	Control subjects (n=15)	*F* value	*P* Value
Whole brain	913.3 (±73.9)	941 (±70.1)	1.10	0.30°
Right hippocampus	4.03 (±0.5)	4.42 (±0.6)	2.80	0.11
Right hippocampus head	1.61 (±0.3)	1.36 (±0.6)	3.9	0.06
Right hippocampus body	1.63 (±0.4)	1.93(±0.2)	5.45	0.03
Right hippocampus tail	0.81 (±0.2)	1.14 (±0.3)	9.63	0.005
Left hippocampus	4.27 (±0.6)	4.45(±0.7)	0.16	0.70
Left hippocampus head	1.58 (±0.4)	1.36 (±0.6)	1.99	0.17
Left hippocampus body	1.73 (±0.4)	1.88 (±0.2)	1.49	0.23
Left hippocampus tail	0.98 (±0.3)	1.20 (±0.3)	3.38	0.07

Tissue volumes are displayed as mean values ± SD in cubic centimetres (cm3).
° Analysis of Covariance (ANCOVA) with age as covariate.

Morphometric data in TBI

There were no significant changes in hippocampal volumes and WBVs at one year post-traumatic brain injury when compared to the volumes at the time of injury. (table 6).

Table 6. volumetric data in mm^3(mean ± SD) comparison at two time.

Volumes	Baseline	Follow Up	*F* value	*P* Value
Whole brain (MM)	1121030,42±122227	1139218,67±108541	0,17	O,69°
Right hippocampus	2636,67±219	2513,5±300	0,01	0,9 ª
Right hippocampus head	758,75±207	668,00±218	0,07	0,8 ª
Right hippocampus body	1103,67±207	1062±202	0,36	0,56 ª
Right hippocampus tail	774,25±127	786,83±151	0,30	0,59 ª
Left hippocampus	2409,42±292	2419±236	1,77	0,21 ª
Left hippocampus head	558,17±181	616,67±231	0,13	0,72 ª
Left hippocampus body	1073,58±230	1038,67±237	3,19	0,11 ª
Left hippocampus tail	777,58±163	763,67±163	0,6	0,46 ª

ª age and WBV as covariates; d.f. = 1.8
º age as covariates; d.f. = 1.10.

Automated method relative to the stereological method

With regard to intra-rater reliability, the intraclass correlation coefficients (ICCs) were all excellent (stereological method: right hippocampus = 0.996, left hippocampus = 0.996, automated method: 1.000 for all brain ROIs). For the test of agreement between the volumetric methods, the ICCs were as follows: right hippocampus = 0.840 and left hippocampus = 0.879. Verification analysis of the quality of the tracing by FreeSurfer found favourable segmentation of the left and right amygdala, whereas the hippocampus and caudate nucleus were located outside of their natural boundaries (i.e., the drawing of the hippocampus appeared on the superior and lateral boundaries of the amygdala).(Fig 11)

