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Stressed-out stress systems: dysregulated stress-systems in the pathophysiology of stress-related disorders

Bauduin, S.E.E.C.

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Chapter 8

Long-term effects of Cushing's disease on visuospatial planning and executive functioning

S.E.E.C. Bauduin^{1,2*}, F.M. van Haalen^{2,3*}, E.J. Giltay¹, Onno C. Meijer^{2,3}, A.M. Pereira^{2,3},
N.J.A. van der Wee^{1,2}, S.J.A. van der Werff^{1,2}

1 Department of Psychiatry, Leiden University Medical Center (LUMC), The Netherlands

2 Leiden Institute for Brain and Cognition, Leiden, The Netherlands

3 Department of Medicine, Division of Endocrinology, and Center for Endocrine Tumors, Leiden University Medical Center, Leiden, The Netherlands

*both authors contributed equally to the manuscript

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Abstract

Background

Patients with remitted Cushing's Disease (CD) often present persisting impairments in executive and cognitive functioning domains. Little research has been conducted regarding the functional neural correlates of an important executive functioning skill, namely the ability to plan, in these patients. We used functional magnetic resonance imaging (fMRI) to examine visuospatial planning related brain activity in patients with remitted CD and matched controls.

Methods

fMRI scans were made using a 3-Tesla scanner while remitted CD patients (n=21) and age-, gender-, and education matched healthy controls (HCs; n=21) completed a parametric Tower of London (ToL) task. Psychological and cognitive functioning were assessed using validated questionnaires. Clinical severity was assessed retrospectively using the Cushing's syndrome Severity Index (CSI).

Results

CD Patients were on average 45.1 (SD=7.1) years old, 81% female, and in remission for mean 10.68 (SD=7.69) years. No differences were found in number of correct trials, response times per ToL trial, or in the region of interest analyses. Exploratory whole-brain analyses found that CD patients showed more activation in several brain regions associated with higher cognitive processes on 2-, 3-, and 5-step trials compared to HCs. Over-recruitment of the right parietal operculum cortex in the patients was significantly negatively associated with the prior active disease state on the CSI ($r=-0.519$, $p=0.02$).

Conclusions

The increased brain activation during the ToL in remitted CD patients versus controls signals over-recruitment of certain brain areas involved in higher cognitive processes. CD may thus result in long-lasting, subtle scarring effects during demanding executive functioning tasks, despite remission.

Introduction

Cushing's disease (CD) is characterized by hypercortisolism caused by a pituitary adenoma secreting excessive amounts of adrenocorticotrophic hormone (ACTH; Nieman & Ilias, 2005). A variety of psychiatric symptoms can be induced by hypercortisolism, whereby the most common is major depressive disorder. However, mania, anxiety, and cognitive dysfunction also often co-occur (Sonino & Fava, 2001). Although CD can be effectively treated (usually by means of transsphenoidal surgery), increased mortality (van Haalen et al., 2015), residual psychopathological and physical morbidity (Resmini, 2014; Tiemensma et al., 2010; Ragnarsson et al., 2012), and reduction in quality of life (Van Aken et al., 2005) often remain. Furthermore, several important skills within the cognitive functioning domain have also often been found to remain impaired (Ragnarsson et al., 2012, Hook et al., 2007; Tiemensma et al., 2012).

It is likely that these residual symptoms are associated with the detrimental effects of long-term exposure to hypercortisolism on brain function and structures (Andela et al., 2013). Several neuroimaging studies have observed changes in both brain structure and function in patients with current CD (e.g. Starkman, Gebraski, Berent, & Scheingart, 1992; Andela et al., 2013; Maheu et al., 2008). With regard to the structural changes of the brain, certain abnormalities appear to persist after successful treatment of CD. The often found decreased hippocampal volume in patients with current CD seems to normalize after remission of the disease (e.g. Starkman et al., 1999; Tiemensma et al., 2010; Van der Werff et al., 2014). In contrast to this, altered gray matter volumes of certain brain regions, such as the anterior cingulate cortex (ACC), tend to persist after remission (Andela et al., 2013; Bauduin et al., 2020).

Regarding functional brain alterations in patients with remitted CD specifically (i.e. not in patients with remitted Cushing's syndrome), functional magnetic resonance imaging (fMRI) studies have revealed abnormalities in brain activity in this patient population in comparison to healthy controls (HCs). An fMRI study using an emotional faces paradigm found remitted CD patients had less activation in the medial prefrontal cortex in comparison to HCs (Bas-Hoogendam et al. 2015). Resting-state functional MRI (rs-fMRI) studies with remitted CD patients have found increased resting-state functional connectivity (RSFC) between the limbic network and the ACC, the default mode network in the left lateral occipital cortex (Van der Werff et al., 2015), and elevated RSFC in the medial temporal lobe, the hippocampus, and the

prefrontal cortex networks (Stomby et al., 2019). In contrast to this, the functioning of the executive control network in these studies was found to be similar in remitted CD patients and HCs. This could be explained by the fact that a rs-fMRI does not include specific goal-oriented tasks that requires high cognitive effort. Thus, it may be the case that differences in functional activity within this network only manifest in the remitted CD population when the cognitive demands are higher, as is the case in patients with other stress-related psychopathologies, such as depression and post-traumatic stress disorder (PTSD, Wang et al., 2008; Aizenstein et al., 2009; Daniels et al., 2010).

