Glucocorticoids and cortical decoding in the phobic brain

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Conflict of Interest

The authors declare no competing financial interests.

Abstract

Glucocorticoids—stress hormones released from the adrenal cortex—reduce phobic fear in anxiety disorders and enhance psychotherapy, possibly by reducing the retrieval of fear memories. Glucocorticoid signaling in the basolateral amygdala can influence connected fear and memory related cortical regions, but this is not fully understood. Previous studies investigated specific pathways moderated by glucocorticoids, for example, visual-temporal pathways, however, these analyses are limited to a priori selected regions. Here, we performed whole-brain pattern analysis to localize phobic stimulus decoding related to the fear-reducing effect of glucocorticoids. We re-analyzed functional magnetic resonance imaging (fMRI) data from a previously published randomized, double-blind, placebo-controlled study with spider-phobic patients while they were looking at spider images. The patients either received oral glucocorticoids (20 mg of hydrocortisone) or a placebo. Patients with phobia had higher decoding in the anterior cingulate cortex (ACC) and the left and right anterior insula compared to controls. Decoding in the ACC and the right insula showed the highest correlations with experienced fear and explained 40% of the variance across all participants. Patients with cortisol reported a reduction of fear by 10–13% and showed decoding of phobic images in the precuneus, the cerebellum, and the opercular cortex. Patients in the placebo group with increased fear showed decoding in the insula, the ACC and the right frontal lateral pole which have been shown to be related to the fear circuitry and episodic memory. This study demonstrates phobic decoding in fear-related frontal regions and suggests that cortisol administration alters these fear-specific processing areas.

Key words: phobia, anxiety disorder, glucocorticoids, fMRI, pattern analysis

Introduction

Anxiety is a common disorder with a lifetime prevalence of 8%–16% [Magee et al., 1996; Vicente et al., 2006] in the North and South America population. Among anxiety disorders, specific phobias are the most common, with a lifetime prevalence of 12.5%, and affect both female and male individuals of all ages [Kessler et al., 2005]. The phobia can be related to fear from high altitudes, airplane travel, enclosed spaces, terror attacks, or animals such as snakes and spiders. Reactions can range from personal distress to panic. Individuals with phobia avoid the stimulus or the situation to reduce the fear, which entails considerable restrictions in their lives. Confrontation with the phobic stimulus or situation (or even its anticipation) almost invariably provokes the retrieval of past phobic memories, which consequently leads to a fear response [Cuthbert et al., 2003; de Quervain and Margraf, 2008]. This mechanism supports the consolidation of additional fear memories and ultimately strengthens these fear memory traces [Sara, 2000]. As a consequence, the retrieval and consolidation of fearful memories seems to be an important factor in the maintenance of phobic disorders.

Evidence shows that cognitive-behavioural therapy (CBT), including exposure and cognitive restructuring, is efficacious and reorganizes processing in key regions including the amygdala, insula, and cingulate cortex [Hauner et al., 2012; Shin and Liberzon, 2010]. However, up to one-third of the patients with anxiety disorders do not respond to CBT [Cuthbert, 2002; Heimberg, 2002; Hofmann and Smits, 2008]. Drugs with the potential to enhance memory extinction processes are therefore promising candidates for enhancing exposure therapy. Over the last decade, various studies have demonstrated that glucocorticoids are involved in memory regulation [for an overview de Quervain et al., 2017]. Specifically, glucocorticoids impair emotional long-term memory retrieval [de Quervain et al., 1998] while enhancing the consolidation of new memories [de Quervain et al., 2009]. Previous studies demonstrated that acute administration of glucocorticoids reduces phobic fear in patients with anxiety disorders [Aerni et al., 2004; Soravia et al., 2006] and improves extinction-based psychotherapy [de Quervain et al., 2011; Soravia et al., 2014]. Similarly, stress-induced cortisol elevation can reduce negative affect after stress [Het et al., 2012]. Thus, glucocorticoid treatment in combination with exposure therapy is a novel and promising approach for a more effective treatment of phobia [Bentz et al., 2010].

Glucocorticoids interact with the noradrenergic system in the basolateral part of the amygdala which has projections to temporal brain areas , such as the hippocampus [de Quervain et al., 2009]. Thus, anxiolytic effects

from glucocorticoids can induce changes in the amygdala that affect connected cortical areas, i.e., influence the retrieval and processing of phobic information in the temporal lobe. In our recent work, we focused on the the visual temporal pathway, including the lingual gyrus, fusiform gyrus, and the amygdala [Nakataki et al., 2017]. In this dynamic causal modeling (DCM) study, we found that the amygdala in phobic patients has a driving visual input mediated by the pulvinar nucleus, and that glucocorticoid administration normalizes these additional amygdala inputs. However, others have demonstrated that the amygdala-hippocampus connectivity is important during the processing of emotionally salient information [Fastenrath et al., 2014], and that the anterior cingulate cortex can suppress the amygdala activity during the processing of emotionally conflicting information [Etkin et al., 2006], showing that additional brain regions can be involved. The latter study suggests that emotional conflict is resolved through top-down inhibition of the amygdala by the cingulate cortex. In the present study, we re-analyze the data from Nakataki et al. [2017] to investigate the relevance of the anterior cingulate cortex in processing of phobic information after glucocorticoid administration. However, unlike the study by Nakataki et al. [2017] that focused only on a few regions of interest, we implemented whole-brain multivoxel pattern analysis called searchlight MVPA [Kriegeskorte et al., 2006], a sensitive multivariate analysis in order to investigate differences in decoding of phobic material throughout the whole brain. The dataset we analyzed contained behavioural and functional MRI (fMRI) data from a double-blind, placebo-controlled, randomized pre-registered clinical study. Spider phobic patients received 20 mg of cortisol or placebo orally one hour before a picture task provoking phobic fear while fMRI images were acquired. During the experiment, participants viewed spider and non-phobic pictures and rated their experienced subjective fear. We hypothesized that decoding of spider images in the limbic and frontal areas, including the amygdala, the insula, and the cingulate cortex, may be correlated with subjective fear. We also hypothesize that changes in fear may be associated by the effects of glucocorticoids in suspected brain areas, such as the anterior cingulate cortex.

