Elevated resting-state connectivity in the medial temporal lobe and the prefrontal cortex among patients with Cushing's syndrome in remission

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Abstract

Objective: Cushing's syndrome is associated with long-term cognitive deficits and affective symptoms such as depression and anxiety. The alterations in brain function underlying these deficits after Cushing's syndrome are unclear and therefore we aimed to explore alterations in resting-state functional connectivity in patients with Cushing's syndrome in remission.

Design: Cross-sectional case–control study.

Methods: Nineteen women with Cushing's syndrome in remission for a median time of 7 years (IQR: 6–10) and a mean age of 45 years were included at three university clinics. These patients and 38 age-matched female controls underwent brain imaging at a single center. The main outcome measure was functional connectivity at rest, measured with functional magnetic resonance imaging.

Results: The medial temporal lobe (MTL) and prefrontal cortex networks, exhibited elevated functional connectivity among patients compared to controls. The degree of elevated functional connectivity in the MTL was negatively associated with time in remission.

Conclusions: Resting-state functional connectivity within glucocorticoid receptor-rich regions, particularly the MTL and medial prefrontal cortex, was increased in patients. These differences in connectivity may provide a neural basis for the cognitive deficits and affective symptoms commonly experienced by patients with Cushing's syndrome in remission.

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Introduction

Glucocorticoid receptors are distributed throughout the brain, with the highest concentration in the hippocampus and prefrontal cortex (1, 2, 3). Several animal models indicate that chronically elevated cortisol levels, either

by direct administration in the CNS, or stress exposure, influence neuronal functions in these brain regions (4, 5, 6). Similarly, human neuroimaging studies have shown that hypercortisolism alter brain function in

various stress-related disorders such as depression (7, 8) and in Cushing's syndrome (CS) (9, 10, 11).

Hypercortisolemia caused by CS offers a model to study the effects of chronically elevated cortisol on the human brain. Indeed, several studies suggest that CS is associated with long-term cognitive impairments, increased anxiety, fatigue and depressive symptoms that still persist several years after remission has been achieved (12, 13, 14).

The most commonly affected cognitive domains in CS include short- and long-term memory as well as attention (12, 13). Notably these functions are primarily controlled by the prefrontal cortex and the hippocampus (15, 16), thus matching the brain areas with abundant expression of the glucocorticoid receptor (17). Furthermore, depressive symptoms and anxiety that are common complications to CS have been linked to increased connectivity in the medial prefrontal cortex and the limbic system including the amygdala and hippocampus (18, 19, 20).

Alterations in brain structure and function have been suggested to provide a substrate for the psychiatric and neurocognitive symptoms among patients with CS in remission. Patients with active Cushing's disease have reduced hippocampal volumes, which seems to normalize after treatment (21, 22). However, among patients with CS in remission and memory impairments, reduced hippocampal volumes persists (23). In addition, smaller volume in neocortical regions such as the anterior cingulate cortex have also been found among patients with CS in remission (24). Moreover, a functional neuroimaging study showed a reduced functional brain activity during memory encoding in the lateral prefrontal cortex in patients with CS (11).

Resting-state functional magnetic resonance imaging (rsfMRI) is an emerging tool used to map the functional architecture of the brain (25, 26). By measuring coherent spontaneous activity (i.e. functional connectivity (FC)) at rest, several resting-state network (RSNs) have been identified (25, 26, 27, 28, 29, 30, 31). These RSNs are altered in several neurological conditions such as Alzheimer's disease, Parkinson's disease and traumatic brain injury, suggesting that measures of RSNs might serve as a sensitive biomarker for these conditions (32). Alterations in some RSNs have been reported in patients with CS in remission (10, 33). More specifically, in comparison with the healthy control, CS patients showed elevated FC within the default mode network (DMN), but also between anterior cingulate cortex and the limbic network. The DMN contains distinct subsystems (34, 35, 36), including hippocampus, which might be differentially affected in different conditions

such as aging (37). On this view, along with the previous observation that a high concentration of glucocorticoid receptors is found in the hippocampus, it is reasonable to expect that elevated FC might be more pronounced in the hippocampus subsystem in patients with CS in remission as compared to controls.