Cognitive functioning has been examined by means of standard neuropsychological testing in current CD patients (Ragnarsson et al., 2012; Tiemensma et al., 2010), as well as in remitted CD patients after a follow-up period of up to 18 months (Hook et al., 2007). These studies found that cognitive and executive functioning (i.e., psychomotor functioning, visuoconceptual tracking, processing speed, auditory attention, auditory working memory, verbal fluency, reading speed, and brief attention) are (and perhaps remain) impaired in active and remitted CD patients. Cognitive planning encompasses the neurological processes that are involved with the strategy formulation, coordination, evaluation, and selection of a thought sequence, and the necessary actions that are needed in order to achieve that goal (Morris et al., 1997). Reductions of these cognitive abilities in patients with remitted CD may lead to lasting effects on planning abilities, affecting one's daily functionality, psychological state, and quality of life. Although these aforementioned studies have found a number of impairments in the cognitive functioning domain, it is currently unclear as to whether impairments are also detectable in the brain activity patterns of this patient population.

In this study, we examined whether patients with remitted CD display altered performance and brain activity patterns in comparison to healthy controls (HCs) with regard to cognitive planning and executive functioning using the Tower of London (ToL) task (Shallice, 1982). Based on previous research on cognitive functioning in CD patients, we hypothesized that remitted CD patients will complete less trials correctly, complete less trials in total, and take more time to complete a ToL trial in comparison to healthy controls. Furthermore, taking the differences in brain activation found in earlier studies with CD patients into account (i.e. Andela et al., 2013; van der Werff et al., 2015), we hypothesized increased activation in the ACC, an area involved in several complex cognitive functions and critically active when engaging in a cognitively demanding task (Fincham & Anderson, 2006), in

comparison to matched HCs. In addition, we performed an exploratory whole-brain analysis to examine whether other task-related differences in activation can be identified. Furthermore, potential associations between brain activity, psychological, cognitive, and clinical measures were explored.

Methods

Subjects

Participants were all remitted CD patients (aged 18-60 years) who were being monitored at the Leiden University Medical Center (LUMC). Of the 49 invited participants, 96% responded to the invitation, and based on primary in- and exclusion criteria, 31 patients were ultimately screened for further study eligibility (details with regard to this study protocol have previously been published elsewhere; Andela et al., 2013). HCs were recruited via advertisements in grocery stores and internet. HCs were matched to each patient based on gender, age, and level of education. A HC specific exclusion criterion was a history of or current psychiatric disorder. Further exclusion criteria for both the remitted CD and HC groups were neurological problems, MRI contraindications, (history of) drug or alcohol abuse, and/or left-handedness. Six remitted CD patients were excluded due to one of these exclusion criteria. Finally, one remitted CD and their matched HC were excluded because behavioral data was not recorded, leaving the final sample to consist of 24 remitted CD patients and 24 matched HCs.

Biochemical, radiological, and clinical observations conform current international guidelines were used to diagnose CD. Detailed information with regard to these criteria have previously been published elsewhere (Tiemensma et al., 2010). All CD patients received transsphenoidal surgery following the diagnosis of active CD. After surgery, CD remission was confirmed by means of clinical evaluation and multiple biochemical test outcomes (for example, normal 24-hr urinary cortisol excretion rates (<220 nmol/24-hr), normal midnight saliva cortisol (below 5.7 nmol/L), and normal overnight suppression of plasma cortisol levels (<50 nmol/l) by dexamethasone (1 mg)). Patients with remaining glucocorticoid dependency were substituted with hydrocortisone (on average 20 mg/day, divided over three doses), and evaluated twice yearly. Prior to study participation, persistent biochemical cure of CD was confirmed in concurrence with the abovementioned diagnostic tests. Disease duration was identified as the moment earliest somatic signs were presented in a patient's history. Duration of remission was calculated from either the date of curative transsphenoidal surgery or from the date of normalization

of biochemical tests in the case of initial persistent disease persistence following surgery. Written informed consent was obtained from all participants and the study protocol was approved by the medical ethical committee of the LUMC. The protocol was written in accordance with the principles of the Helsinki declaration. Patient and treatment characteristics were obtained from patient medical records.

Behavioral and clinical severity assessment

Psychopathology and cognitive functioning were assessed using the following scales: the 10 item Montgomery-Åsberg Depression Rating Scale (MADRS; Montgomery & Åsberg, 1979), and the 28 item Inventory of Depression Symptomatology (IDS; Rush et al., 1986) to assess the severity of depressive symptoms. An interviewer assessed the MADRS, all other scales used were self-report. Anxiety was evaluated using the blood injury phobia, social phobia, blood injury subscales, and total score of the 15 item Fear Questionnaire (FQ; Marks & Mathews, 1979), and the 21 item Beck Anxiety Inventory (BAI; Beck et al., 1988). The 14 item Irritability Scale (IS) and the 14 item Apathy Scale (AS) were used to assess the severity of irritability and apathy, respectively (Starkstein et al., 2001; Chatterjee et al., 2005). Participants with total scores of more than 14 points were considered to be irritable or apathetic. Failures in memory, motor function, and perception were assessed using the 25 item Cognitive Failure Questionnaire (CFQ; Broadbent et al., 1982). Higher sum scores indicate greater symptom severity.