Materials and Methods

Participants

Thirty-six right-handed patients who fulfilled ICD-10 criteria for specific phobia for spiders and 29 healthy control participants were included in the study. The patients were selected from a cohort of our previously

clinical trial, investigating the effectiveness of cortisol treatment and cognitive behavioral therapy on spider phobia treatment [Soravia et al., 2014;ClinicalTrials.gov, NCT01574014]. Diagnosis of spider phobia was based on the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV). We used a computer-based structured clinical interview (DIA-X), which was based on the Composite International Diagnostic Interview [CIDI; Robins et al., 1988]. We assessed fear of spiders using the German version of the Spider Phobia Questionnaire [SPQ; Watts and Sharrock, 1984] and the Fear of Spider Questionnaire [FSQ; Szymanski and O'Donohue, 1995]. This assessment was to confirm that patients has significantly more spider phobia symptoms than the control group. Healthy participants were screen by the SCL-90-R (The Symptom Checklist-90-Revised) questionnaire [Franke, 1995] and the SPQ and FSQ to confirm that the participants had no mental illness, including spider phobia. We also confirmed no previous history of mental illness. The exclusion criteria included any of the following conditions: history of head injury, acute, or chronic medical conditions, a recent history of systemic or oral glucocorticoid therapy, psychiatric disorders other than specific phobia, psychotropic drug treatment, smoking of > 15 cigarettes per day, neurological diseases, current drug or alcohol abuse, pregnancy, use of hormonal contraceptives, current behavioural therapy, and any contraindications to MRI. Female participants were evaluated during the luteal phase of their menstrual cycle as previous studies showed that amygdala activation in response to psychological stressor depends on menstrual cycles [Chung et al., 2016] cortisol responses to stress are comparable between females in a luteal phase and males [Kirschbaum et al., 1999]. After assessment, the spider-phobic patients were randomly assigned to two groups according to a double-blind, placebo-controlled design.

We carefully performed data quality checks and excluded three individuals in the cortisol group, three in the placebo group, and five controls from data analysis due incomplete fMRI or behavioral data (12% excluded), or head movements larger than 4 mm of translation or 4 degrees of rotation (5% excluded); for details, see Supplementary Figure 1A. The justification for excluding individuals with head movements is that these can introduce spurious effects [Power et al., 2012]. After exclusions, we analysed a final sample of 54 participants: 15 patients in the cortisol group, 15 patients in the placebo group, and 24 healthy controls (see Table 1 for demographic details). After providing a complete description of the study to the participants, written informed consent was obtained. The study was approved by the ethics committee of the Canton of Bern, Switzerland (Nr. 161/07) in accordance with the principles of the Declaration of Helsinki and the Swiss authority for

pharmaceutical drugs (Swissmedic). All patients were offered to attend an exposure-based short-term group therapy after the study.

Design and procedure

The experiments were conducted at the Bern University Hospital between 2 PM and 5 PM each day. Patients and healthy controls underwent the same experimental procedure except, for the diagnostic interview, substance administration, and collection of saliva samples, which only included the patients. Saliva samples were collected to control the effectiveness of the cortisol administration. Upon arrival, participants were informed about the procedure, asked to fill out the Spielberger State Anxiety Inventory [Spielberger et al., 1970], and rate their actual subjective fear, physical discomfort, and avoidance behaviour on a visual analogue scale (VAS) ranging from 0 (no symptoms) to 100 (maximal symptoms). The first saliva sample was collected in the patient group using a Salivette (Sarstedt Inc., Rommelsdorf, Germany). Participants were instructed regarding the picture task, and performed a few practice trials on the computer to become familiar with the rating procedure. After the oral administration of cortisol (20 mg; Galepharm, Küsnacht, Switzerland) or placebo to the patients, a resting period of 30 minutes followed. Sixty minutes after substance administration, the second saliva sample was collected, before the beginning of the fMRI task, to control for the cortisol level increase. Functional MRI images were acquired during the picture task (24 minutes). After the scanning session, the third saliva sample was collected from the patients; the level of cortisol was therefore measured at 3 time points. All participants completed questionnaires regarding anxiety (FSQ and SPQ) before the scan. Additionally, participants were asked to retrospectively rate experienced fear on a visual scale from 0–100 while looking at the spider images in the scanner. A further questionnaire asked about side effects and whether the patient believed that he/she received cortisol or placebo. The saliva samples were stored at -20°C until required for biochemical analysis.