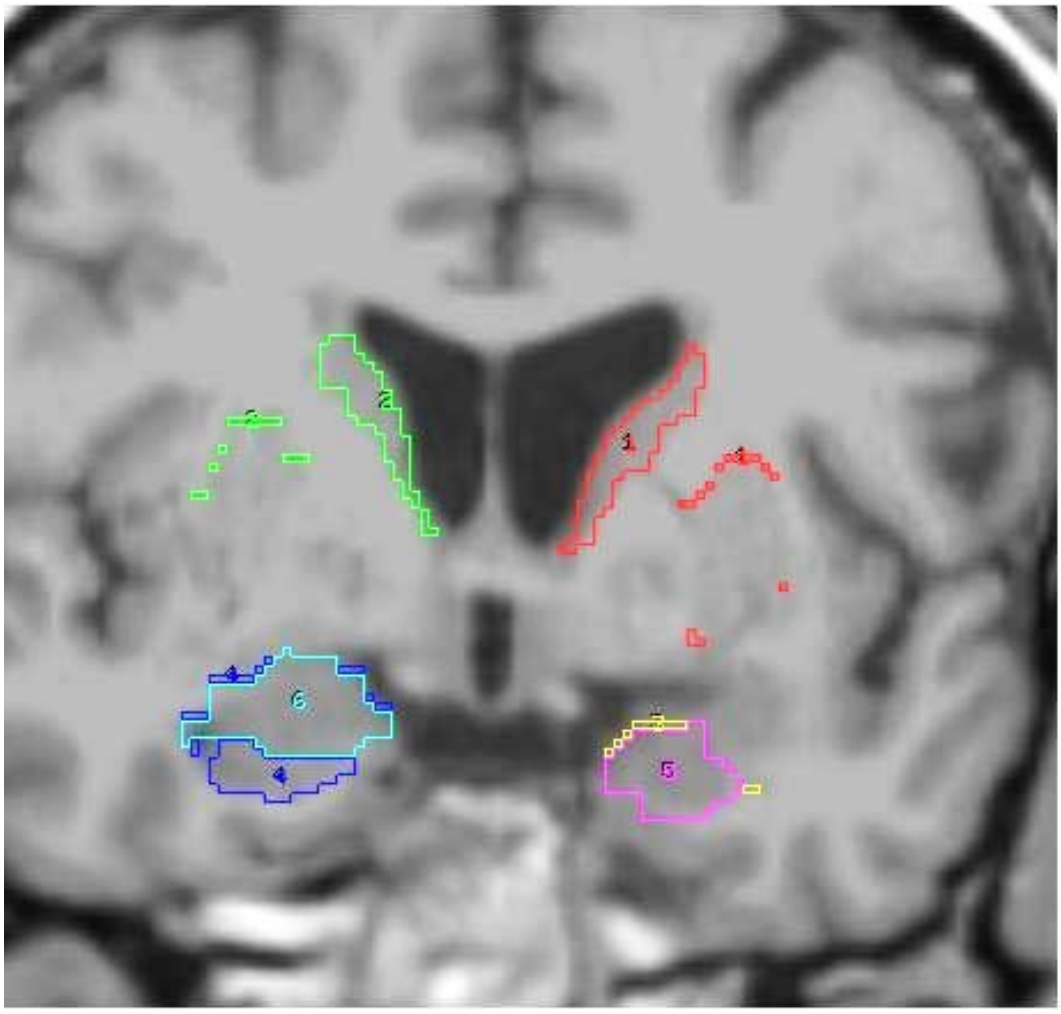

Figure11: Delimitation of the ROIs with automatic method

DISCUSSION

This study investigated hippocampal volume changes that occur in three patients' sample: CD patients following the selective surgical resection of adrenocorticotropic hormone (ACTH)-secreting pituitary micro-adenomas using a trans-sphenoidal approach, subjects with rFPDD versus healthy controls and patients a year after closed-head mild TBI using different methods. Furthermore, we used a stereological (manual) method (using Analyze software) to validate an automated method using FreeSurfer software. There were four major findings of this study.First, we found a significant increase in right and left HH volumes in patients with CD after trans-sphenoidal surgery. Our data are consistent with previous preclinical data concerning hippocampal sensitivity to GCs/cortisol changes (Patil et al., 2007). Moreover, a clinical study on CD demonstrated that cortisol damage to the HC was reversible, and plasticity was maintained 17.2 ± 10.1 months after the surgery (Starkman et al., 1999). To our knowledge, this is the first study to investigate HC volume along its longitudinal axis in CD. Glucocorticoids induce damage in HC by mechanisms that are only partially known. However, four processes have been suggested : 1) decreased glucose uptake with selective vulnerability of the dentate gyrus to hypoglycaemia; 2) increased action of excitatory amino acids (glutamate), particularly on CA3 cells; 3) reduced neurotrophic factors (nerve growth factor-β and brain-derived neurotrophic factor); and 4) reduced neurogenesis (McKinnon, 2009, Szeszko, 2006). The stronger sensitivity to GCs damage in HH versus HB and HT might be related to its specific cytoarchitecture. HH has a higher excitatory cell density and a lower inhibitory cell density (Barbas and Blatt, 1995); thus, the CA1 neurons in this area are more vulnerable to ischemic insults, as this segment of the HC is quite vascularized (Szesko et al., 2008, Szesko, 2006). The focal abnormality in the anterior HC might suggest an anterior-posterior gradient of GC damage and of reversible atrophy along the axis of the HC. The anterior-posterior hippocampal segments differ not only with respect to cytoarchitecture, but also in neural connectivity patterns. In detail, the posterior HC, which encapsulates the