CD symptom severity during the active and the remitted disease state were established using the 8 item Cushing's syndrome Severity Index (CSI; Sonino et al., 2000). The CSI score during active disease was estimated retrospectively. The remission score was based on the last annual evaluation. Total CSI scores were used for both active and remitted disease states and scores on this index can range between 0 and 16 (higher total score indicates greater symptom severity). The necessary information in order to score the CSI was obtained from the patient's clinical history and medical records. The index was scored by two independent raters that reached consensus in case of discrepancy. Finally, prior to and after the fMRI ToL task, anxiety levels were monitored by means of a Visual Analogue Scale (VAS; Huskisson, 1974) ranging from 0 to 100, where a higher score indicates a higher level of anxiety.

Task paradigm

An event-related parametric version of the ToL was used. A detailed description of this task has been previously published (van den Heuvel et al., 2003). In brief,

participants were presented with either a baseline or test trial. In the baseline trials, participants were requested to count the number of yellow and blue beads presented on the screen. In the test trials, participants were requested to count the minimum number of steps from the 'start' condition to the 'goal' condition. The test trials ranged from 1 to 5 steps (see Figure 1 for examples). The task was pseudorandomized and self-paced, with a maximum response duration of 60 seconds for each trial. The trial was presented by means of E-Prime software (Psychological Software Tools, Pittsburg, PA, USA). Responses and response times were logged by means of button boxes. No feedback was given with regard to the answers. Image Acquisition

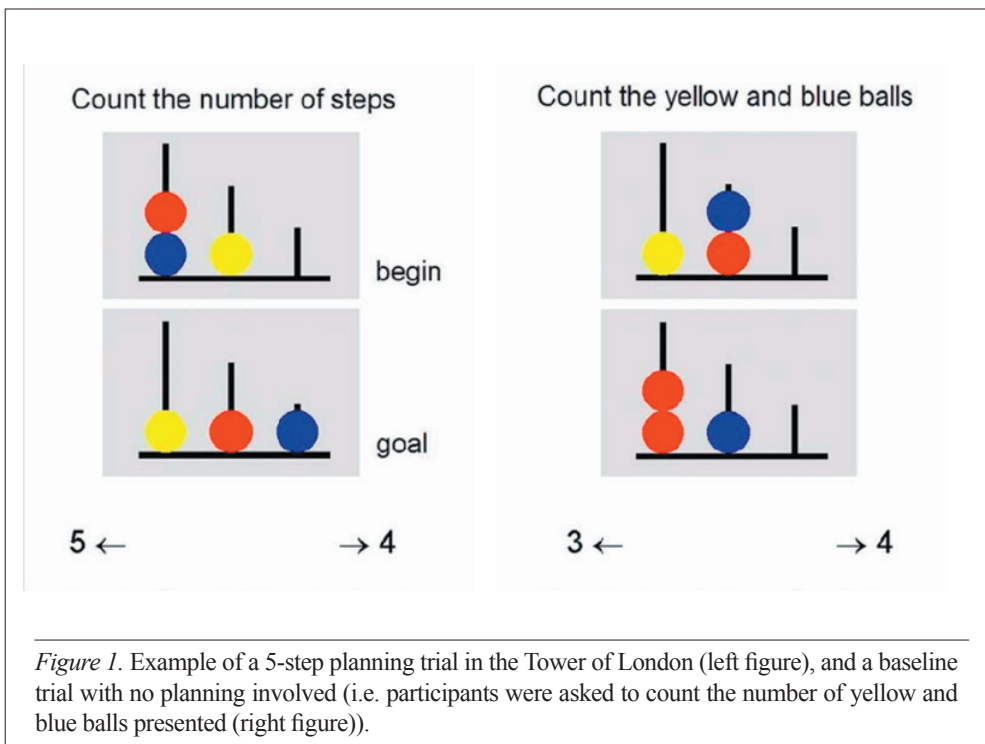


Figure 1. Example of a 5-step planning trial in the Tower of London (left figure), and a baseline trial with no planning involved (i.e. participants were asked to count the number of yellow and blue balls presented (right figure)).

Image Acquisition

The ToL paradigm was part of a larger fMRI protocol, which included a resting-state scan and an emotional faces paradigm. In each session, the ToL was administered as the first fMRI paradigm in each session. The task duration was 17 minutes and 36 seconds. Imaging data were acquired in the LUMC using a Philips 3T system (Philips Healthcare, Best, The Netherlands; software version 3.2.1). A SENSE-32 channel headcoil was used for transmission and reception of radio frequencies. For each

subject, anatomical imaging was acquired by means of a transvers 3D gradient-echo T1-weighted sequence (repetition time (TR) = 9.8, echo time (TE) = 4.6 ms, flip angle = 8°, Field of view (FOV) matrix size = 256 x 256, voxel size = 1.17x1.17x1.2 mm, 140 slides), which were examined by a neuroradiologist blinded for patient details. Other than age-related white matter intensities and effects of post-transsphenoidal surgery, no further macroscopic abnormalities were detected. ToL fMRI echo-planar images (EPI) were acquired using a T2*-weighted gradient-echoplanar imaging sequene (EPI) (TR = 2200 ms, TE = 30 ms, flip angle = 80°, 38 transverse slices, no slice gap, FOV = 220 × 220 mm, voxel size = 2.75 × 2.75, 3 mm slice thickness), which was then registered to the MNI T1-template brain.