Paradigm

During the event-related experiment, participants viewed 80 randomized pictures from the International Affective Picture System [ISAP; Lang et al., 2008]. We presented four categories (20 trials each) of images: phobic (spiders), other animals, negative, and neutral. The presentation time was five seconds, with inter-stimulus intervals (ISI) between 10.1–13.7 s (Supplementary Figure 1B). All participants rated their subjective fear after each trial on an analogue scale between 1 (no fear) and 4 (maximum fear).

Statistical analysis

Baseline variables and anxiety self-ratings were analysed using Kruskal-Wallis and Wilcoxon rank-sum tests because of small sample size and the distribution of the data. Cortisol levels were investigated using a 2 × 3 repeated measures ANOVA (group cortisol vs. placebo, and 3 time points). MVPA was performed with the "Searchmight" toolbox [Pereira and Botvinick, 2011] and a nonparametric ANOVA with SnPM (v. 13.1.05). For correlations Spearman's rho was used. Statistical analysis was performed in R (v. 3.4.4); A P-value of < 0.05 was considered statistically significant and all test were two-tailed. All voxel-wise tests were corrected for multiple comparisons using FDR of 0.05. The analysis pipeline is illustrated in Supplementary Figure 1C.

Hormone analysis

We analysed free cortisol concentrations in saliva using commercially available chemiluminescence immunoassays (cortisol: CLIA; IBL-Hamburg, Germany). The inter- and intra-assay coefficients of variation were below 10%. The samples of all subjects were analysed in the same run to reduce error variance caused by intra-assay variability.

MRI data acquisition and pre-processing

Functional images were acquired with a 3T Siemens Magnetom Trio and a 12-channel head coil, using an interleaved EPI sequence (579 volumes; 37 slices; voxel, 3.6 × 3.6 × 3 mm^3; gap thickness, 0 mm; matrix size, 64 × 64; FOV, 230 × 230 mm^2; TR, 2500 ms; TE, 30 ms). For structural images, a high-resolution 3D T1-weighted imaging protocol (modified driven equilibrium Fourier transform, MDEFT) was used (176 sagittal slices; thickness, 1.0 mm; FOV, 256 × 256 mm^2; matrix size, 256 × 256; voxel, 1 × 1 × 1 mm^3; TR, 7.92 ms; TE, 2.48 ms). Pre-processing is illustrated in Supplementary Figure 1C. We performed standard pre-processing using SPM8 (http://www.fil.ion.ucl.ac.uk/spm) and normalized to MNI space (2 × 2 × 2 mm^3), except that data were not smoothed due to subsequent pattern analysis.

Multivoxel pattern analysis

We used whole-brain multivoxel pattern analysis (searchlight MVPA) with a classifier to investigate individual stimulus decoding on the subject level [Kriegeskorte et al., 2006]. This step was performed within-subject for all

individuals to identify brain voxels that contain information to classify between phobic images and non-phobic images (negative, animal, and neutral images). The resulting classification accuracy maps, one per subject, represent brain areas that decoded phobic content versus the three other categories and contain values from 0–1, with 1 indicating 100% accuracy in classification of the phobic images. A major benefit of MVPA is the increased power to detect cognitive states [Haynes and Rees, 2006; Norman et al., 2006] compared to the standard mass-univariate approach. Multivariate approaches use the BOLD signal from multiple voxels (patterns) to predict stimulus category. Therefore, we can detect smaller regional differences compared to classical approaches. This shifts the interest of whether a single voxel responds differently in two conditions (mass-univariate) to whether a pattern of activity carries enough information to distinguish between two conditions (multivariate). Prior to MVPA, a GLM was performed for each trial, including a regressor for the single trial and a regressor coding for all remaining trials, which is best practice for the subsequent MVPA [Mumford et al., 2012]. We also included a CSF, a WM, and six movement parameters and their first-order derivatives in the model as nuisance regressors. The resulting beta estimate maps of the individual trials were subjected to a whole-brain MVPA using a searchlight approach [Kriegeskorte et al., 2006] that involved a Gaussian Naive Bayes classifier with leave-one-sample-out cross-validation [Pereira and Botvinick, 2011]. In other words, the testing set (a single image) was independent from the training images. The searchlight involved a cube of $3 \times 3 \times 3$ voxels ($6 \times 6 \times 6$ mm^3). Classification was performed between 20 spider pictures and 20 randomly sampled pictures from the three other categories (negative, animal, and neutral). The classification step was performed 60 times (bootstrapping) using the same 20 spider images but always a different randomly sampled set of 20 negative, animal, and neutral pictures (with a random images left out for testing) and a whole-brain mean accuracy map was created for each subject. As already stated, this accuracy map is the average percentage (across the bootstrapping) of correct classification of spider pictures versus that for the three other categories and can simply be interpreted as the neuronal decoding of spider pictures.