body and the tail, is connected to sensory cortical areas, including the parietal cortex , it plays a role in spatial learning andmemory) and shows higher density of pyramidal cells, concentration of metabolites and smaller density of granular cells. In contrast, the anterior HC or head has connections with medial prefrontal regions and amygdala, regulates the hypothalamo-pituitary-adrenocortical axis (HPA)with a negative feedback and might be associated with explicit memory].(Fanselow and Dong, 2010). Further preclinical and clinical studies are needed to support the hypothesis of a longitudinal molecular segmentation of the hippocampus along with the better known functional segmentation (Szeszko et al., 2008). This finding may increase our knowledge of the relationship between structural and functional hippocampal abnormalities observed in stress-related neuropsychiatric disorders. In our sample, the lack of DSM-IV axis I psychiatric disorders suggests that an association between chronic exposure to high cortisol levels and structural alterations in HH may not be sufficient to determine psychiatric syndromes. Other factors (genetic and environmental) are probably needed for their development, as previously suggested in other studies in which focal abnormalities in the head of the hippocampus have been reported in healthy adults (Szeszko, 2006) as well as patients with schizophrenia (Szeszko, 2008) and PTSD (Vythilingam et al., 2005). Additionally, the selective damage of the HH induced by cortisol might explain the explicit memory deficits observed in patients with Cushing's disease (Sapolsky, 2000); however, this association must be demonstrated. Short-term explicit memories are a function of the HH and, as long as they become more enduring, are more posteriorly localised (Strange, 2009). Subsequent studies using tests of explicit memory (e.g., memory for names test, paired associations learning test, prose memory test, California verbal learning test, Rey complex figure test and Corsi block-tapping test) will be required to determine if the volume of bilateral HHs mediates an early decline in CD patients and whether appropriate surgical treatment results in the improvement in these cognitive functions.

Second, we found a significant reduction in right hippocampal body (HB) and tail (HT) volumes relative to matched controls in women suffering from primary recurrent familial depressive disorder (rFPDD). Our data are in line with previous studies and a meta-analysis that demonstrated significantly smaller hippocampal volumes in patients with MDD, especially when the depression was recurrent and of long duration, when compared with healthy control subjects (McKinnon 2009, Campbell et al., 2004; Videbech and Ravnkilde, 2004, Steffens et al., 2000; Mervaala et al., 2000; Bremner et al., 2000; Frodl et al., 2002). Moreover, we found a selective reduction of the most caudal segment of the right hippocampus. Although hippocampal head volume was preserved, hippocampal body volume showed a significant ($p \leq 0.03$), and hippocampal tail volume was strongly reduced ($p \leq 0.005$) in females with rFPDD. Tentative interpretations of our results have implications for both preclinical and clinical data. In fact, a selective vulnerability of the hippocampal tail is present both in preclinical models of stress and in clinical samples of depressed patients. Precliclinical studies have found that prolonged chronic stress or glucocorticoids cause retraction and simplification of apical dendrites in the laminar CA3 region of the hippocampus (Mc Ewen, 2008; Woolley et al., 1990; Reagan and McEwen, 1997). This effect leads to a reduction in the number of neuropils without neuronal loss, similar to that found in the post-mortem hippocampus of patients with MD (Stockmeier et al., 2004). It is noteworthy that the CA3 subfields are over-represented in the mid-posterior rather than in the anterior sections of the hippocampus (Posener et al., 2003). Another well-established mechanism of stress-induced volume reduction is the impairment of neurogenesis in both the rodent and primate hippocampus (Pittenger and Duman, Dranovsky and Hen, 2006), although no clear-cut data on a differential anterior-posterior gradient effect on neurogenesis are currently available.

Neuroanatomical and functional imaging studies suggest that the posterior versus the anterior hippocampus have distinct neuroanatomical projections and functional correlates (Strange and Dolan, 1999). In normal subjects functional segregation within the human hippocampus has been demonstrated along its anterior-posterior axis Perceptual novelty enhanced activation in the left anterior

hippocampus, whereas retrieval activated the posterior hippocampal regions, where memory traces seem to be organised in a topographical manner (Strange and Dolan, 1999). Studies in patients with unmedicated symptomatic depression (Porter et al., 2003) as well as patients in remission from MDD (Weiland-Fiedler et al., 2004) showed neurocognitive deficits involving spatial learning and memory. These deficits were generally linked to posterior rather than to anterior hippocampal dysfunction (Moser et al., 1993). More recently, clinical data on posterior vs. anterior involvement in MDD have been reported. Neumeister et al. (2005) found evidence of smaller posterior portions of the hippocampal complex in patients with recurrent MDD. Selective damage of the hippocampal tail in a sample of MDD patients was confirmed (Maller et al., 2007), and larger pre-treatment posterior (body and tail) volumes have been reported as predictors of remission (McQueen, 2008).