Data analysis

Task performance and clinical characteristics

Psychometric and task performance data were analyzed using IBM SPSS Statistics for Windows version 24 (IBM Corp. Armonk, N.Y., USA). If the data did not meet the assumptions required for parametric analyses, the appropriate nonparametric tests were performed (i.e. Mann-Whitney U test). VAS scores and performance were analyzed using paired samples t-tests. Proportion correct scores and mean response times per trial were entered as dependent factors in the analyses.

Image processing

Preprocessing and analyzing of the ToL data was conducted using FSL v.5.0.8. Preprocessing included artefact removal with FSL FIX (Salimi-Khorshidi et al., 2014), motion correction (realignment), grand mean scaling, and spatial smoothing with 6mm Gaussian kernel. ICA-AROMA (Pruim et al., 2015) was used for motion artefact removal, and high-pass filtering was used. FSL FEAT was used to create first-level statistical parametric maps.

The fMRI ToL paradigm was modelled in an event-related manner with regressors (i.e. explanatory variables) made by convolving each event-related stimulus function (baseline, 1-5 step trials), with a canonical hemodynamic response function, and then modulated using reaction times. Low-frequency noise was stripped by applying a high-pass filter (set at a cut-off of 128 seconds) to the time series at every voxel.

Main effects of task and between-group comparisons

Analysis were conducted in line with the ToL analysis approach reported in van Tol et al. (2011). Contrast images for task load, which ranged from trial type 1-5 with weighting [-1.5, -1.0, -0.5, 1, 2] based on level of cognitive demand respectively, were

calculated per subject on a voxel-by-voxel basis and then entered into a second-level analyses for between group comparisons (remitted CD and HC). Thus, in all cases, activation of the regions specified were modulated by the complexity of the task.

Thresholds for the main effects of the task and between group comparisons were corrected using a cluster z -threshold of 2.3 with $p < 0.05$. Between group comparisons were conducted using the ACC as a region of interest (ROI). This region was defined using the Harvard-Oxford Cortical Structural Label Atlas implemented in FSLeves (version 5.0.10). This was followed by an exploratory whole-brain analysis per trial step. Both the ROI- and the exploratory analyses were carried out using FSL FEAT fMRI analysis and analyzed as matched pairs in order to identify differences in planning activity between the remitted CD population and the HCs (Beckmann, Jenkinson, & Smith, 2003; Woolrich et al., 2004). Significant clusters per trial step were tested for correlations between measures of psychiatric symptom severity, cognitive functioning and clinical severity and corrected for multiple testing. As the questionnaires used for the behavioral assessment show considerable overlap, correction for multiple testing using the Benjamini-Hochberg (Benjamini and Hochberg, 1995) method with an FDR set at 5% was considered too stringent. For this reason, we corrected for multiple testing using an FDR set at 20%. We report the uncorrected Pearson's correlations for normally distributed data, and the Spearman's rho for data that is not normally distributed.

Results

Sample characteristics

Three subjects (1 remitted CD patient and 2 HCs) and their respective matched pairs (thus $n = 6$), were excluded from the analyses because they did not meet the prespecified overall performance percentage of more than 75% correct responses. This was done in order to increase the likelihood of capturing task-based planning activity and to reduce possible non-task related bias, resulting in a total of 21 pairs of participants. Remitted CD patients and the HCs were well-matched as they did not differ significantly in gender, age, education, and intracranial volume (ICV). Mean MADRS, IDS, BAI, and AS scores differed significantly between remitted CD and HC groups (all $p < 0.02$), whereas mean scores on the total FQ score and its subscales, and the IS did not (see Table 1 for further details). Mean disease duration in the remitted CD was 7.6 years and duration of remission, 10.7 years. Mean scores on the CSI were 7.95 (SE = 0.428) during the active phase and 2.33 (SE = 0.340) upon remission of CD.

Table 1. Demographic and clinical characteristics remitted CD (RCD) patients and matched HCs. Data are presented as mean \pm standard deviation or number (%), with a significance level set at $p < 0.05$.