First, we performed a voxel-wise one-way ANOVA with the three group labels as levels (non-parametric permutation/randomisation test with SnPM) and a FDR correction of 0.05 to identify regions that differed between the three groups; data were smoothed beforehand (FWHM = 8 mm). This is the classical analysis approach but does not take into account that high spatial resolution in MVPA. In other words, this analysis was only to generally test the hypothesis that fear relates regions are involved, i.e. to validate that we actually measure decoding of phobic stimuli. It is important to note that group analyses in MVPA can be challenging as

large smoothing kernels are not appropriate, for example if patterns are located within a same relatively large structure such as cingulate cortex of insula, but the patterns of activation do not spatially overlap across individuals. Canonical analysis approaches do not take into account these individual spatial differences; hence summed binary significance maps have been suggested (Pereira and Botvinick, 2011). We performed this analysis for the cortisol and placebo group to investigate treatment effects. We corrected the unsmoothed individual decoding maps with a FDR correction of 0.05 to get above chance decoding accuracies and applied a small smoothing kernel (FWHM = 2 mm); in these significant decoding maps, the average above chance decoding accuracies for the 30 patients was 72% (range: 66% to 85%). We binarized the individual FDR corrected accuracy maps and summed them for each patient group, resulting in two count maps demonstrating brain areas that exhibited significant individual decoding in a specific number of subjects. To directly compare decoding in the cortisol to the placebo group, we subtracted the two maps, resulting in a brain map with relative increase/decrease in the number of subjects with significant decoding (difference map). To threshold this map and test for significant differences we performed a randomization test: we randomly sampled two groups each n = 15 from the pooled cortisol and placebo sample, with each group containing individuals from both groups. We summed the maps and created two count maps, one for each group, and subtracted the maps. We performed this k = 500 times to created a distribution under the null hypothesis of no difference between the cortisol and placebo group. To control the alpha error, we determined the upper and lower percentile (2.5% and 97.5%); thus only in 5% or less of the cases the the data are as extreme as these boundaries (alpha level). These boundaries were determined voxel-wise and used to threshold the original difference map. The average upper and lower alpha level thresholds across voxels were +3.6 subjects (SD: 0.51) and -3.4 subjects (SD: 0.54). We used the Harvard-Oxford cortical atlas to identify and label the significant brain regions.

Results

Demographics, baseline fear and endocrine measures

Demographics are shown in Table 1. The three groups did not significantly differ in age between the three groups (Kruskal-Wallis test, χ^2 = 0.80, df = 2, P = .67). There were a higher proportions of females in the two patient groups, however, the three group did not differ significantly regarding gender (χ^2 = 2.73, df = 2, P =

.26). There was also no difference regarding body mass index (Kruskal-Wallis test, χ^2 = 1.03, df = 2, P = .60). The cortisol and placebo groups did not differ regarding spider phobia symptoms assessed by FSQ and SPQ at baseline before the experiment (Wilcoxon rank-sum test; FSQ: W = 99, P = .98; SPQ: W = 121.5, P = .29). At baseline the patients had significantly higher scores in spider phobic symptoms compared to healthy controls, who had no fear (Kruskal-Wallis test; FSQ: χ^2 = 36.6, df = 2, P < .0001; SPQ: χ^2 = 37.0, df = 2, P < .0001; Table 1). The cortisol group had 5.2 times higher cortisol levels at the beginning of the fMRI experiment, and 4.3 times higher levels at the end of the experiment compared to their baseline, while the placebo group showed no increase over time (Table 1) which was confirmed by a significant interaction group × time (repeated measures ANOVA; $F_{4,98}$ = 46.6, P < .0001).

Subjective fear

During the fMRI task, patients (cortisol and placebo group) exhibited 2.1 times higher subjective fear in response to spider pictures compared to controls (Kruskal-Wallis rank sum test, χ^2 = 28.8, df = 2, P < .0001), see Figure 1A. Patients with cortisol treatment had a 9.8% decrease in subjective fear while looking at spider pictures compared to patients with placebo (Wilcoxon rank sum test, W = 47.5, P = .021), see Figure 1A. We found a 2.4 times higher subjective fear in patients with spider-phobia compared to controls when subjects were asked to retrospectively rate their perceived fear in the scanner after the experiment (χ^2 = 23.2, df = 2, P < .0001), see Figure 1B, and patients with cortisol had a subjective fear reduction of 13.3% compared to the placebo group (W = 42.5, P = .011). The three groups did not differ with respect to subjective fear while looking at the negative pictures (χ^2 = 4.57, df = 2, P = .10), see Figure 1C.

Cortical decoding of spider images

We first performed a voxel-wise test for group difference with the null hypothesis that decoding of phobic images is equal in all the three groups (non-parametric one-way ANOVA, FDR 0.05). We found the strongest group effects in the left anterior insula, the right anterior insula, and in the anterior cingulate gyrus (ACC) (Figure 2). These areas showed a higher median decoding in both patient groups (cortisol: insula left 57%, insula right 56%, ACC 59%; placebo: insula left 56%, insula right 55%, ACC 56%) compared to healthy controls (insula left 50%; insula right 50%, ACC 50%), see Figure 3A. These decoding values are averages across across individuals and across the area of the anterior insula and the ACC, however these decoding values

are found to be much higher locally and individually, i.e., above to 80% accuracy, see Supplementary Figure 2. Decoding in all three areas correlated with subjective fear rated during the scanner and explained 36%–40% of the variance (Spearman's rank correlation; insula l., $\rho = .60$, $P < .0001$; insula r., $\rho = .64$, $P = .0001$; ACC, $\rho = .64$, $P < .0001$), see Figure 3B.