In this case-control study, we compared patients with CS in remission with age-matched control using fMRI at rest. A data-driven, independent component analysis (ICA), was used to identify RSNs. We hypothesized that the patients display elevated FC in the medial and lateral prefrontal cortex as well as the medial temporal lobe (MTL) including the hippocampus, brain regions with a high glucocorticoid receptor density.

Subjects and methods

Study design

This was a multicenter, cross-sectional, case-control study on 19 women with CS in remission and 38 age-matched female controls. The patients were studied on two occasions with ≤ 12 weeks (median 6, range 0–11) between the visits. During an inclusion visit, medical history was reviewed, a physical examination was performed and blood and urine samples were collected. The second visit took place at the Department of Radiation Sciences (Diagnostic Radiology) in Umeå where a MRI session including rsfMRI was conducted as described previously (11).

Ethical considerations

Informed oral and written consent was obtained from all participants. The study was approved by the Local Ethical Committees at the University of Gothenburg, University of Oslo and Umeå University. The study was conducted according to the Declaration of Helsinki.

Patients

Nineteen right-handed women diagnosed and treated for CS at three university hospitals (Sahlgrenska University Hospital and Umeå University Hospital, both in Sweden, and Oslo University Hospital-Rikshospitalet in Norway) participated in the study (Table 1). Eligible for participation were women, 20–70 years of age, in remission for 2–15 years after treatment for either adrenocorticotrophic hormone-secreting pituitary adenoma (Cushing's disease (CD)) or cortisol-producing adrenal adenoma. Patients receiving

Table 1 Characteristics of the women with Cushing's syndrome in remission and controls. Data is presented as mean (s.d.), *n* (%) or median (IQR).

	Patients (<i>n</i> = 19)	Controls (<i>n</i> = 38)	<i>P</i> value
Age at diagnosis	36 ± 12		
Age at fMRI	45 ± 11	45 ± 12	0.9
Duration of active CS	5 (3.5–6.5)		
Duration of remission	7 (6–10)		
Educational level			0.02
Elementary school	1 (5%)	2 (5%)	
Upper secondary education	11 (58%)	8 (21%)	
University education	7 (37%)	28 (74%)	
Smoking habits			0.9
Non-smoker	12 (63%)	22 (58%)	
Ex-smoker	5 (26%)	11 (29%)	
Smoker	2 (11%)	5 (13%)	
Antidepressant treatment	3 (16%)	2 (5%)	0.3

hormone replacement treatment had to have a stable dose for at least 6 months. Patients previously treated with pituitary radiation therapy, and patients receiving more than 20 mg of daily hydrocortisone, or equivalent dose of cortisone acetate, were not included. Also, patients with any other chronic disease potentially affecting cognitive function and patients with clinically evident depressive symptoms were not considered eligible.

Fourteen of 19 patients (74%) had previously been diagnosed with CD, all treated by transsphenoidal pituitary surgery. Two patients had been operated on two occasions, one due to recurrence 8 years after a successful operation and one since the first operation was unsuccessful. None had been treated with bilateral adrenalectomy. Five patients (26%) had been diagnosed with cortisol-producing adrenal adenomas, all successfully treated with unilateral adrenalectomy.

Remission was confirmed in all patients based on 24-hour urinary free cortisol concentration within the normal reference range and serum cortisol concentration ≤50 nmol/L following a 1-mg overnight dexamethasone suppression test. Pituitary and adrenal functions were evaluated clinically and by measuring serum-free thyroxine, thyroid-stimulating hormone, estrogen, DHEAS, androstenedione, gonadotropins and insulin-like growth factor I. In patients treated for CD, growth hormone status was evaluated with insulin tolerance test (*n*=8), combined arginine and growth hormone-releasing hormone (*n*=5) or an arginine stimulation (*n*=1).