	RCD patients (n=21)	HCs (n=21)	<i>p</i> -value
Gender (female, (%))	17(81%)	17(81%)	1.00 ^a
Age (years \pm SD)	45.9 \pm 7.1	44.6 \pm 7.7	0.57 ^b
Education (years \pm SD)			1.00 ^a
Low	5 (23.8%)	5 (23.8%)	
Medium	10 (47.6%)	10 (47.6%)	
High	6 (28.6%)	6 (28.6%)	
ICV mm ³ (mean \pm SD)	1.51·10 ⁶ \pm 1.41·10 ⁵	1.52·10 ⁶ \pm 1.69·10 ⁵	0.76 ^b
MADRS (mean \pm SD)	5.43 \pm 3.91	1.38 \pm 1.80	<0.001 ^c
Inventory of Depressive Symptomatology (mean \pm SD)	45.55 \pm 12.60	36.10 \pm 6.07	0.02 ^c
Beck Anxiety Inventory (mean \pm SD)	28.15 \pm 6.10	24.05 \pm 3.34	0.02 ^c
Fear Questionnaire (mean \pm SD)	22.85 \pm 17.10	14.52 \pm 9.94	0.07 ^b
Agoraphobia subscale	5.30 \pm 6.69	2.67 \pm 3.26	0.52 ^c
Blood injury phobia subscale	6.45 \pm 9.04	3.76 \pm 4.28	0.73 ^c
Social phobia subscale	11.10 \pm 7.33	8.10 \pm 4.89	0.13 ^b
Irritability Scale	11.90 \pm 8.99	8.52 \pm 6.45	0.23 ^c
Apathy Scale	13.6 \pm 6.6	7.8 \pm 3.8	0.002 ^c
Cognitive Failures Questionnaire	35.60 \pm 14.17	29.0 \pm 9.46	0.09 ^b
Disease duration (years)	7.55 \pm 8.39		
Duration of remission (years)	10.68 \pm 7.69		
Cushing's Syndrome Severity Index (CSI)	7.95 \pm 1.96		
Active phase (total)			
Remission phase (total)	2.33 \pm 1.56		

ICV = Intracranial volume; MADRS = Montgomery-Åsberg Depression Rating Scale

^a *p*-values were tested with χ^2 test

^b *p*-values were tested with independent samples *t*-test

^c *p*-values tested with Mann-Whitney *U* test

Behavioral results

Mean VAS scores, mean accuracy scores, and mean response types per trial type are reported in Table 2. Remitted CD patients reported significantly higher levels of anxiety in comparison to healthy controls ($p = 0.02$) both before and after the task ($p = 0.006$). Overall, mean accuracy decreased with increasing task load. This did not differ significantly between the groups on any of the step trials. Also, performance speed increased as task load increased in both groups, although no differences in response times on any of the trial steps were found. An overview of the mean number of trials per trial type and group can be found in Appendix 1. Although the remitted CD group completed less trials per step in comparison to the HC group, this did not differ significantly between groups on any of the trial steps.

Table 2. Overview of proportion correct answers and response times (in seconds) per group (remitted CD (RCD) and Healthy Controls (HC)).

Variable	RCD (n = 21)	HC (n = 21)	Mean difference	p-value
VAS prior to ToL (total score)	37.86 (29.63; 46.09)	19.81 (11.68; 27.94)	18.05 (3.87; 32.22)	p=0.01
VAS after ToL (total score)	34.05 (27.03; 41.07)	16.14 (9.04; 23.24)	17.90 (5.78; 30.03)	p=0.006
Proportion correct:				
- Baseline	0.98 (0.97; 0.99)	0.97 (0.96; 0.98)	0.00 (-0.01; 0.02)	p=0.56
- 1 step	0.93 (0.91; 0.95)	0.96 (0.94; 0.98)	-0.04 (-0.10; 0.03)	p=0.23
- 2 steps	0.91 (0.89; 0.93)	0.92 (0.90; 0.94)	-0.02 (-0.08; 0.05)	p=0.58
- 3 steps	0.91 (0.87; 0.95)	0.91 (0.87; 0.95)	-0.01 (-0.08; 0.06)	p=0.85
- 4 steps	0.79 (0.75; 0.83)	0.83 (0.79; 0.87)	-0.05 (-0.14; 0.05)	p=0.30
- 5 steps	0.73 (0.67; 0.79)	0.80 (0.74; 0.86)	-0.07 (-0.19; 0.05)	p=0.24
- Total	0.92 (0.91; 0.93)	0.93 (0.92; 0.94)	-0.17 (-0.49; 0.15)	p=0.27
Response time(s):				
- Baseline	3.26 (2.95; 3.57)	3.24 (2.81; 3.67)	0.12 (-0.52; 0.54)	p=0.96
- 1 step	4.86 (2.84; 6.88)	5.00 (2.47; 7.53)	-0.14 (-1.01; 0.73)	p=0.74
- 2 steps	6.73 (5.67; 7.79)	6.01 (5.30; 6.72)	0.72 (-0.74; 2.19)	p=0.31
- 3 steps	8.44 (7.24; 9.64)	8.62 (7.33; 9.91)	-0.18 (-2.24; 1.88)	p=0.86
- 4 steps	13.15 (10.72; 15.58)	12.15 (10.29; 14.01)	1.00 (-2.72; 4.72)	p=0.58
- 5 steps	15.32 (13.73; 16.91)	16.23 (13.76; 18.70)	-0.91 (-4.41; 2.59)	p=0.59

* p-values were tested using paired samples t-tests
 **VAS: Visual Analogue Scale measuring anxiety prior to the ToL task and after the ToL task

fMRI results

Main task effects

No participants were excluded from the analyses due to movement or scanning artifacts. The task effects across all participants identified two significant activity clusters: (i) in the superior frontal gyrus and the frontal pole, and (ii) in the cingulate gyrus (posterior division) and the precuneus cortex ($p < 0.05$ for both clusters after cluster correction; see Table 3 and Figure 2). No main effects of increasing task load were found in both the remitted CD group and the HC group. This is likely due to a lack of power due to less trials in the more difficult steps (Appendix I). No significant activity clusters were found in the ROI. Significant activity clusters were found in the parietal operculum cortex on 2 step trials ($z = 3.75, p = 0.005$ after cluster correction), and in the supramarginal gyrus on 3 step trials in the remitted CD patient group ($z = 3.27, p = 0.02$ after cluster correction) in comparison to HCs. Group comparisons on the other trial steps did not reveal further significant differences (see Table 3). However, at a lower threshold (1.9), significant activity clusters were found in the remitted CD group on 4 and 5 step trials (see Appendix II).