We investigated significant decoding accuracies on the subjects level (FDR 0.05 corrected) and transformed these into binary maps that we summed across individuals of the placebo and the patient group (count maps, Supplementary Figure 3). We subtracted the count maps of the placebo and cortisol group to show group differences, and thresholded this map with a randomization test and alpha level of $p = 0.05$ (Figure 4AB, for more axial slices, see Supplementary Figure 4). In the cortisol group, an increased number of individuals showed decoding of spider images in the right precuneus cortex (+8 individuals; 12 cortisol; 4 placebo), the left central opercular cortex (+6; 8 cortisol; 2 placebo), the left cerebellum (+7; 8 cortisol; 1 placebo), the right parietal opercular cortex (+7; 9 cortisol; 2 placebo), see Table 2 for a complete list. In the placebo group, an increased number of individuals demonstrated higher decoding in the insula (+6; 10 placebo; 4 cortisol), the ACC (+5; 7 placebo; 2 cortisol), and the frontal pole (+6; 6 placebo; 0 cortisol), see Table 3 for a complete list.

Discussion

In this study, we investigated cortical decoding of spider images related to the administration of glucocorticoids in patients with spider phobia. The anxiolytic effect of glucocorticoids in phobic fear and anxiety disorders has previously been demonstrated [Aerni et al., 2004; Soravia et al., 2006] which can be beneficial for patients not responding to standard therapy. In this study, subjective fear was reduced by 10–13% in the cortisol group during viewing of phobic images compared to the placebo group. Such a reduction can be clinically relevant and, in combination, improves extinction-based psychotherapy [Bentz et al., 2010; de Quervain et al., 2011; Soravia et al., 2014]. In a previous study, we investigated a visual-temporal pathway with 3 subcortical nodes, the lingual gyrus, fusiform gyrus, and amygdala and we found that the amygdala in phobic patients has a driving visual input mediated by the pulvinar nucleus, and that glucocorticoid administration normalizes these additional amygdala inputs [Nakataki et al., 2017]. However, the literature suggests that other areas may also play a key role this process, such as the hippocampus that processes salient information, or the cingulate cortex that can suppress the amygdala activity and a fear response [Etkin et al., 2006; Fastenrath et al., 2014].

The aim of this study was to perform a whole-brain multivariate pattern analysis to investigate the relevance of the anterior cingulate gyrus and other potentially relevant areas that demonstrate changes in phobic information decoding after glucocorticoid administration. Decoding of phobic images in the anterior insula and the ACC was higher in the patients compared to the controls and was correlated with subjective fear. This involvement of the limbic system and the fact that the decoding magnitudes correlated with subjective fear supports the idea that our brain-derived phenotype, the decoding (classification accuracies from MVPA), is a valid measure and closely linked to phobic information processing. These areas are well-known key players in the processing of emotions and part of the fear network [Greco and Liberzon, 2016], particularly in specific phobia [Del Casale et al., 2012], and it was shown that exposure therapy reduces activity specifically in these regions, the cingulate cortex and the insula [Hauner et al., 2012].

We compared the treatment (cortisol) and the placebo group. In the placebo group, a larger number of individuals showed decoding in frontal regions: the insula, the ACC and the right lateral frontal pole. It has been demonstrated that these regions are involved in fear and memory processes: the insula and ACC are key player in the fear circuitry and its role has been well demonstrated in anxiety disorders [Shin and Liberzon, 2010]. The frontal pole is involved in various cognitive functions, such as mentalizing, multitasking, but also episodic memory [Christoff and Gabrieli, 2000; Gilbert et al., 2006]. It has been shown that the lateral frontal pole co-activates with the ACC and the anterior insula [Gilbert et al., 2010]. Our result are consistent with the findings that symptom reduction achieved through CBT was associated with lower responsiveness in the bilateral insula and the ACC and a reduction in cerebral blood flow [Hauner et al., 2012; Soravia et al., 2016].

The cortisol group showed decoding in the precuneus, the anterior cerebellum and in the opercular cortex. The precuneus is a hub involved in multimodal, attentional and memory processes, and is also involved in the default mode network. Functional connectivity mapping of this hub showed negative connectivity with the amygdala [Zhang and Li, 2012]. Thus, it may be possible that glucocorticoid related changes in the amygdala may moderate the precuneus cortex, but this needs to be further investigated. The additional areas are not directly known to be involved in fear processing but can be related: anterior parts of the cerebellum, such as the vermis, are involved in memory formation and fear conditioning [Sacchetti et al., 2002]. The left opercular cortex is related to auditory processing and speech.

A recent study with healthy participants showed that cortisol disrupts ventromedial prefrontal cortex functioning and its communication with other brain regions such as the cingulate cortex and parahippocampal gyrus [Kinner

et al., 2016]. This is a potential mechanism behind our finding that the cortisol group showed reduced decoding in the ACC. The insula is particularly involved in processing of emotionally salient information as it is strongly connected with limbic structures, the cingulate cortex, the amygdala, and also prefrontal regions. Additionally, several fMRI studies showed that successful cognitive-behavioral therapy (CBT) for spider phobia is accompanied by reduced CBF in the bilateral insula [Schienle et al., 2007; Schienle et al., 2009]. Hence, the acute administration of glucocorticoids and similarly CBT can reduce the hyperactivation in response to phobic stimuli in brain regions that are crucially involved in identifying fearful stimuli and generating fear response, such as the insula [Duval et al., 2015].

