The handle http://hdl.handle.net/1887/53164 holds various files of this Leiden University dissertation.

Author: Andela, C.D.
Title: Understanding clinical outcome in patients with pituitary disease: a biopsychosocial approach
Issue Date: 2017-09-28
CHAPTER 3

Smaller grey matter volumes in the anterior cingulate cortex and greater cerebellar volumes in patients with long-term remission of Cushing’s disease: a case-control study

Cornelie D. Andela*, Steven J.A. van der Werff*, J. Nienke Pannekoek, Susan M. van den Berg, Onno C. Meijer, Mark A. van Buchem, Serge A.R.B. Rombouts, Roos C. van der Mast, Johannes A. Romijn, Jitske Tiemensma, Nienke R. Biermasz, Nic J.A. van der Wee, and Alberto M. Pereira

*Equally contributed

ABSTRACT

Objective: Patients with long-term remission of Cushing’s disease (CD) have persistent psychological and cognitive impairments. It is unknown whether, and to what extent, these impairments are accompanied by structural abnormalities in the brain. We aim to investigate structural changes in the brain in a sample of patients with predominantly long-term remission of CD and to examine whether these changes are associated with psychological and cognitive dysfunction, and clinical severity.

Design: A cross-sectional, case control study.

Methods: In 25 patients with predominantly long-term remission of CD and 25 matched healthy controls, grey matter volumes in the regions of interest (hippocampus, amygdala, anterior cingulate cortex) and in the whole brain were examined, using 3T Magnetic Resonance Imaging and a voxel-based morphometry approach. Psychological and cognitive functioning were assessed using validated questionnaires and clinical severity was assessed using the Cushing’s syndrome Severity Index.

Results: Compared to controls, patients had smaller grey matter volumes of areas in the anterior cingulate cortex (on average 14%, P<0.05) and greater volume of the left posterior lobe of the cerebellum (on average 34%, P<0.05). As expected, patients with remitted CD reported more depressive symptoms (P=0.005), more anxiety (P=0.003), more social phobia (P=0.034), more apathy (P=0.002) and more cognitive failure (P=0.023) compared to controls, but the differences in grey matter volumes were not associated with psychological or cognitive measures, nor with clinical severity.

Conclusion: Patients with predominantly long-term remission of CD showed specific structural brain abnormalities, in the presence of psychological dysfunction. Our data form a basis for future work aimed at elucidating the relation of the structural brain abnormalities and the sustained psychological deficits after long-term exposure to high cortisol levels.
INTRODUCTION

Cushing’s disease (CD) is caused by excessive endogenous cortisol exposure (1). After successful surgical correction of hypercortisolism, the physical, psychological and cognitive symptoms improve substantially (2;3). However, despite curative treatment of the adenomas per se, multiple physical, psychological and cognitive complaints may persist and morbidity and mortality remain increased, even in case of long-term remission (4;5).

Cortisol is the main hormonal mediator of the stress response and acts via stimulation of both the mineralocorticoid (MR) and glucocorticoid receptors (GR) in the central nervous system. HPA-axis activity is regulated by limbic structures such as the hippocampus and amygdala and the anterior cingulate cortex (ACC) (6). These areas are also important target areas for glucocorticoid hormones via activation of MR and GR. In accordance, long-term exposure to elevated cortisol levels has been linked to functional and structural changes of these limbic structures both in humans and preclinical studies (7;8). For example, prolonged cortisol elevations predict memory dysfunction and reduced volume of the hippocampus and ACC during aging (9;10). Moreover, in patients with Cushing’s syndrome, hypercortisolism was associated with smaller hippocampal volumes and overall brain atrophy (11-13), with increasing hippocampal volumes and improving emotional and cognitive functioning after correction of hypercortisolism (11;14-18).

To date, the long-term effects of chronic overexposure to cortisol, such as in CD, on the brain has been evaluated in only one study (19). In that study, focusing on memory function and hippocampal volume in 33 patients with Cushing’s syndrome and 34 matched healthy controls, no overall differences in hippocampal volume between patients and controls were found (19). However, there was a considerable heterogeneity within the patient group in terms of disease status and treatment. Both patients with active CD and patients with CD in remission, with either pituitary or adrenal disease, were included and analyzed as one group (19), precluding definite conclusions. Furthermore, volumetric analyses were limited to the hippocampus and did not include other brain regions known to be important in emotional and cognitive functioning.