Our result of predominantly right hippocampal volume decrease is consistent with previous studies revealing reductions in right hippocampal volume (Videbech and Ravnkilde, 2004; Steffens et al., 2000; Janssen et al., 2004) and right hippocampal gray matter density (Bell-McGinty et al., 2002) in both early-onset and geriatric, long-term depression. A recent study in familial early-onset MDD showed that in a sample of male and female paediatric patients with MDD (age 8-21), both left and right hippocampal volumes were reduced (MacMaster et al., 2008). However, the study concluded that sex differentially affects abnormal hippocampal maturation in females with familial MDD. In fact, affected females had greater reductions in right hippocampal volume when compared to males, although this difference was not present in female versus male controls. In contrast, left hippocampal volumes were smaller in female versus males in both patients and controls. Lateralised sex-specific maturational changes in the volumes of the medial temporal structures have been reported by Giedd et al. (1996). Between the ages of 4 and 18, the left amygdala increases significantly only in males, whereas the right hippocampus increases significantly only in females. Therefore, the right hippocampal volume reduction seen in our sample of patient with familial recurrent depression that was not correlated with the severity or number of episodes (see below) could be linked to an

endophenotype that precedes the onset of familial recurrent MD. Thus, it could be the expression of an altered sex-related developmental maturation that predisposes females to recurrent major depression. Moreover, in a previous study (Frodl et al., 2004), reduced right hippocampal volumes predicted poor response to treatment in patients with nonremitted depression at baseline and at 1-year follow-up, suggesting that such lateralised reduced hippocampal volumes may predispose patients to a poor clinical outcome without full remission from depression (Frodl et al., 2004). An alternative explanation of our finding of smaller right posterior volumes when compared to those on the left is that the study had low statistical power, as not all studies have detected this right versus left difference, and a recent meta-analysis did not confirm the result of Videbech's meta-analysis of right smaller volumes in MDD (McKinnon 2009). Thus, larger studies comparing males and females with familial recurrent depression are needed. Our results may contribute to a better understanding of the complex relationship between limbic alterations and MDD when considering the peculiarity of the population we have selected. Female gender and recurrence are two well-identified risk factors for MDD. In fact, the majority of studies have reported a greater sensitivity to the depressogenic effect of stressful life events in women (Post et al., 1995), whereas other studies demonstrate that illness duration and recurrent episodes are predictors of hippocampal volume changes (MacQueen et al., 2003). A possible link between these two phenomena is represented by the "kindling hypothesis" for recurrent MDD (Monroe and Harkness, 2005). Monroe and Harkness (2005) suggest that the kindling model and behavioural stress sensitisation may be more than simply analogous mechanisms, as hippocampal atrophy may facilitate the loss of inhibition of HPA stress-related hormone release, with a consequent decrease in blood and liquoral levels of neuroprotective factors, such as brain-derived neurotrophic factor (BDNF). These phenomena may represent key factors for recurrence in recurrent unipolar depression, as suggested by many authors (Maes et al., 2009; Post, 2007; Perini and Battistin, 2005). The literature supports a neuroendocrinological model of hypercortisolemic stress-related disorders in which the hippocampus may be the most vulnerable and neuroplastic structure of the brain (Duman and Monteggia 2006; Sapolsky, 2000a). Moreover, the well-

documented GC-mediated inhibition of neurogenesis may also explain the presence of hippocampal atrophy (McEwen, 2001). These observations highlight one of the principal limitations of our study, which is the absence of an evaluation of the cortisol levels. Thus, we were not able to verify the hypothetical link between depression, hypercortisolemia and glucocorticoid-induced hippocampal damage/remodelling. In general, the literature suggests that the hippocampus is one of the primary glucocorticoid target sites within the brain. The HPA axis dysregulation associated with melancholic depression may modulate a glutamatergic cascade with neurotoxic effects (Sapolsky, 2000b) on hippocampal neurons.