In this study, we used pattern analysis, a method applied on the individual subject level. This yields individual decoding pattern that only partially overlap due to local functional decoding differences among individuals. It can therefore be challenging to find consistent results in classical voxel-wise group analyses, and such group analyses are generally not suggested with MVPA [Stelzer et al., 2013]. We addressed this issue using count maps [Pereira and Botvinick, 2011] that were thresholded on the individual, and on the group level with a randomization test, providing robust statistics but also accounting more thoroughly for individual variation in decoding.

Some limitations of this study merit discussion. First, we present data from a sample with small subgroups, but this is not uncommon in neuroimaging studies involving clinical samples and drug administration. We excluded some subjects due to head movements; it is well known that head movements can confound results in neuroimaging [Deen and Pelphrey, 2012; Power et al., 2012]. Correlations and functional connectivity seem especially sensitive to movement and can exhibit false effects in response. It is unknown to what extent pattern analysis can be confused by this, but it is possible in cases where the movement correlated with the experimental design. It is not surprising that some phobic patients moved their head while confronted with spider images. It is important to realize that small sample sizes may overestimate effects and have low reproducibility [Button et al., 2013], and careful statistics are required to control false positives. We addressed this problem by using a FDR 0.05 on the subject-level decoding maps and a randomization test on the group level (with FDR 0.05 as well). Non-parametric test are suitable for small samples, do not make the assumption of normality, and are generally more conservative in showing significant effects. The gender imbalance is a limitation of this study: there are 46% males in the control group, but only 33% in the cortisol and 20% in the placebo group. An explanation is that spider phobia may be more common among females, and therefore, the control group should have been

more carefully matched. However, our main result is the comparison between the cortisol and the placebo group, which both had a lower proportion of males that did not significantly differ.

Conclusions

In sum, this study elucidates cortical decoding patterns of phobic stimuli related to glucocorticoid treatment. Glucocorticoids can improve the treatment of patients because they inhibit the retrieval of fear memories while they enhance the consolidation of new corrective experiences achieved through exposure therapy [de Quervain et al., 2009; de Quervain et al., 2011; Soravia et al., 2014]. We have identified posterior decoding (precuneus, cerebellum, and operculum) in the treatment group (cortisol), and more frontal decoding (insula, ACC and frontal pole) in the placebo group which provides new insight into the information processing of the phobic brain after glucocorticoid administration.

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Figures

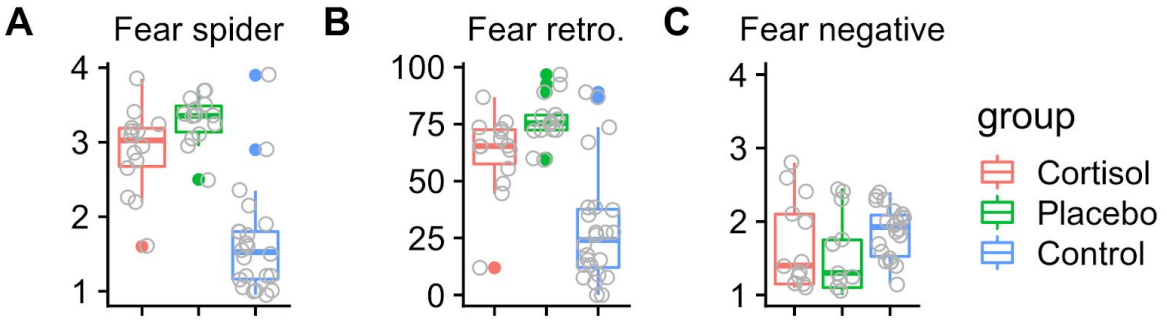

Figure 1. Subjective fear during the fMRI experiment. **(A)** The cortisol group exhibits a 9.8% reduction of fear towards spider pictures compared to placebo (P = .021). Patients (cortisol and placebo groups) had a 2.1 times higher subjective fear compared the controls (P < .0001). **(B)** Retrospective fear during the experiment assessed after the experiment: the cortisol group shows a 13% reduction of fear from spiders (P = .011). Patients had a 2.4 times higher fear compared the controls (P < .0001). **(C)** No significant group differences were found regarding emotional negative pictures (P = .10).