Table 3. Mean task activation and planned paired comparisons of activity related to increasing task load at threshold 2.3.

Mean task activation/ paired comparison	Area	Side	Cluster size	Peak Voxel (MNI)			<i>p</i>
				<i>x</i> (mm)	<i>y</i> (mm)	<i>z</i> (mm)	
Mean task activation	Superior Frontal Gyrus/ Frontal Pole	L	801	-6	56	26	0.004**
	Cingulate gyrus, posterior division/ Precuneus Cortex	R	554	2	-40	44	0.03**
ROI							
ACC RCD*>HC	None						
ACC RCD<CD	None						
1 Step							
RCD>HC	None						
RCD<HC	None						
2 steps							
RCD>HC	Parietal Operculum Cortex	R	664	60	-36	26	0.005**
RCD<HC	None						
3 steps							
RCD>HC	Supramarginal Gyrus	R	527	58	-42	20	0.02**
RCD<HC	None						
4 steps							
RCD>HC	None						
RCD<HC	None						
5 steps							
RCD>HC	Occipital Fusiform Gyrus/ Lingual Gyrus	R	599	20	-74	-18	0.01**
RCD>HC	Supramarginal Gyrus	R	478	58	-38	28	0.04**
RCD<HC	None						

*RCD = remitted Cushing's Disease patients

*Thresholded using Cluster correction $z = 2.3$; $p < 0.05$.

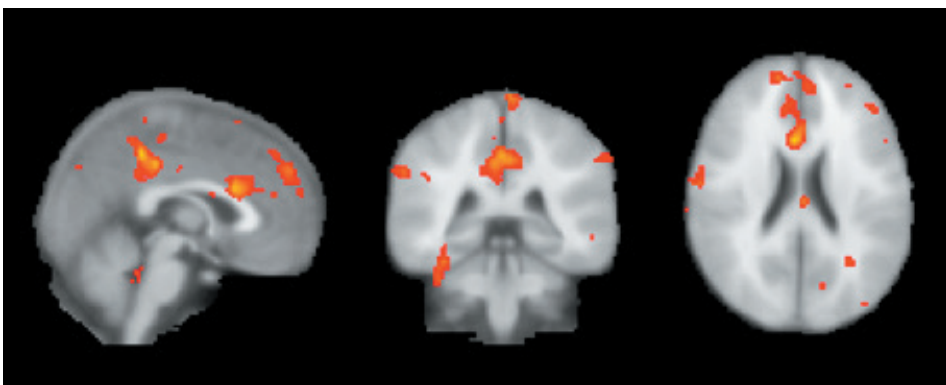


Figure 2. Mean activity during task performance across the subjects displayed at cluster z -threshold of 2.3 with $p < 0.05$ [-39, 36, 30].

Correlation analyses

After adjusting for multiple comparisons using the Benjamini-Hochberg procedure (Benjamini & Hochberg, 1995) using an FDR set at 20%, activation in of the right parietal operculum cortex in remitted CD patients was found to be significantly negatively associated with the prior active disease state on the CSI ($r = -0.519$, $p = 0.02$). No other significant associations between significantly activated clusters and scores on behavioral scales, measures of disease duration, duration of remission, and clinical disease severity were found.

Discussion

In this study, we investigated whether patients with remitted CD displayed altered performance and brain activity patterns in comparison to HCs using the ToL, a parametric visuospatial planning task. No differences in performance were found between the groups, neither in the number of trials completed correctly, nor in the number of trials completed in total, nor in the amount of time needed to complete the trials. Although mean task activation was identified in two brain clusters, our ROI analysis of the ACC did not yield any significant difference in activation between the groups. However, an exploratory whole-brain analysis identified areas of increased brain activity in the remitted CD group on 2-, 3-, and 5-step trials. Finally, a negative association between activation of the right parietal operculum cortex in remitted CD group with the prior active disease state as measured on the CSI was found. These findings indicate that CD may result in subtle scarring effects despite long-term remission in certain brain areas when completing demanding executive functioning tasks.

As mentioned earlier, previous research conducted with remitted CD patients found impairments in multiple domains of neurocognitive functioning (i.e. Hook et al., 2007; Ragnarsson et al., 2012; Tiemensma et al., 2010; Zarino et al., 2019). We therefore hypothesized that remitted CD patients would also present impairments on the ToL task by completing less trials correctly, completing less trials in total, and taking more time to complete a ToL trial in comparison to HCs. Surprisingly, remitted CD patients showed no cognitive and executive functioning deficits in comparison to HCs as measured on the ToL. As several studies have identified visuospatial impairments in active CD patients (Siegel et al., 2020), albeit using other measurement instruments, our findings suggest that certain visuospatial impairments may improve upon remission of CD. However, further insight into whether remission of CD also remits all or most visuospatial impairments should be

confirmed in longitudinal studies comparing performance on the ToL in the active disease state with the long-term remission state.