Three patients received hydrocortisone replacement therapy (5, 10 and 20 mg per day, respectively), 8 had

levothyroxine and 7 were receiving growth hormone replacement. Of 12 premenopausal women, one had hypogonadotropic hypogonadism and was receiving estrogen and progesterone as replacement therapy.

Estimation of the duration of active CS was obtained by review of medical records and by asking the patients to estimate for how long they had symptoms related to CS before diagnosis.

Controls

Data for the 38 age-matched women in the control group was obtained from an ongoing prospective cohort study at Umeå University (Table 1; 29, 38). The control group was examined with MRI between 2008 and 2010 on the same MRI scanner with an identical sequence. No subject from the control group suffered from dementia, which was excluded by a clinical investigation. One control was being treated with prednisolone due to rheumatic disease and two with antidepressants.

Image acquisition

The rsfMRI was conducted on a 3T Discovery MR750 (General Electric) with a 32-channel headcoil. An echo planar imaging sequence was used (37 transaxial slices, thickness; 3.4 mm, gap; 0.5 mm, repetition time; 2000 ms, echo time; 30 ms, flip angle; 80°, field of view; 25×25 cm, 170 volumes). Ten dummy scans were collected before the experimental image acquisition. During scanning, participants were instructed to lay still, watch a white cross on a black background, stay awake and be quiet.

Resting-state data analysis

rsfMRI images were preprocessed using the Statistical Parametric Mapping Software (Wellcome Department of Imaging Science, Functional Imaging Laboratory, University College London). Preprocessing included correction for differences in slice-time acquisition within each volume, motion correction, alignment of functional and structural images using within-subject rigid registration, out regression of movements with the Friston 24-parameters model (39), normalization of the realigned nuisance-corrected images using a sample-specific template created with Diffeomorphic Anatomical Registration using Exponentiated Lie Algebra, alignment of images to the Montreal Neurological Institute template and smoothing with a 6 mm full-width at half-maximum Gaussian filter.

The GIFT toolbox (30) was used to analyze the preprocessed rsfMRI data with temporal concatenation-independent component analysis (ICA) as previously described (29, 34, 37). ICA is a multivariate method that extracts patterns of the brain (i.e., components) that are spatially independent but temporally coherent. A time series of each voxel was initially intensity normalized to improve test-retest reliability of subsequent ICA output (Allen et al. 2011), and then normalized data for all participants were concatenated across time. Using minimum description length algorithm, 40 independent components (ICs) were estimated. Two-step data reduction was carried out using principal component analysis. The Infomax ICA algorithm (40) was conducted to extract 40 ICs. The latter procedure was repeated 20 times using the ICASSO toolbox (41) and the resulting components were clustered to estimate the reliability of the ICs. The reliability index (Iq; ranges from 0 to 1) was greater than 0.90 for all except one component (Iq=0.78) which was not among components of interest. Finally, GICA3 method was used for a back-reconstruction (42). As such, time courses (TCs) and spatial maps (SMs) in the unit of percent signal changes were computed for each subject. After visually inspecting the group average maps, 32 RSNs, including MTL and PFC, were identified. These RSNs showed peak activation in the gray matter and exhibited low spatial overlap with the topology of potential artifacts (e.g., vascular, ventricle, motion and susceptibility artifacts).

For each component, we computed two ICA-driven measures that reflect distinct but complementary facets of RSNs: (1) Component SMs reflected the level of coactivation (connectivity) within a network in a voxel-wise manner (voxel-wise connectivity). An SM was created after the back-reconstruction step of the group ICA, which creates subject-specific maps from a corresponding group-level IC. The value of each voxel in a SM represents the degree to which the voxel is correlated with a given networks' mean TC. (2) Global indices of FC reflected a subject-specific connectivity within the entire network. As suggested by Glahn and colleagues (43), individual 3D subject-specific SMs for each network were concatenated into a single 4D map, and the first principal eigenvector representing the subject's connectivity was calculated within a study-specific mask of the corresponding network. If differences in FC are so widespread across a network, both global and voxel-wise estimates should reflect a significant group difference. However, if group difference is only expressed

in a specific portion of a network, a significant difference in voxel-wise, but not global, connectivity might be observed. On this view, networks with differences in both global and voxel-wise connectivity are those with strongest group difference.