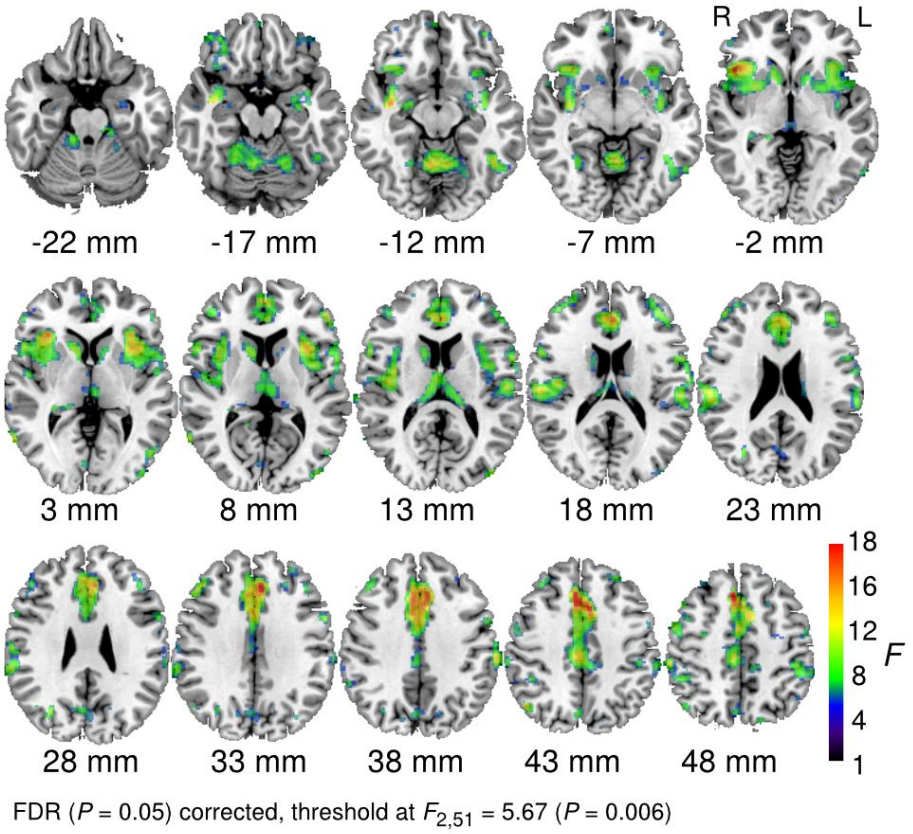

Figure 2. Areas showing group effects (differences of means between the cortisol, placebo and control group) in phobic decoding (spider vs. negative, animal and neutral images). Most prominent regions are the left anterior insula, the right anterior insula, the anterior cingulate cortex (ACC).

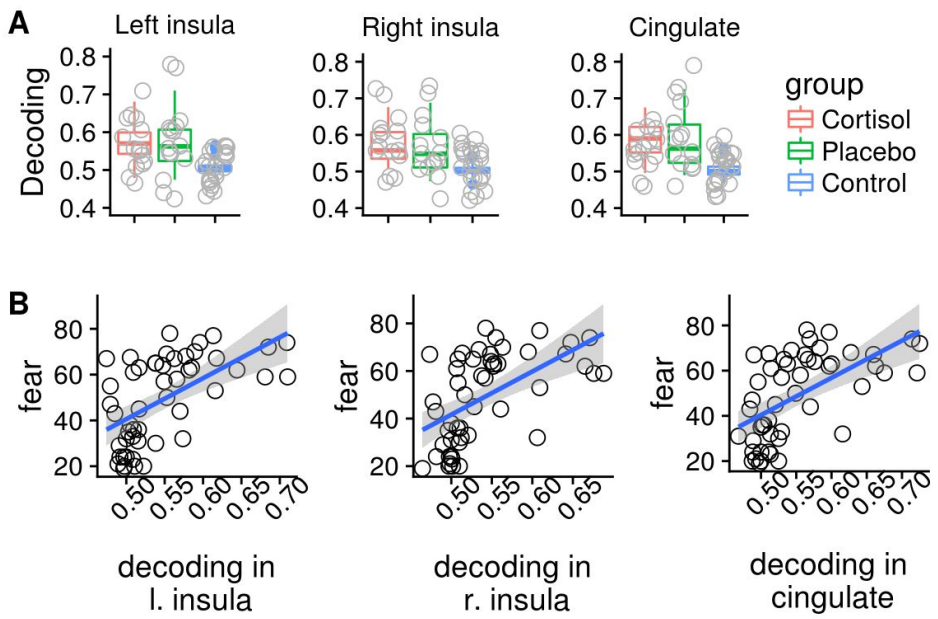

Figure 3. **(A)** Patients (cortisol and placebo) show higher decoding accuracies compared to controls in the left anterior insula, the right anterior insula, and the anterior cingulate gyrus (ACC). **(B)** Decoding of spider images in these three regions correlated positively with subjective fear from spiders and explained 36%–40% of the variance (R^2; gray areas around regression line is the 95% CI).