Recently, we performed a large cross-sectional study in a well-characterized cohort of patients with predominantly long-term biochemical remission of pituitary-dependent CD. We found a decreased quality of life (20;21), a higher prevalence of psychopathology (e.g. depression, anxiety, apathy) (22), maladaptive personality traits (22) and subtle cognitive impairments (23) despite long-term cure. The results of these studies suggest irreversible effects of longer periods with glucocorticoid excess on brain function and possibly brain structure. These findings were associated with clinical characteristics (e.g. hydrocortisone dependency).

The primary aim of the present cross-sectional study was to investigate whether this cohort of patients with predominantly long-term biochemical remission of pituitary-dependent CD shows structural brain abnormalities, using a voxel-based morphometry approach. In particular, given the results of our previous study, we aimed to evaluate structural changes in
important cerebral regions of the limbic system, i.e. the hippocampus, the amygdala, but also in a cerebral key region for both cognitive and emotional functioning: the ACC. Furthermore, we performed an explorative whole brain analysis to detect possible structural changes in areas outside this a priori defined regions of interest. In addition, we aimed to explore associations between structural changes and measures of psychological and cognitive dysfunction and to take clinical characteristics, such as hydrocortisone dependency into account.

**SUBJECTS AND METHODS**

**Subjects**

All patients in long-term remission of CD of pituitary origin monitored at our institute (N=49) and between 18 and 60 years of age, were invited by letter and those who did not respond were contacted by phone. The response rate was 96% and 31 patients were screened for eligibility. Exclusion criteria were (history of) drug- or alcohol abuse, neurological problems, contraindications for undergoing a MRI scan and left-handedness. 25 CD patients and 25 matched healthy controls were included in the present study. All CD patients had been treated by transsphenoidal surgery, two patients (8%) additionally underwent bilateral adrenalectomy, whereas six patients (24%) had received additional radiotherapy. One patient (4%) used antidepressants. Healthy controls were pair-wise matched for gender, age, and education and recruited by advertisements in grocery stores and via Internet. Inclusion criteria for healthy controls were age between 18 and 60 years, right handedness, no current or prior drug- or alcohol abuse, no present and past history of psychiatric or neurological disorders, no use of psychotropic medication and no contraindications for MRI scanning.

The diagnosis of CD had been confirmed in all patients. ACTH dependent Cushing’s syndrome had been diagnosed based on internationally agreed guidelines, with clinical manifestations and positive biochemical tests, including increased urinary excretion rates of free cortisol, decreased overnight suppression by dexamethasone (1 mg) and elevated midnight salivary cortisol values. Cure of CD had been achieved by transsphenoidal surgery and, if necessary, followed by repeated surgery and/or postoperative radiotherapy. Cure of CD was defined by normal overnight suppression of plasma cortisol levels (< 50 nmol/liter) after administration of dexamethasone (1 mg) and normal 24-h urinary excretion rates of cortisol (<220 nmol/24 h). Hydrocortisone independency was defined as a normal cortisol response to CRH or insulin tolerance test (> 500 nmol/L). Patients were followed at our department with yearly intervals, and pituitary hormone substitution was prescribed in accordance with the results of the yearly evaluation. In patients who were glucocorticoid dependent after treatment, recovery of the pituitary-adrenal axis was tested twice a year. The dose of hydrocortisone was on average 20mg/d divided into 2-3 dosages. After withdrawal of hydrocortisone replacement for 24 hours, a fasting morning blood sample was taken for
the measurement of serum cortisol concentrations. Patients with serum cortisol concentration <120 nmol/liter were considered to have ongoing glucocorticoid dependency, and hydrocortisone treatment was restarted. Patients with serum cortisol levels of 120-500 nmol/liter were tested by ACTH stimulation tests (250µg). A normal response to ACTH stimulation was defined as a stimulated cortisol >550 nmol/liter. When the cortisol response to ACTH was normal, patients were tested by ITT or CRH stimulation test. When cortisol responses to these tests were <550 nmol/liter, hydrocortisone treatment was restarted. Evaluation of growth hormone (GH) deficiency was done by insulin-tolerance test or arginine-GHRH test only in patients under the age of 70 years and only after at least 2 years of remission. Patients with an inadequate stimulation of GH by one of these tests were treated with recombinant human GH, aiming at IGF-1 levels between 0 and +2 SD values. In addition, the twice yearly evaluation consisted of measurement of free T₄ and testosterone levels (in male patients). If results were below the lower limit of the respective reference ranges, substitution with L-thyroxin and/or testosterone was started. In the case of amenorrhea and low estradiol levels in premenopausal women, estrogen replacement was provided. Persistent cure of CD was documented by normal values of a dexamethasone (1 mg) suppression test, urinary cortisol excretion rates, and midnight salivary cortisol levels before participation in the current study.