Statistical analysis

The global connectivity within each RSN was compared between groups using ANCOVA with age as a covariate since age was significantly associated with the global connectivity in all but one RSN. The chi-square test or Fisher's exact test were used to compare proportions and an independent samples *t*-test was used to compare other parameters between groups. The Pearson correlation coefficient was calculated to test associations between connectivity in the RSNs and time in remission, as well as estimated duration of active CS. A *P* value <0.05 was considered significant in these analyses. For the voxel-wise analysis of rsfMRI data a *P* value <0.05, corrected for the false discovery rate (FDR (44)) and an extent threshold of five voxels were considered significant.

Results

Mapping RSNs

Our primary results stem from ICA decomposition of rsfMRI data at an ICA dimensionality of 40. Thirty-two of the 40 components were considered to reflect RSNs. These networks displayed a similar spatial pattern as presented among healthy individuals in a previous rsfMRI study (30). These networks included the sensorimotor, visual, cerebellar, auditory and fronto-parietal networks. Critically, we found frontal networks as well as the DMNs, including the MTL network. The frontal networks included the medial PFC as well as ventrolateral and dorsolateral PFC. The MTL network included left and right hippocampus and parahippocampal gyri.

Stronger within-network connectivity in the prefrontal cortex and MTL

Consistent with our hypothesis, patients with CS had stronger FC than the controls in both prefrontal and MTL networks. For the prefrontal and MTL networks, the results of voxel-wise analyses showed an increased connectivity in patients compared to controls and

Figure 1

Statistical parametric maps of resting-state networks with significantly increased connectivity among patients compared with controls, (A) medial temporal lobe, (B) medial prefrontal cortex and anterior cingulate gyrus and (C) lateral prefrontal cortex. Green color represents the networks (T-value >8) and red color represents voxels with a significantly increased connectivity ($P < 0.05$ FDR) within each network. The bar charts represent the average connectivity in the whole network (green color in SPM) among patients (black) and controls (white). Significance for the differences in global average connectivity was tested with ANCOVA.

converged with the global connectivity indices (Fig. 1). More specifically, voxel-wise analyses in the MTL network revealed that patients had increased connectivity in the right hippocampus and parahippocampal gyrus (Table 2, Fig. 1A). Moreover, voxel-wise analyses of the prefrontal components showed increased within-network connectivity in large parts of the anterior cingulate gyrus-medial prefrontal cortex (Table 2, Fig. 1B) as well as the left and orbital gyrus located in the lateral PFC (Table 2, Fig. 1C).

Weaker within-network connectivity in the parietal lobe and sensory networks

Using a more exploratory analytic approach, other RSNs were also investigated. These analyses showed that some portions of the DMN, dorsal attentional network, visual and auditory networks showed lower FC in patients compared to controls (Table 3, Fig. 2A and B)

FC in relation to clinical parameters

Duration of remission was negatively associated with the global average within-network connectivity of the MTL (Fig. 3). Thus, patients with shorter time in remission had greater FC within the MTL. There were no significant associations between duration of remission and/or duration of CS before diagnosis, with connectivity in the other networks.

Sensitivity analysis excluding patients on antidepressant treatment

Three patients, one in remission for 4 years and two for 7 years, were on treatment with antidepressants (Citalopram, Mirtazapine and Mirtazapine and Sertraline, respectively). Furthermore, two controls were treated with Sertraline and Citalopram. Since previous studies indicate that antidepressants may alter FC in

Table 2 Resting-state networks with significantly ($P < 0.05$ FDR) increased within-network connectivity in a voxel-wise comparison between patients and age-matched controls.