Third, we did not find significant changes in hippocampal volumes or WBVs in subjects with TBI. The lack of differences in brain volumes in this study may be due to the relatively young age of the sample and the severity of the injury. Clinical volumetric magnetic resonance imaging (vMRI) studies have observed hippocampal volume reduction in subjects with more severe head injury, as measured by the initial Glasgow Coma Scale (GCS) (Bigler et al 1997, 2002; Tate et al. 2000; Himanen et al. 2005; Jorge et al. 2006; Levine et al. 2008). The two mechanisms that play a role in the neurotoxic effects of TBI include a) neuronal and glial cell death and b) traumatic axonal injury. Neuronal and glial loss might progress for weeks to months after the initial insult in selectively vulnerable regions remote from the site of impact, such as the hippocampus, thalamus and cerebellum (Raghupathi 2004). The hippocampus seems to have stronger sensitivity to this process, as suggested by preclinical data. This occurs because a) there is higher N-methyl d-aspartate (NMDA) receptor and voltage-dependent Ca^{2+} channel density in the HC (Geddes *et al.* 2003); b) there are many unidirectional connections between its different sub regions and associated brain areas (McCarthy *et al.*, 2003); c) there is a large number of glucocorticoid (GC) receptors and finally d) the HC is a site of neurogenesis. Necrotic cell death by release of excessive excitatory neurotransmitters is probably only the first step in neuronal death, followed by a shift in the balance between pro- and anti-apoptotic factors towards the expression of proteins that promote death (Raghupathi 2002). Moreover, the inhibition of neurogenesis due to

direct damage to precursor cells of the dentate gyrus and/or hyper-activation of the hypothalamo-pituitary-adrenal (HPA) axis after exposure to a head injury may be the basis for temporal and spatial progression of damage in the HC. Elevated glucocorticoid levels could also cause inhibition of the genesis of new neurons and/or glia by direct damage to stem cells and/or reductions in brain-derived neurotrophic factor (BDNF) expression. In contrast, recent studies suggest that in addition to triggering destructive processes, an endogenous reparative response may be produced after TBI. BDNF is important in the process, as it promotes the survival and differentiation of neurons and inhibits cellular apoptotic death. It is unclear why the neurodegenerative process may take precedence over the neuroprotective process or vice versa. A comparison between the hippocampus volumes in our sample and the uninjured control group are necessary to elucidate whether head injury has an effect on brain structures in subjects with traumatic brain injury.

Fourth, the automated method shows favourable agreement with the stereological method. The results, however, indicate that the automated method should be improved with respect to the calculation of hippocampal volume. After the implementation of FreeSurfer, the experiment will be repeated and followed by verification of construct validity; this will be done by comparing the volumetric data of patients with those of controls. When the outcomes of these two processes are positive, FreeSurfer will be ready for use for the efficient and accurate analysis of volumetric data in brain ROIs.

The primary limitation of this study was the use of relatively low-resolution scans. It is very difficult to achieve high resolution images using a 1.0-T scan. However, we obtained high inter-rater reliability (ICC $\geq$ 0.89). The high ICC value achieved may primarily depend on the following two factors: (1) the training of two raters who were experienced in hippocampal delimitations and the use of a very rigorous segmentation protocol and (2) a specific software tool that allows for work on three orthogonal cross-sections simultaneously and permits the delineation of difficult boundaries (e.g., the transition of the

hippocampus to the amygdala in anterior slices). For good reliability, the magnetic field strength is only one of many image acquisition parameters. Others factors that influence reliability are as follows: precision of the anatomical guidelines, presence of an automated delineation and measurement protocol and selection criteria, as reported in a recent paper on reliability and practical guidance for hippocampal volumetry (Jeukens et al., 2009). All of these factors are present in our study.

In conclusion, we have investigated the hippocampal volume of different neuropsychiatric disorders using semiautomatic and stereological methods. We report a pre-post surgery difference on HH volume that could contribute to a better understanding of the pathophysiology of CD as an *in-vivo* model for stress-related hypercortisolemic neuropsychiatric disorders. We examined females with familial depression and healthy comparison subjects and found right posterior hippocampal volume to be decreased in subjects with depression. This could be a core structural endophenotype for rFPDD. Prospective follow-up studies in rFPDD that begin with the onset of depressive symptoms are needed to confirm this finding and to elucidate whether a smaller right posterior hippocampal volume in females is a specific, reversible, acquired state marker or whether it is a structural endophenotype for recurrent depressive disorders. In this case, it should also be present before the onset of the disease and in first-degree female relatives as a structural vulnerability trait, thus representing a negative predictor of course and treatment outcome. No significant differences in hippocampal volumes were found in subjects with mild traumatic brain injury. A higher sample of subjects and functional imaging studies are necessary to make conclusions concerning changes in brain structures among subjects with mild traumatic brain injury.

Finally, we aimed to validate FreeSurfer, an automated software program developed at the Martinos Center for Biomedical Imaging (Harvard University, Boston, USA). This aim is in line with the current research for determining the volume of brain areas, which has been geared towards the development of automated methods of volumetric

analysis that are highly reproducible, accurate and potentially more efficient than the current gold standard of the manual technique.

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