The estimated duration of disease was determined through patients’ history by looking for the earliest physical/somatic signs. Duration of remission was calculated from the date of curative transsphenoidal surgery, or in case of persistent disease, from the date of normalization of biochemical tests after postoperative radiotherapy. Patient and treatment characteristics were collected from the patient records.

Written informed consent was obtained from all participants prior to the clinical assessment and the MRI-scan session. Our institutional review board approved the study protocol. This study was in accordance with the principles of the declaration of Helsinki.

**Study design**

We scheduled a single study visit of approximately two hours for MRI scanning (60 minutes) and an interview for the evaluation of the clinical data and the assessment of psychological and cognitive functioning. Scan sessions took place between 9.00 am and 12.00 am. After the examination, participants were asked to complete several self-rating questionnaires at home for the assessment of psychopathology and cognitive functioning and to return them within a week.

**Assessment of psychopathology and cognitive functioning (Appendix 1)**

Presence and severity of depressive symptoms were evaluated using the Montgomery-Åsberg Depression Rating Scale (MADRS) (24,25), which was the only scale that was assessed by the interviewer, and the Inventory of Depression Symptomatology (IDS) (26). Anxiety was evaluated using the Beck Anxiety Inventory (BAI) (27) and the Fear Questionnaire (FQ) (28).
Apathy and irritability were assessed using the Apathy Scale (AS) and the Irritability Scale (IS), respectively (29;30). The Cognitive Failures Questionnaire (CFQ) was used to assess failures in perception, memory, and motor function (31).

**Cushing’s syndrome Severity Index (CSI)**

The Cushing’s syndrome Severity Index (CSI) (32) was used to assess current severity of symptoms and to retrospectively estimate (clinical) severity at the time of active disease. The CSI contains eight clinical features and can be scored on a 3-point scale, ranging from 0 to 2. A higher total score on the CSI indicates greater severity, with a range of 0–16. The information necessary for completing this index was derived from clinical history and medical files. Two raters, who reached consensus on each feature in case of discrepancy, scored the CSI. For the active phase, the CSI was scored retrospectively. The current score was evaluated based on the last yearly evaluation. The total score of the active phase and the total score of the remission phase were used in the analyses.

**MRI data acquisition**

Images were acquired on a Philips 3T magnetic resonance imaging system (Philips Healthcare, Best, The Netherlands), (software version 3.2.1). A SENSE-32 channel head coil was used for radio frequency transmission and reception. For each subject, anatomical images were obtained using a sagittal 3-dimensional gradient-echo T1-weighted sequence (repetition time (TR) = 9.8 ms, echo time (TE) = 4.6 ms, matrix size 256 x 256, voxel size 1,17 x 1,17 x 1,2 mm, 140 slices, scan duration 4:56 minutes) as part of a larger imaging protocol. A neuroradiologist, blinded for the clinical details of the subjects, examined all anatomical images. Apart from incidental age-related white matter hyperintensities and effects of the post-transsphenoidal surgery in the perisellar area, no other macroscopic abnormalities were observed in the patients and controls.

