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Short communication

Volumetric MRI analysis of hippocampal subregions in Cushing's disease: A model for glucocorticoid neural modulation

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ABSTRACT

Several preclinical studies have demonstrated neuronal effects of glucocorticoids on the hippocampus (HC), a limbic structure with anterior–posterior anatomical and functional segmentation. We propose a volumetric magnetic resonance imaging analysis of hippocampus head (HH), body (HB) and tail (HT) using Cushing's disease (CD) as model, to investigate whether there is a differential sensitivity to glucocorticoid neuronal damage in these segments. We found a significant difference in the HH bilaterally after 12 months from trans-sphenoidal surgical selective resection of the adrenocorticotropic hormone (ACTH)-secreting pituitary micro-adenomas. This pre–post surgery difference could contribute to better understand the pathopysiology of CD as an in vivo model for stress-related hypercortisolemic neuropsychiatric disorders.

1. Introduction

Cushing's disease (CD) represents a unique in vivo model to investigate morphological effects of cortisol on the human hippocampus (HC), using magnetic resonance imaging (MRI) [23]. HC is a limbic structure involved in several neuropsychiatry disorders, such as major depression disorder (MDD), post-traumatic stress disorder (PTSD), schizophrenia, mild cognitive impairment (MCI), Alzheimer's disease (AD) and traumatic brain injury (TBI) [14,5,24,9,11]. Many preclinical findings have stressed that chronic elevated levels of glucocorticoids (GCs) could cause neuronal damage within the HC, through stimulation of glucocorticoid receptors in this area [13]. More specifically, studies in experimental animals have demonstrated that an interaction between GCs and glutamate is involved in the hippocampal atrophy [22] by a decrease of neuropil volume, a loss of cells (glia and neurons) and an inhibition of neurogenesis [6]. In contrast, a body of researches has established that toxic effects of GCs could be suppressed and the HC atrophy could be reversed, by increasing neurogenesis [20]. All these findings suggest that the HC shows a certain degree of plasticity [13]. Also recently, neuroimage and histological studies have observed that HC is a structure, along its anterior–posterior axis, heterogeneous for neuroanatomical connections, functions [25], cytoarchitectura [3] and metabolite concentrations [12]. In detail, the posterior HC, which encapsulates the body and the tail, is connected to sensory cortical areas, including the parietal cortex [15], it plays a role in spatial learning and memory [8]) and shows higher density of pyramidal cells, concentration of metabolites and smaller density of granular cells [12]. In contrast, the anterior HC or head has connections with prefrontal regions, regulates the hypothalamo-pituitary-adrenocortical axis (HPA) with a negative feedback [29] and might be associated with explicit memory [26]. Therefore the HC should not be considered as a single entity but should be studied in all its three anatomical segments: hippocampus head (HH), body (HB) and tail (HT). The goal of our study was to investigate whether the hippocampal volume in patients with CD differs along its longitudinal axis after a time period of 12 months from pituitary micro-adenoma trans-sphenoidal surgical selective resections, knowing the anterior–posterior differential sensitivity of HC to cortisol and glutamate effects [2].

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2. Patients and methods

2.1. Subjects

Eight women and two men with CD (see for criteria [1]), at the time of admission to treatment, were recruited in the Neurosurgery Unit at the University-Hospital of Padova. Mean age was 38.2 years (S.D. = ±13.1). Means estimated duration of illness and school education were, respectively, 3.5 (S.D. = ±1.1) and 12.4 years (S.D. = ±3.6), based on an assessment of the patient's history. All subjects had no current or previous psychiatric illnesses as determined from the structured Mini-International Neuropsychiatric Interview (MINI) [21]. Exclusion criteria included other acute or chronic medical conditions, neurological diseases, head trauma, history for 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. The ethical approval has been received by the University of Padova. All patients received a MRI, shortly before the surgical selective resection of the adrenocorticotropic hormone (ACTH)-secreting pituitary microadenomas by trans-sphenoidal approach and after an interval of one year following surgery, to obtain volumetric measurements of the brain regions of interest (ROIs).