Network	Location for peak	Volume (cm ³)	T _{max}	X	Y	Z	P*
Anterior cingulate cortex	Anterior cingulate cortex	11.3	8.3	-4	34	-6	0.023
Medial temporal lobe	R parahippocampal gyrus/R hippocampus	1.5	5.0	34	2	-32	0.039
Lateral prefrontal cortex	L/R orbital gyrus	0.3	5.0	-34	52	0	<0.001

*P was calculated by using ANCOVA, comparing the average within-network connectivity for the whole network (not peak) between patients and controls. L, left; R, right.

Table 3 Resting-state networks with significantly ($P < 0.05$ FDR) decreased within-network connectivity in a voxel-wise comparison between patients and age-matched controls.

Network	Location for peak	Volume (cm ³)	T _{max}	X	Y	Z	P*
Posterior default mode network	L Precuneus	18.2	6.2	−6	−40	16	<0.001
Dorsal attentional network	L Precuneus	3.0	5.6	−4	−44	72	0.001
Visual network	R Fusiform gyrus	5.8	6.1	24	−74	−6	0.005
Auditory network	L Superior temporal gyrus	3.1	4.3	−50	−32	12	0.016

*P was calculated by using ANCOVA, comparing the average within-network connectivity for the whole network (not peak) between patients and controls. L, left; R, right.

corticolimbic networks (45), we performed sensitivity analyses excluding these participants. Fifteen patients and 30 controls were included. The difference between patients and controls in global average connectivity in the anterior cingulate cortex decreased (patients=0.14 vs controls=0.11, between group difference: $P=0.072$), as well as the difference in the MTP (patients=0.13 vs controls=0.10, between group difference: $P=0.093$). Otherwise, there were no significant alterations compared with the original analysis in the other RSNs.

Discussion

We investigated alterations in resting-state FC among CS patients in remission for several years using ICA and dual regression. We found that the MTL and PFC networks exhibited elevated FC in patients compared to healthy controls. Interestingly, the degree of elevated FC in the MTL was negatively associated with duration of remission.

Thus, our results provide novel evidence of alterations in functional architecture of the brain among CS patients in glucocorticoid receptor-rich regions.

Increased cortisol levels in CS seem to cause functional alterations in the MTL, which may improve slowly over several years after remission has been achieved. Increased hippocampal FC was reported in previous studies of aging in which older adults were found to exhibit stronger connectivity within the MTL network including the hippocampus (29, 37). This was found to be deleterious for efficient episodic memory processing since excessive elevation of FC within MTL may prevent engagement of additional regions during task performance (29, 37). Thus, based on studies of memory function in aging individuals, our findings suggest that increased resting-state FC of MTL is linked to the memory impairments commonly found among CS patients.

In line with our *a priori* hypotheses, the within-network connectivity was increased in a RSN located in the anterior cingulate gyrus and medial prefrontal cortex.

Figure 2

Statistical parametric maps of resting-state networks with significantly decreased connectivity among patients compared with controls, (A) Posterior default mode network and (B) dorsal attentional network. Green color represents the networks (T-value >8) and blue color represents voxels with a significantly decreased connectivity ($p < 0.05$ FDR) within each network. The bar charts represent the average connectivity in the whole network (green color in SPM) among patients (black) and controls (white). Significance for the group difference in global average connectivity was tested with ANCOVA.

Figure 3

The association between time in remission of CS and the global average within-network connectivity of the medial temporal lobe ($r = -0.52$, $P = 0.022$, $n = 19$).

A previous study among patients with CS in remission found increased connectivity at rest between the limbic system and anterior cingulate gyrus (10). Thus, our results, by including a larger portion of the medial prefrontal cortex, confirm and extend these previous findings. The anterior cingulate and medial prefrontal cortex modulate activity in the limbic system and the hypothalamic-pituitary-adrenal axis (46, 47). Notably, conditions with increased endogenous cortisol production (48) such as treatment-naïve patients with depression and anxiety also have increased connectivity in the anterior cingulate gyrus at rest (49, 50). Thus, our results suggest that long-standing, but transient hypercortisolemia due to CS, may cause increased connectivity in the anterior cingulate gyrus, which in turn may be linked to anxiety and depressive symptoms, features that are common among patients with CS (14). However, to confirm this suggested cause and effect relationship among hypercortisolemia, brain connectivity and depression, a prospective study is needed.