**Statistical analyses and data preprocessing**

The first analysis comprised the voxel-based comparison of grey matter volumes in the regions of interest (ROI) (i.e. hippocampus, amygdala, ACC) and across the whole brain between patients with predominantly long-term remission of CD and their matched healthy controls. Structural data was analyzed with FSL-VBM, a voxel-based morphometry style analysis (FMRIB’s Software Library) (33). First, structural images were brain-extracted and grey matter-segmented (34). The resulting grey matter partial volume images were then aligned to MNI152 (T1 standard brain average over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada) standard space, using affine registration (35), followed by nonlinear registration. The resulting images of all participants were averaged to create a study-specific template, to which the native grey matter images were then non-linearly re-registered.
The Jacobian of the warp field obtained in this registration reflects the voxel-wise relative volume change between the original and the study specific template (i.e. a Jacobian of 5 indicates that a volume in the original image has been shrunk by a factor of 5). In order to correct for local expansion or contraction, the registered partial volume images were then modulated by dividing by the Jacobian of the warp field. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. The Gaussian outputs a weighted average of each voxel’s neighborhood, with the average weighted more towards the value of the centrally located voxels. The application of this type of smoothing reduces the noise in the data substantially.

The Harvard-Oxford Cortical and Subcortical Structural Atlases implemented in FSL were used to create masks for our regions of interest: the bilateral hippocampus, the bilateral amygdala and the ACC. Probability range was set to 50-100% for all three structures. The study-specific template was then applied to this mask to create a study specific template of the grey matter values in the regions of interest only. Finally, groups were compared using a general linear model (GLM) including age, gender and level of education as confound regressors. A voxel-wise GLM was applied using permutation-based (5000 permutations) non-parametric testing, correcting for multiple comparisons across space. First, groups were compared in our regions of interest, using the created mask. Second, an exploratory whole brain VBM analysis was done, using the study-specific grey matter image as a mask to investigate whether any unpredicted differences existed between CD patients and controls. To explore possible differences between patients with hydrocortisone substitution (n=13) and patients without substitution (n=12), these two steps were repeated contrasting these two groups. Threshold-free Cluster Enhancement was used for finding clusters in the data (36), with thresholds for both the regions of interest comparison as well as the whole brain analysis set on P<0.05, corrected. In addition to the VBM analysis, we used FMRIB’s integrated registration and segmentation tool (FIRST) to perform an automated segmentation of the amygdala and the hippocampus, allowing both shape and volume analyses.

The second analysis compared patients with predominantly long-term remission of CD and their matched healthy controls, on measures of psychological and cognitive functioning. Data from questionnaires were analyzed using SPSS for Windows version 20.0 (SPSS Inc., Chicago, IL). All data are presented as numbers and percentages, means and standard deviations (SD) or median and interquartile range (IQR). The assumption of normal distribution was tested using the Kolmogorov-Smirnov Test and the assumption of equal variances with a Levene’s Test. With respect to psychological and cognitive functioning, normally distributed continuous variables between patients and matched controls were compared using t-tests, and non-normally distributed continuous variables (MADRS, IDS, BAI, FQ, AS, CFQ) using Mann-Whitney U tests. Considering the overlap in phenomenology assessed by the questionnaires, a strict correction for multiple testing might be too conservative, therefore all tests were two-sided with P<0.05 uncorrected.
A third analysis was conducted in the patient group. In this analysis, we examined voxel-wise correlations of behavioural and clinical characteristics with grey matter volume in the areas resulting from the ROI analysis and the whole brain analysis. The possible influence of radiotherapy could not be properly examined, considering the small number of patients that had received radiotherapy. The level of significance was set at P<0.05.

RESULTS

Patient characteristics
As expected, patients and matched healthy controls did not differ in age, gender and education. The mean estimated duration of active disease was 7.9 ± 7.9 years (range 0.8 - 37.0). The mean duration of remission was 11.2 ± 8.2 years (range 0.8 - 29.4). Hydrocortisone replacement therapy was given to 13 patients (52%). The mean CSI score during active disease was 8.1 ± 2.0, and 2.5 ± 1.5 at the time of evaluation (i.e. long-term remission) (Table 1).