2.2. Magnetic resonance imaging study

MRI scans of whole brain were obtained by using a Polaris-Marconi Medical Systems 1.0 T resonance scanner. T1-weighted sagittal three-dimensional sequences were acquired for volumetric estimations (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). Volumetric measures were performed on Acer PC workstation using the DCM View software (Padova Ricerche, Italy) in which ROIs were manually outlined on consecutive coronal slices in an anterior–posterior direction and verified from axial and sagittal orientations, by two independent raters (P.A. and T.T.). Calculations of the volumes were computed automatically excluding voxels with density unwanted (e.g. the fimbria white matter and sporadic fluid in the hippocampus complex). Hippocampi were traced referencing to an anatomical atlas [6] and a segmentation's protocol for the HC [18], and included the cornu ammonis, dentate gyrus and subiculum. The most posterior slice (HT) was defined as the slice where the hippocampus first appeared adjacent to the trigone of the lateral ventricle. The first anterior slice was established where the hippocampus (HH) began to show a characteristic triangular shape from the overlying amygdala and the caudate and the third ventricle could be seen. To obtain the distinction between HT and HB, we used the first slice on which the aqueduct of Sylvius became fully visible, while the slice, where the cerebral peduncles were easily recognizable, represented the boundary between HB and HH. 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. Intra-rater and inter-rater reliabilities (intra-class correlation coefficients) were greater than 0.89 for hippocampal measures and 0.92 for WBV.

2.3. Statistical analysis

All data were statistically analyzed using Statistical Package for the Social Sciences version 11.5 for Windows (SPSS, Chicago, Illinois, USA). The Kolmogorov-Smirnov test was applied to test for normal distribution of morphometric data. To investigate the differences between pre- and post-surgery, volumes analysis of covariance (ANCOVA) was used. Covariates included age, education and duration of illness to control for differences of all volumes (WBV, HC, HH, HB and HT). We also used WBV as covariate to determine the effect of surgery on the hippocampal volumes (HC, HH, HB and HT). The level of significance was established at $P < 0.05$.

3. Results

All morphometric data followed a normal distribution. ROIs volumes and ANCOVA's data are shown in Table 1. As compared to before surgery, 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. No significant differences were observed for other hippocampal volumes and WBV ($P > 0.05$).

4. Discussion

The principal finding of our study is a significant increase in right and left HH volumes in patients with CD after trans-sphenoidal surgery. Our data are consistent with previous preclinical researches on hippocampal sensitivity to GCs/cortisol changes [20]. Moreover, a clinical study on CD demonstrated that cortisol damage on HC was reversible and plasticity after 17.2 ± 10.1 months following surgery was maintained [23]. To our knowledge, this is the first study that investigates the HC volume along its longitudinal axis in CD. Glucocorticoids induce damage in HC by mechanisms only partially known. However, four processes have been suggested:

Table 1
Hippocampal and whole brain volumes data of patients with Cushing's disease compared after 1 year.

	Patients with Cushing's disease		ANCOVA	
	Pre-treatment	Post-treatment	*F*	*P*
Right hippocampus volume (cm^3)	3.56 ± 0.91	3.85 ± 0.48	0.80[a]	0.39[a]
Hippocampus head (HH)	0.77 ± 0.36	1.27 ± 0.53	5.83[a]	0.03[a]
Hippocampus body (HB)	1.72 ± 0.25	1.78 ± 0.32	0.43[a]	0.52[a]
Hippocampus tail (HT)	0.90 ± 0.37	0.87 ± 0.46	0.39[a]	0.54[a]
Left hippocampus volume, cm^3	3.63 ± 0.90	4.22 ± 0.89	2.31[a]	0.15[a]
Hippocampus head (HH)	0.71 ± 0.39	1.26 ± 0.59	4.87[a]	0.04[a]
Hippocampus body (HB)	1.82 ± 0.35	1.84 ± 0.32	0.02[a]	0.90[a]
Hippocampus tail (HT)	1.10 ± 0.47	1.00 ± 0.36	0.48[a]	0.50[a]
Whole brain volume (WBV) (cm^3)	932.93 ± 88.30	935.43 ± 91.20	0.01[b]	0.96[b]

Volumes are given as mean ± S.D. Level of significance $P < 0.05$.
[a] Age, education, duration of illness and WBV as covariates; d.f. = 1.14.
[b] Age, education and duration of illness as covariates; d.f. = 1.15.