With regard to fMRI brain activation patterns on the ToL, an earlier study with HCs aimed at validating the ToL paradigm for fMRI found mean task activation in a number of brain areas (i.e., in the dorsolateral prefrontal cortex, the cingulate cortex, the cuneus, the supramarginal and angular gyrus in the parietal lobe, and the frontal opercular area of the insula, Lazeron et al., 2000). We were unable to replicate these results in current sample of HCs as it was too small, however we did investigate mean task activation over the whole sample. Although we did not expect to find precisely the same activation in all of the brain areas found in the aforementioned study due to the differences in our study populations and MRI scanners, we did expect to find a certain amount of overlap. We identified mean group activation in two separate brain clusters: (i) the superior frontal gyrus and the frontal pole, and (ii) the posterior division of the cingulate gyrus and the precuneus cortex. Our first cluster (i.e., the superior frontal gyrus (part of the dorsolateral prefrontal cortex), and the frontal pole), partially overlapped with one of the activated areas found in the Lazeron et al. (2000) paper (i.e. the dorsolateral prefrontal cortex). This area has been found to be involved in the management of uncertainty, where increasing uncertainty leads to increased activation (Volz, Schubotz, & von Cramon, 2005), and the frontal pole has been implicated in cognition, perception, and working memory (Bludal et al., 2014). With regard to the second cluster identified (i.e. the cingulate gyrus (an area in the cingulate cortex) and the precuneus cortex), this largely overlaps with an area found in the Lazeron et al. (2000) study (i.e. the cingulate cortex and the precuneus). These overlapping findings increase the validity of our current findings, and provide further evidence regarding the specific brain areas that are recruited during visuospatial planning tasks.

Considering the differences in brain structure and activation found in earlier studies with this same population of remitted CD patients (i.e. Andela et al., 2013; van der Werff et al., 2015), we hypothesized to find increased activation in the ACC, an area involved in several complex cognitive functions and critically active when engaging in a cognitively demanding task (Fincham & Anderson, 2006), in comparison to matched HCs. However, we did not find any differences in activation between the groups. This indicates that although the ACC has previously been implicated in displaying altered resting-state brain activity, altered gray matter volumes, and altered white matter integrity in this same patient group (van der Werff et al., 2015; Andela et al., 2013; Van der Werff et al., 2014), they are not overrecruited during

the ToL task. It could, however, be the case that both patients and HCs overrecruit the ACC in this type of visuospatial planning and executive functioning task, as it is an area involved in several complex cognitive functions (Stevens et al., 2011).

As mentioned earlier, certain regions were not identified in our mean group activation that were identified in the Lazeron et al. (2000) paper. Interestingly, several of these areas were found to be more activated in the remitted CD group on a number of the trial steps. Increased right parietal operculum cortex recruitment was found as a function of increased planning load on 2-step trials in the remitted CD group. This is an area involved in mathematical thought, visuospatial cognition, and imagery of movement, among other functions (Witelson, Kigar, & Harvey, 1999). Also, increased right supramarginal gyrus recruitment was found as a function of increased planning load on 3- and 5-step trials. This brain area has been found to be involved in complex cognitive functions, such as calculation and visuospatial awareness (De Schotten et al., 2005). These findings indicate that remitted CD patients need to overrecruit these brain regions to attain a similar performance level as the HCs. Moreover, increased recruitment in the occipital fusiform implicated in higher processing for visual information such as the processing of color information, word recognition, and working memory capacity, amongst others (Ramachandran, 2011; McCandliss, Cohen, & Dehaene, 2003; Brunyé, Moran, Holmes, Mahoney, & Taylor, 2017), and lingual gyri was found on the 5-step trials. Both regions (i.e. the occipital fusiform and lingual gyri) have demonstrated to play an important role in color perception (i.e. Sakai et al., 1995; Sereno et al., 1995). In sum, this indicates that remitted CD patients primarily characterize themselves in increased recruitment of the abovementioned brain regions on certain trial steps, and not in executive and cognitive functioning as measured in the number of trials answered correctly or the time needed to answer each trial.

Although no differences in altered brain activity were found on the 4-step trials, we believe this was likely due to lack of power (i.e. too few trials to be able to identify a possible effect). We therefore ran further exploratory whole-brain analyses at a lower threshold (i.e. 1.9) for all trial steps (see Appendix II). Although we cannot interpret these results as we interpret the results set at the more stringent and accepted threshold, we did find increased activation in the precuneus of the remitted CD group, an area that was also found to be overrecruited in the mean task activation. Moreover, activation in this area was also observed in 5-step trials at this lower threshold. Previous studies have shown activation in the precuneus during action generation tasks (Allendorfer et al., 2012), as well as in visuospatial and -motor imagery (Cavanna & Trimble, 2006; Kawashima, Roland, & O'Sullivan,

1995). It has also been suggested to be involved in the direct visual route from vision to action, functioning in extracting visual-motor and spatial relationship features (Wang et al., 2019).