Table 1. Clinical characteristics of patients with predominantly long-term remission of Cushing’s disease (n = 25)

<table>
<thead>
<tr>
<th></th>
<th>CD patients (n=25)</th>
<th>Matched controls (n=25)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>4/21</td>
<td>4/21</td>
<td>1.000*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45 ± 8</td>
<td>47±7</td>
<td>0.471b</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td>0.946*</td>
</tr>
<tr>
<td>Low</td>
<td>6 (24%)</td>
<td>6 (24%)</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>12 (48%)</td>
<td>11 (44%)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>7 (28%)</td>
<td>8 (32%)</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>25 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transsphenoidal adenomectomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral adrenalectomy</td>
<td>2 (8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>6 (24%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>7.9 ± 7.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of remission (years)</td>
<td>11.2 ± 8.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypopituitarism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any axis</td>
<td>14 (56%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>10 (40%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH/FSH</td>
<td>9 (36%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSH</td>
<td>10 (40%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADH</td>
<td>3 (12%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone substitution</td>
<td>13 (52%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone dose (mg/d)</td>
<td>20.0 (0.0 – 20.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P values were tested with: *Chi-square test, bindependent-sample t-test.
Data are presented as mean ± SD or number (%) or by median IQR.
MRI Analyses

Region of interest analyses
The VBM-analysis, in patients in with predominantly long-term remission of CD showed smaller grey matter volumes in a large part of the bilateral ACC in comparison with controls. Closer examination of the data revealed that the patients had an average of 14% smaller grey matter volumes in the ACC compared to matched healthy controls. There were no grey matter volume differences in the bilateral hippocampus and amygdala (Figure 1A). We observed no greater grey matter volumes in any of the ROIs in CD patients, compared to controls. Furthermore, within the patient group no differences were found in grey matter volumes between patients with hydrocortisone substitution and patients without substitution. The FIRST analysis showed similar results, with no differences in both shape and volume of the bilateral amygdala and bilateral hippocampus, between patients and controls.

Whole brain analysis
Patients with predominantly long-term remission of CD showed smaller grey matter volumes in the left perigenual region (Brodmann’s area between BA 32 and BA 12) of the ACC, compared to controls (Figure 1B). Greater grey matter volumes were found in the posterior lobe of the left cerebellum in CD patients compared to controls (Figure 1C). On average patients showed 34% larger grey matter volumes in the left posterior lobe of the cerebellum compared to controls (P<0.05). When the threshold was lowered to P<0.10 an additional similar effect was observed in grey matter volumes of the right posterior lobe of the cerebellum. Within the patient group, no differences were found in grey matter volumes between patients with hydrocortisone substitution and patients without substitution.

Psychopathology and cognitive functioning among patients and controls
Table 2 shows that patients with predominantly long-term remission of CD had more depressive symptoms (P<0.005) compared to controls, as assessed with the MADRS and the IDS. The mean total score on the MADRS was 6.3, indicating mild depressive symptoms. Furthermore, CD patients experienced more anxiety (P=0.003), more social phobia (P=0.034), and a greater degree of apathy (P=0.002), with 44% of patients having a score of 14 or higher, which is indicative of clinically relevant apathy. On the Irritability scale, 36% of the patients had a score of 14 or higher, which is indicative of clinically relevant irritability. In addition, CD patients reported more cognitive failure (P=0.023) compared to controls. No other significant between-group differences were found. Within the patient group, no significant differences were found in psychopathology and cognitive functioning between patients with hydrocortisone substitution and patients without substitution.
Figure 1. VBM analysis results.
A): Results of regions of interest analysis, with grey matter volumes in patients < controls (P < 0.05; 617 voxels, 2mm isotropic).
B): Results of whole brain analysis with grey matter volumes in patients < controls (P < 0.05; 37 voxels, 2mm isotropic).
C): Results of whole brain analysis with grey matter volumes in patients > controls (P < 0.05; 323 voxels, 2mm isotropic). Effects are presented on the MNI-152 1mm standard brain at a threshold of P < 0.05. Coordinates are x = -4, y = 42, z = 0 for Fig. 1a and 1b and x = -29, y = -66, z = -56 for Fig. 1C. The left hemisphere corresponds with the right side of the image.
Furthermore, in the patient group no significant associations between grey matter volumes in the ACC and cerebellum, and scores on the distinguishing psychometric instruments (MADRS, IDS, BAI, AS, CFQ) were found using a voxel-wise correlation approach. Also, no significant associations between grey matter volumes of the areas of effect and clinical characteristics (i.e. estimated disease duration, duration of remission, Clinical Severity Indexes; active and remission subscale) (data not shown) were found.