The validity and the mechanistic link between CS and our findings is supported by the fact that the increased FC matches the glucocorticoid receptor expression in the human brain (1, 2, 3). Furthermore, animal studies in both rodents and primates suggest that increased corticosteroid levels and stress influence hippocampal neuronal function (51), including reduction of hippocampal neurogenesis (52) and reduces the number of dendritic spines in the medial PFC (5). Although these cross-sectional data should be interpreted with caution, we find it interesting that the normalization of connectivity within the MTL seems to

be a slow process occurring several years after remission is achieved. Future prospective studies could provide more data on the temporal pattern of this suggested normalization of MTL connectivity and its relation to cognitive functions.

In addition to the expected alterations in the prefrontal cortex and MTL, we also found decreased within-network connectivity in the dorsal DMN and the dorsal attentional network. Specifically, within the DMN we found lower connectivity between precuneus, which is a critical brain hub (53), and other cortical DMN regions. To the best of our knowledge, the effects of glucocorticoids on these brain areas have not been elucidated previously. However, increased anxiety among previously depressed patients is also associated with weaker connectivity in the posterior DMN and precuneus (50). Thus, this is a novel finding and the impact of decreased connectivity in relation to affective symptoms should be further elucidated in future studies.

Since CS is a rare condition and several patients had to be excluded due to comorbidities, the number of participants in this study was limited. Three patients with a relatively short time in remission and two controls were on treatment with antidepressants. When these patients and the corresponding controls were removed from the analyses the difference between groups in the MTL and medial PFC RSNs did not reach significance. This may be explained by (a) a smaller sample size with lower power, (b) removal of patients with short time in remission and more pronounced brain changes or (c) an effect of the antidepressant treatment *per se*. In future studies, it is therefore important to take the possible effect of antidepressant into account since this is a commonly used treatment in patients with CS. Notably though, participants with clinically evident depressive symptoms were excluded from this study. In addition, one participant in the control group was treated with prednisolone. This individual was not an outlier in any of the studied RSNs and removing the individual from our analyses did not alter the results (data not shown). Furthermore, a greater proportion of the participants in the control group had a university education. However, we find it unlikely that this difference would affect our results, particularly since higher education has been associated with stronger rather than weaker FC in most RSNs (54). Finally, the patients in this study had higher scores for fatigue, depressive symptoms and anxiety, compared to controls (for details see Ragnarsson *et al.* (11)). Future studies should therefore adjust for these factors and include cognitive testing to be able to test if

there is an association between alterations in functional brain connectivity and cognitive impairments.

To conclude, despite remission, patients treated for CS have increased FC in the PFC and the MTL and decreased FC in the parietal lobe. These alterations may provide a pathophysiological background to both cognitive impairments and affective symptoms. Critically, increased connectivity was negatively associated with time in remission suggesting that these changes might be normalized over time, a finding that should be tested in future prospective studies.

Declaration of interest

J A E has received lecture fees from Novartis Norway AS and Pfizer Norway AS. J B has received unrestricted research grants from Novartis Norway AS and Pfizer Norway AS, and consultant fees from Shire and Novartis. G J has served as consultant for Shire and Astra Zeneca and has received lecture fees from Eli Lilly, Ipsen, Novartis, Novo Nordisk, Merck Serono, Otsuka and Pfizer. A S received research grant from Knut and Alice Wallenberg foundation, Swedish Research Council and StartNeuro. O R has received lecture fees from Novo Nordisk, Ipsen, Sandoz and Pfizer, an unrestricted research grant from HRA-pharma and consultancy fees from Novartis and HRA-pharma. Gudmundur Johannsson is on the editorial board of EJE. Gudmundur Johannsson was not involved in the review or editorial process for this paper, on which he/she is listed as an author. The other authors have nothing to disclose.

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