A negative association was found between the right parietal operculum cortex in remitted CD patients and the CSI score of the prior active disease state, although this result should be interpreted with caution as the FDR rate used was adjusted due to overlap in the behavioral assessment measures. However, this association indicates that the more severe the active disease state (as was measured using the CSI), the less activation in the right parietal operculum cortex, a region that has been found to be involved in mathematical thought, specifically in the knowledge of numbers and their relations (Blackmore & Firth, 2005). This seems to imply that this brain region may be less proficient in increasing activation in remitted CD patients who have experienced a more severe active phase of CD. There were no further significant associations found between activated brain clusters and scores on behavioral scales, measures of disease duration, and duration of remission.

The hypothesis has been posited that studying patients with remitted CD could offer further insight into the effects of prolonged cortisol exposure on, amongst others, the brain, as these findings may in turn be (partially) generalizable to other remitted stress-related disorders (such as depression and/or anxiety), as well as to conditions treated with synthetic glucocorticoids. A previous study investigating the neural correlates of the ToL task in out-patients with (remitted) depression and anxiety found that only patients with a current moderate or severe depression had increased dorsolateral prefrontal cortex activation as a function of increasing task load, whereas patients with current mild or remitted depression, with a current diagnosis of anxiety disorder(s) (such as generalized anxiety disorder and/or panic disorder and/or social anxiety disorder) did not, in comparison to HCs (van Tol et al., 2011). Thus, it seems that the prolonged excess exposure of endogenous cortisol on the brain in the magnitude as is the case with CD, leads to seemingly permanent alterations in brain activation of certain brain regions after long-term disease remission in contrast to, for example, patients with remitted depression.

Due to the cross-sectional nature of this study, causal conclusions cannot be drawn as we cannot be certain whether the found differences in psychopathology or brain activity were present prior to the onset of CD. Also, we cannot know for certain whether other factors that occurred over the course of the seven-year remission period influenced the observed changes in the neural networks. A further possible

limitation of this study is the use of the CSI to evaluate disease severity during the active phase. Although this instrument has been validated repeatedly, it does make use of retrospective assessments, which may lead to less accurate estimations. Study strengths were the homogeneity of the patient population (i.e. all of the patients included in the study were treated by means of transphenoidal surgery), and the selection of the age-, gender-, and education matched HCs. Nevertheless, heterogeneity was present in the remitted CD patient group regarding duration of the disease and duration of remission, and this therefore may have decreased the precision of the effect estimates of the study.

In conclusion, we found no evidence for pervasive cognitive impairments for the domain of visuospatial planning and executive functioning as measured on the ToL task in remitted CD patients. We did find differences in brain activation in the remitted CD patient group on 2-, 3- and 5-step trials, namely an over-recruitment of a number of brain regions predominately involved with higher cognitive functioning. This increased activation implies that remitted CD patients require increased effort of certain brain regions to successfully complete this visuospatial planning task, although they do not need more time to do so accurately, indicating a subtle scarring due to CD. In the future, longitudinal studies are necessary to provide further insight with regard to the onset and course of alterations in cognition and brain activity patterns in the CD patient population during the active disease state and the transition into remission.

Appendix I. Overview of number of trials per step per group (remitted CD (RCD) and Healthy Controls (HCs) on the Tower of London task.

	RCD (n=21; no. of trials, (%))	HCs (n = 21; no. of trials, (%))	Rounded mean no. of trials per participant remitted CD group (%)	Rounded mean no. of trials per participant HC group (%)
Baseline	1376 (40.6)	1410 (40.5)	66 (40.7%)	67 (40.4%)
1 step	647 (19.1)	669 (19.2)	31 (19.1%)	32 (19.3%)
2 steps	535 (15.8)	539 (15.5)	25 (15.5%)	26 (15.7%)
3 steps	347 (10.2)	358 (10.3)	17 (10.5%)	17 (10.2%)
4 steps	292 (8.6)	303 (8.7)	14 (8.6%)	14 (8.4%)
5 steps	195 (5.7)	200 (5.7)	9 (5.6%)	10 (6.0%)
Total	3392 (100.0)	3479 (100.0)	162 (100%)	166 (100%)

Appendix II. Effects of paired testing per trial step at threshold 1.9 for remitted CD (RCD) and Healthy Controls (HCs).

Paired comparison	Area	Side	Cluster size	Peak Voxel (MNI)			<i>p</i> *
				x (mm)	y (mm)	z (mm)	
1 step							
RCD>HC	Cingulate Gyrus, posterior division	R/L	1361	6	-48	20	0.005
RCD<HC	None						
2 steps							
RCD>HC	Supramarginal gyrus, posterior division/ parietal operculum cortex	R	2242	60	-36	26	6.53e-05
RCD<HC	None						
3 steps							
RCD>HC	Supramarginal Gyrus (posterior division)/	R	1105	58	-42	20	0.02
RCD>HC	Intracalcarine Cortex	L	1008	-16	-70	4	0.03
RCD<HC	None						
4 steps							
RCD>HC	Precuneous Cortex	R	965	0	-44	56	0.03
RCD<HC	None						
5 steps							
RCD>HC	Lingual Gyrus/Occipital Fusiform Gyrus	R	5398	-2	-36	-10	1.01e-09
RCD>HC	Supramarginal Gyrus, posterior division	R	1692	58	-38	28	<0.001
RCD>HC	Precentral Gyrus	L	1289	-58	4	36	0.007
RCD>HC	Precuneous Cortex	R	1265	2	-44	66	0.008
RCD<HC	None						

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