**DISCUSSION**

The present study demonstrates that structural abnormalities in the brain are present in patients cured from CD, despite long-term remission. The data indicate that in comparison to matched healthy controls, volumes of areas in the ACC were smaller, whereas grey matter volumes of the left posterior lobe of the cerebellum were larger in patients. There were no significant differences in grey matter volumes in the hippocampus or amygdala between the two groups. These findings may support the hypothesis that the increased prevalence of depressive symptoms, anxiety, apathy and cognitive impairments observed in patients with long-term cured CD (22;23) is associated with structural brain changes. However, in these patients no significant correlations were found between psychological dysfunction and clinical characteristics on the one hand, and the grey matter volumes of the ACC and left posterior lobe of the cerebellum on the other hand.

**Table 2. Psychopathology and cognitive failure questionnaires: patients with predominantly long-term remission of Cushing’s disease vs. matched controls**

<table>
<thead>
<tr>
<th></th>
<th>Cushing’s disease (n = 25)</th>
<th>Matched controls (n = 25)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MADRS</td>
<td>6.3 ± 5.5</td>
<td>1.4 ± 1.8</td>
<td>0.000b</td>
</tr>
<tr>
<td>Inventory depression scale (IDS)</td>
<td>46.8 ± 13.0</td>
<td>36.3 ± 5.8</td>
<td>0.005a</td>
</tr>
<tr>
<td>Beck Anxiety inventory (BAI)</td>
<td>28.4 ± 5.7</td>
<td>24.0 ± 3.1</td>
<td>0.003b</td>
</tr>
<tr>
<td>Fear Questionnaire (FQ)</td>
<td>24.5 ± 17.4</td>
<td>14.2 ± 10.0</td>
<td>0.051a</td>
</tr>
<tr>
<td>agoraphobia subscale</td>
<td>6.1 ± 7.9</td>
<td>3.4 ± 4.7</td>
<td>0.477ab</td>
</tr>
<tr>
<td>blood injury phobia subscale</td>
<td>6.2 ± 8.3</td>
<td>3.2 ± 4.1</td>
<td>0.118b</td>
</tr>
<tr>
<td>social phobia subscale</td>
<td>12.2 ± 8.0</td>
<td>7.6 ± 4.9</td>
<td>0.034b</td>
</tr>
<tr>
<td>Irritability scale (IS)</td>
<td>12.1 ± 8.7</td>
<td>8.0 ± 6.1</td>
<td>0.066ab</td>
</tr>
<tr>
<td>Total score &gt; 14</td>
<td>9 (36%)</td>
<td>6 (24%)</td>
<td></td>
</tr>
<tr>
<td>Apathy scale (AS)</td>
<td>13.6 ± 6.6</td>
<td>7.8 ± 3.8</td>
<td>0.002b</td>
</tr>
<tr>
<td>Total score &gt; 14</td>
<td>11 (44%)</td>
<td>2 (8%)</td>
<td></td>
</tr>
<tr>
<td>Cognitive failure questionnaire (CFQ)</td>
<td>38.0 ± 16.5</td>
<td>27.6 ± 9.7</td>
<td>0.023a</td>
</tr>
</tbody>
</table>

P values were tested with: * independent-sample t-test, ** Mann-Whitney U test.
Level of significance was set at P<0.05 and significant values are in bold.
Data are presented as mean ± SD or number (%).
We confirmed our hypothesis that the ACC would be affected in cured CD. The amygdala and hippocampus are connected to the anterior regions of the ACC and constitute a neural circuitry for stress reactivity and modulation (37). Dysfunction of this circuitry is implicated in mood and anxiety disorders (38). In addition, patients with stress-related psychopathology show a reduced volume of the ACC (39;40). In accordance, reduction of ACC volume is also found in animals exposed to hypercortisolism (8) and in elderly humans with dysregulation of the HPA-axis (10). Importantly, the ACC is involved in cognitive-affective processes such as assessing the projection of emotional and motivational stimuli and the regulation of emotional responses (41), and mediates ongoing behavioral adaptation (42). Therefore, the identified abnormalities of the ACC may be involved in disturbances of cognitive and emotional functioning identified in CD (43) and in patients after long-term remission of CD (4;22;23). However, in the current study we were not able to demonstrate a correlation between the observed brain changes and quantitative estimates of psychopathology. This may be due to power problems or limitations of the clinical rating scales for psychopathology. An alternative hypothesis could be that the identified structural abnormalities may also underlie or reflect abnormalities in functional or structural connectivity.