(1) decreased glucose uptake with selective vulnerability of dentate gyrus to hypoglycemia;
(2) increased action of excitatory amino acids (glutamate) that is explicated in particular on CA3 cells;
(3) reduced neurotrophic factors (nerve growth factor-β and brain-derived neurotrophic factor);
(4) reduced neurogenesis [17,31].

The stronger sensitivity to GCs damage in HH versus HB and HT might be associated to its specific cytoarchitecture. HH has higher excitatory cells density and lower inhibitory cell density [4] and a greater vulnerability of CA1 neurons to ischemic insults, being this segment of HC densely vascular [30,31]. The focal abnormality in the anterior HC might suggest an anterior–posterior gradient of GCs damage and of reversible atrophy along the axis of HC. The anterior–posterior hippocampal segments not only are different in cytoarchitecture, but also in neural connectivity patterns. In HH, these neural connections are mainly directed to the amygdala and to medial prefrontal cortex and they are related to different functions of anterior versus posterior hippocampus (affective/stress responses versus cognitive functions) [7]. 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 [29]. Our finding might have relevance in increasing the knowledge on 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 exposition to high cortisol levels and structural alterations of HH could 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 hippocampus have been reported in healthy adults [27], schizophrenia [28] and PTSD patients [30]. Additionally, the selective damage of HH induced by cortisol might explain the deficit of explicit memory mainly observed in patients with Cushing' disease [19], but this association must be demonstrated. Short-term explicit memories are a function of HH and, as long as they become more enduring, are more posteriorly localized [25].

This study has several limitations that should be acknowledged to interpret our findings. First, the sample size is small. Second, relatively low-resolution scans were utilized: the issue of poor resolution represents one of the major limitations in our study since it is very difficult to achieve high resolution images using a 1.0 T scan. However, we obtain a high inter-rater reliability (ICC $\geq$ 0.89). The high value of ICC achieved may depend mainly on two factors. First, the training of the two raters, who are experienced in hippocampal delimitations and used a very rigorous segmentation protocol as described in a previous published study [16]. Second, the specific software tool that allows the ability to work on three orthogonal cross-sections simultaneously and permits the delineation of difficult boundaries (e.g., the transition of the hippocampus to the amygdale in the anterior slices). For a good reliability, the magnetic field strength is only one of image acquisition parameters. Others factors that influence reliability are: precision of the anatomical guidelines and presence of automated delineation protocol and measures of and selection criteria as reported in a recent paper on reliability and practical guidance of hippocampal volumetry [10]. All these others factors are present in our study. As third limitation, we have not analyzed the correlation between volumetric data and cortisol concentrations before and after trans-sphenoidal surgery, because unfortunately they were not all available at the time of MRI acquisitions. Finally, cognitive tasks are lacking to investigate structure-function relations. Subsequent studies using tests of explicit memory (memory for names test, paired associates learning test, prose memory test, California verbal learning test, Rey complex figure test and Corsi block-tapping test) will be required to investigate if the volume of bilateral HHs mediates an early decline in CD patients and later, after appropriate surgical treatment, an improvement in these cognitive functions. In conclusion, we report a pre–post surgery difference on HH volume that could contribute to a better understanding the pathophysiology of CD as an in-vivo model for stress-related hypercortisolemic neuropsychiatric disorders.

Conflict of interest statement

All the authors declare that they have no conflict of interest.

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