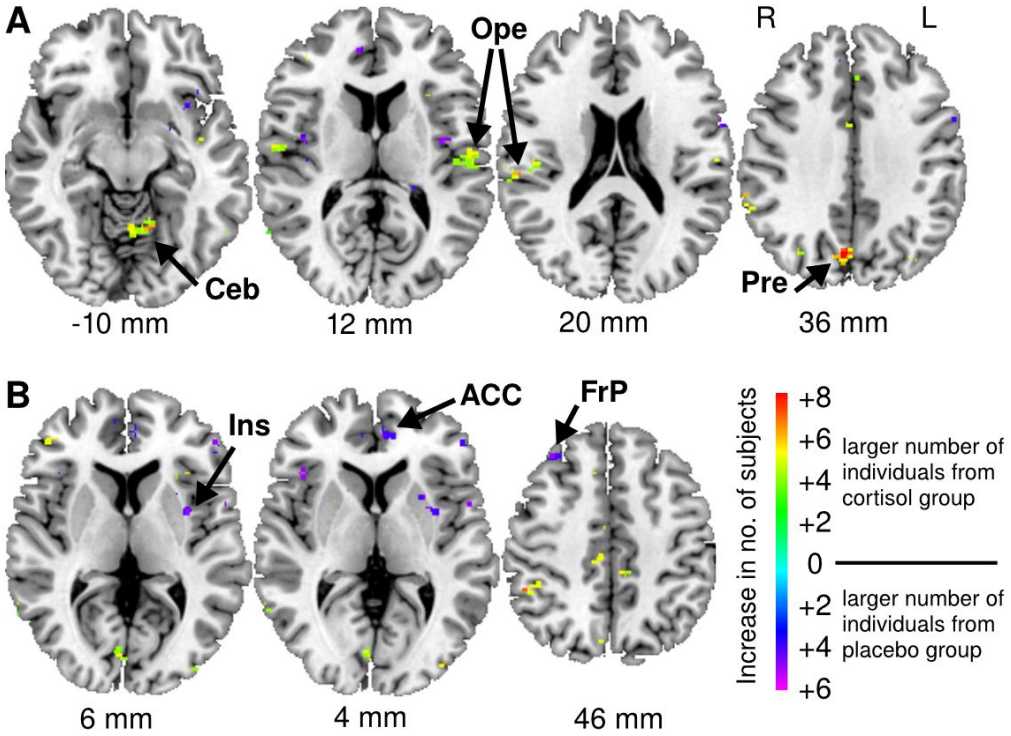

Figure 4. Decoding of spider images. Brain areas with a relative increase in the number of subjects who have significant individual decoding of spider images (FDR 0.05 corrected on the subject-level; randomization test $p = 0.05$ on the group level). **(A)** A larger number of individuals in the cortisol group showed decoding of spider images most prominently in the right precuneus cortex (Pre), the right and left opercular cortex (Ope), and the left cerebellum (Ceb). **(B)** A larger number of individuals in the placebo group showed decoding most prominently in the left insula (Ins), the anterior cingulate gyrus (ACC), and the frontal pole (FrP).

Tables

Table 1. Descriptives of demographics, baseline fear and cortisol levels.

	Patients with cortisol (n = 15)	Patients with placebo (n = 15)	Healthy controls (n =24)
Median age (IQR)	28 (24–40)	29 (22–42)	27 (24–29)
Gender (male/female)	5/10	3/12	11/13
Median BMI (IQR)	22.2 (20.9–24.6)	22.7 (21.3–25.0)	21.9 (20.8–23.8)
Median (IQR) *fear from Spiders at baseline:*			
FSQ	75.0 (69.5–91.5)	77.0 (67.8–84.8)	9.0 (2–16.5)
SPQ	22.0 (20.0–26.0)	21.0 (18.3–22.0)	4.5 (2.8–6.0)
Median (IQR) cortisol concentration of saliva (nmol/l):			
baseline before administration	9.0 (5.9–11.9)	7.1 (6.7–12.5)	–
60 min. after administration	46.7 (17.0–56.2)	7.6 (4.9–10.1)	–
120 min. after administration	38.6 (22.9–75.3)	4.6 (3.0–5.8)	–

IQR: Interquartile range

BMI: Body Mass Index

FSQ: Fear of Spider Questionnaire

SPQ: Spider Phobia Questionnaire

Table 2. Brain regions with a significant larger number of participants in the cortisol group who have above-average decoding compared to the placebo group.

Voxels	Difference in No. of Subjects (Cortisol / Placebo)	MNI x y z	Region (Harvard-Oxford Cortical Structural Atlas)
112	8 (12/4)	6 -74 36	R Precuneus Cortex
70	6 (8/2)	-56 -16 12	L Central Opercular Cortex
68	7 (8/1)	-12 -58 -10	L Cerebellum
53	7 (9/2)	56 -26 20	R Parietal Opercular Cortex
30	6 (10/4)	64 -40 30	R Supramarginal Gyrus, posterior division
27	7 (11/4)	6 -26 42	R Cingulate Gyrus, posterior division
24	5 (7/2)	56 -10 8	R Central Opercular Cortex
20	7 (10/3)	50 -44 46	R Supramarginal Gyrus, posterior division
20	6 (7/1)	48 -20 18	R Parietal Operculum Cortex
20	6 (7/1)	6 -82 8	R Intracalcarine Cortex
20	5 (7/2)	30 -68 24	R Lateral Occipital Cortex
17	6 (10/4)	-8 -36 44	L Cingulate Gyrus, posterior division
15	5 (6/1)	64 -54 2	R Middle Temporal Gyrus
11	6 (10/4)	0 2 40	Cingulate Gyrus, anterior division

L Left; R Right;

Table 3. Brain regions with a significant larger number of participants in the placebo group who have above-average decoding compared to the cortisol group.

Voxels	Difference in No. of Subjects (Placebo / Cortisol)	MNI x y z	Regions (Harvard-Oxford Cortical Structural Atlas)
28	6 (10/4)	-36 0 -2	L Insular Cortex
28	5 (7/2)	-4 46 4	L Paracingulate Gyrus / L Anterior Cingulate gyrus
19	6 (6/0)	32 36 46	R Frontal Pole
14	5 (7/2)	42 -6 12	R Central Opercular Cortex
14	6 (6/0)	8 46 8	R Paracingulate Gyrus / R Anterior Cingulate gyrus
13	6 (9/3)	-62 4 18	L Precentral Gyrus

L Left; R Right;