In the exploratory whole brain analysis we found an enlarged volume of the left cerebellum in patients with predominantly long-term remission of CD. When we lowered the threshold, grey matter volumes of the right cerebellum were also found to be enlarged in patients with predominantly long-term remission of CD, indicating that this effect might be bilateral. Interestingly, the cerebellum is susceptible to increased cortisol levels (44) and it is involved in motor functioning, as well as cognitive and emotional functioning (45). Intriguingly, a study by Spinelli et al. (2009), reported that individuals exposed to an extremely stressful environment developed a larger cerebellum (46). Another research group investigated the effect of chronic stress on cortical and striatal circuits (required for goal-directed behavior and habits) in rats. They found global hypertrophy of the dorsolateral striatum and atrophy of the dorsomedial striatum and suggested that the reorganization of the corticostriatal circuits after chronic stress is bidirectional, based on hypertrophy and atrophy of neuronal dendritic trees (47). This mechanism of bidirectional reorganization could also provide an explanation for the larger volume of the cerebellum in our patients treated for CD.

Contrary to our hypotheses, we did not find alterations in the hippocampus and amygdala. However, it might be that these brain structures were affected during active disease (11-13), but that grey matter volumes increased after biochemical cure. This would be in accordance with the previously found increase in hippocampal volume in CD patients after correction of hypercortisolism (11;14-16) and the well-documented plasticity of hippocampal neurons in animal models (48). Nevertheless, children experienced cognitive decline despite reversal of brain atrophy one year after surgical remission (49) and adult patients with long-term remission of CD still demonstrated impaired memory function (23). Recently, a potential mechanism was provided for this persisted memory impairment, by demonstrating that
in comparison to healthy matched controls, patients in remission of CD show biochemical abnormalities in the hippocampus, without hippocampal volume reduction (50). Studies in animals have documented that other brain areas also show structural changes in response to increased cortisol levels (47). However, the plasticity (in this case the extent of reversibility) of these non-hippocampal structures in CD is still unknown. Since there are no studies that have focused on other brain structures in patients with active CD, like the ACC or amygdala, it is not clear when these structural changes occur and how they develop over time.

For direct effects of glucocorticoids on a brain area, either the MR or GR has to be present in this area. Using data on human brain tissue arrays available from ‘The Allen Institute for Brain Science’, a high expression was demonstrated of both MR and/or GR, not only in the hippocampus, ACC and amygdala, but also in the cerebellum (51). Taken into account the effects found in our study, which were limited to the ACC and cerebellum, one can conclude that expression of MR and/or GR in a brain area is necessary, but not predictive of structural changes following chronic overexposure to glucocorticoids. A possible alternative explanation is that structural changes may also occur via transsynaptic mechanisms. Such mechanisms have been suggested for (transient) morphological changes in the hippocampal CA3 area, which itself expresses very low numbers of GRs, but receives input from the cortisol sensitive dentate gyrus (52).

To our knowledge, our study is the first to show that structural abnormalities in the brain are present in patients cured from CD, despite long-term remission. Strengths of our study are the homogeneity of our patient cohort with regard to treatment (i.e. all patients had been treated with transsphenoidal surgery) and the careful selection of controls. Nevertheless, heterogeneity still existed in the patient group with regard to disease duration and duration of remission, which may have decreased the power of this study. Although a sample size of 25 in both groups is appropriate for the evaluation of structural changes with MRI (53), our study might have been underpowered to detect possible correlations between clinical data, psychological and cognitive measures, and grey matter volumes within the patient group, and to detect grey matter differences between patients with or without hydrocortisone substitution. In addition, cognitive functions were assessed using a questionnaire (i.e. CFQ), and although this questionnaire has been validated repeatedly, it is no substitute for extensive neuropsychological testing, which gives a more accurate representation of cognitive functioning. Furthermore, because of our cross-sectional design it cannot be excluded that structural abnormalities were already present in patients before onset of CD. The use of a longitudinal design in future research could provide more insight into the course of the found abnormalities.

In general, alterations in grey matter volume in adults with pathology have been found to be associated with dysfunctions of specific areas or related circuitry. However, the absence of volumetric differences does not exclude functional alterations in brain areas and circuits. It should also be acknowledged that a volumetric VBM approach does not reveal the underly-
ing changes or pathology in grey matter microstructure, i.e. at the level of neurons or glia cells. Subsequently, at present there are no data available on abnormalities at the level of neurons or glia cells after chronic overexposure to glucocorticoids that may shed more light on the nature of the observed structural abnormalities. Therefore, conclusions about functional alterations in the specific brain areas cannot be drawn based solely on our findings. Exploring functional brain characteristics in our sample would be an important next step to further elucidate the neurobiological basis of psychological dysfunction in patients with remitted CD.

The data presented in this study provide a further perspective towards detailed phenotyping of patients after treatment of CD, who have always been considered cured after long-term remission of hypercortisolism. In agreement with others, CD and possibly Cushing’s syndrome as well, could be a unique model to study the apparently prolonged, or even irreversible, effects of increased cortisol exposure on the brain. It is tempting to speculate that these findings, to a certain extent, could also apply to patients with chronic or recurrent forms of highly prevalent stress-related disorders, and, in addition, to patients chronically treated with exogenous corticosteroids, that are commonly prescribed to suppress the immune system (54).

In summary, the present study demonstrates that patients with long-term cure after treatment for CD have profound structural alterations in the brain, with smaller volumes of an area in the ACC and greater volumes of the left posterior lobe of the cerebellum, and report more depressive symptoms, anxiety, social phobia, apathy, and cognitive failure, compared to healthy controls. The findings suggest possible structural substrates for long-term psychological effects of hypercortisolemia in CD. Clearly, more research is needed to increase our insight in the underlying mechanisms and the trajectory of changes, which may also lead to the identification of ‘critical time windows’ or potential targets for prevention.

REFERENCES

Brain abnormalities after Cushing’s disease


Chapter 3


Brain abnormalities after Cushing’s disease


APPENDIX 1. DESCRIPTION OF USED PSYCHOPATHOLOGY AND COGNITIVE FAILURE QUESTIONNAIRES.

Inventory of Depression Symptomatology (IDS) is a 28-item multiple-choice questionnaire, which is designed to measure severity of depressive symptoms. A higher total score indicates greater severity of depressive symptoms (24).

Montgomery-Åsberg Depression Rating Scale (MADRS) is a 10-item questionnaire which focuses on the most commonly occurring symptoms of depressive illness (i.e. apparent sadness, reported sadness, inability to feel, difficulty in concentration, inner tension, pessimistic thoughts, suicidal thoughts, lassitude, reduced sleep, reduced appetite). A higher total score indicates more severe depression (22), with severity grades: 0-6 = absent, 7-19 = mild, 20-34 = moderate, 35-60 = severe (23).

Beck Anxiety Inventory (BAI) is a 21-question inventory which evaluates anxiety. For each question the participant was asked to chose between 4 answers, ranging from 1 = not at all, to 4 = severely. A higher sum score indicates greater severity of anxiety (25).

Fear questionnaire (FQ) is a 24-item questionnaire assessing phobic anxiety. Each question can be answered on an 8-point Likert scale, ranging from 0 = would not avoid it, to 8 = always avoid it. For the present study, we only used the items necessary for the total phobia score (item 2-16). This total phobia score can be divided into three subscales: the agoraphobia subscale, the blood injury phobia subscale and the social phobia subscale. Higher scores indicate greater severity of phobias (26).

Apathy Scale (AS) consists of 14 questions on a 4-point scale. The items ranges from 0 = no apathetic behavior, to 3 = maximum intensity of apathetic behavior. A total score can range from 0 to 42 points, with higher scores indicating greater apathy. A total score of 14 points or more is being used to characterize subjects as apathetic (27;28).

Irritability Scale (IS) consists of 14 items on a 4-point scale. The items ranges from 0 = no irritable behavior, to 3 = maximum intensity of irritable behavior. Sum scores can range from 0 to 42, with higher scores indicating greater irritability. A total score of 14 point or more is being used to characterize subjects as irritable (28).

Cognitive Failures Questionnaire (CFQ) measure ones failures in perception, memory, and motor function. On this 25-item questionnaire each question can be answered on a 5-point Likert scale, ranging from 0 = never, to 4 = very often. A higher sum score indicates that an individual experiences greater cognitive failure (29).