Glucocorticoids change neural decoding in the middle cingulate cortex accompanied by a reduction of subjective fear in patients with spider phobia

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Abstract

Background. Corticosteroids—stress hormones released from the adrenal cortex—reduce phobic fear in humans and enhance psychotherapy, possibly by reducing the retrieval of fear memory. However, the underlying neural mechanism is not yet fully understood.

Methods. We investigated the neural correlates of the acute fear-reducing effect of glucocorticoid administration in phobia with a randomized, double-blind, placebo-controlled functional magnetic resonance imaging (fMRI) study. We analysed fMRI data of participants diagnosed with spider phobia ($n = 28$) and healthy controls ($n = 18$) using multivoxel pattern analysis (MVPA). The spider-phobic patients received oral glucocorticoids (20 mg of hydrocortisone) or placebos. Participants rated their subjective fear while viewing spider or non-phobic pictures in the scanner.

Results. Patients in the placebo and cortisol group exhibited increased decoding of phobic images in the middle cingulate cortex (MCC) and bilateral anterior insula compared to healthy controls with decoding at chance level. Patients with cortisol had less decoding in the MCC. Decoding of spider pictures in the MCC explained 38% of subjective fear across all individuals. In the placebo group, a causal model explained 12% variance in subjective fear influenced by the right anterior insula and the MCC; this relationship was changed in the cortisol group.

Conclusions. These results suggest that the anterior insula and the MCC are strongly related to the decoding of phobic stimuli. Glucocorticoids seem to modulate the circuitry of MCC and anterior insula leading to a reduction in subjective fear.

Key words: phobia, fear, anxiety, glucocorticoids, fMRI, pattern analysis, middle cingulate cortex, anterior insula

Introduction

Anxiety is a common disorder with a lifetime prevalence of 8%–16% in the American and European populations (Magee *et al.*, 1996; Alonso *et al.*, 2004; Vicente *et al.*, 2006). 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). Such fears can be related to high altitudes, airplane travel, enclosed spaces, 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 can cause considerable constraints on the behaviour and life of the individuals.

Confrontation with the phobic stimulus (or even its anticipation) almost invariably provokes the retrieval of stimulus-associated fear memories, which results in a fear response (Cuthbert *et al.*, 2003; de Quervain and Margraf, 2008). The retrieval of past phobic experiences and the subsequent fear response support the consolidation of the fear memories and finally strengthen the fear memory trace (Sara, 2000). Accordingly, the retrieval and consolidation of fearful memories seems to be an important factor in the symptomatology and maintenance of phobic disorders.

There is extensive evidence 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). It has become the method of choice for the treatment of specific phobia (Hofmann and Smits, 2008; Zlomke and Davis, 2008), but up to one-third of the patients with anxiety disorders do not respond to such treatment (Cuthbert, 2002; Heimberg, 2002; Hofmann and Smits, 2008). Drugs with the potential to enhance 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). Glucocorticoid treatment in combination with exposure therapy is thus a promising approach for the treatment of phobia (Bentz *et al.*, 2010).

There is evidence from fMRI studies showing that the prefrontal cortex and the amygdala contain key circuitry for fear processing (Davidson, 2002). However, the underlying neuronal mechanism of the phobic fear reducing effect of glucocorticoid administration is not yet fully understood. A recent study focused on the the visual cortical pathway, including the lingual, fusiform gyrus, and the amygdala (Nakataki *et al.*, 2017). However, it should be noted that the fear related brain areas are part of a large circuit, the limbic system that involves most prominently the cingulate gyrus, the insula, and the hippocampus. This network is responsible for the generation and modulation of emotional processes. Additionally, other areas can also be involved, for example the cerebellum can contributes to the extinction of conditioned fear (Utz *et al.*, 2015).

Therefore, we investigated whole-brain multivariate analysis to elucidate the neuronal correlates of the anxiolytic effect of glucocorticoids on phobic fear, we collected behavioural and functional MRI (fMRI) data in a double-blind, placebo-controlled, randomized 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 used multivariate pattern analysis (MVPA) to quantify decoding of phobic pictures. MVPA is a well-established method in neuroimaging and has proven to be more sensitive and more informative about functional organization of the cortex (Haxby *et al.*, 2001; Haxby, 2012) compared to the classical univariate approach of the general linear model (GLM). Multivariate approaches use the information from multiple voxels (patterns) to predict stimulus category based on the BOLD signal. 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). Furthermore, results from MVPA and classification/decoding accuracies can be interpreted in a very natural way, as percent classification accuracies, or, the decoding of phobic images in our study. We hypothesized that decoding in the limbic and related regions, including right the amygdala, bilateral insula, and cingulate cortex, which are key areas in the neurocircuitry and the reorganization of fear (Shin and Liberzon, 2010; Hauner *et al.*, 2012), may be correlated with subjective fear and may be specifically modulated by glucocorticoids.

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. 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, 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). Patients were excluded from the study if they met 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 more than 15 cigarettes per day, neurological diseases, current drug or alcohol abuse, pregnancy, use of hormonal contraceptives, or current behavioural therapy. 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) and that cortisol responses to stress are comparable between females in a luteal phase and males (Kirschbaum *et al.*, 1999). After assessment, the included 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 four individuals in the cortisol group, four in the placebo group, and 11 controls from data analysis due incomplete data (12% excluded) or head movements (17% excluded); for details, see Supplementary Figure S1A. It is well-known that head movements can introduce spurious effects (Power *et al.*, 2012). After exclusions, we analysed a final sample of 46 participants: 14 patients in the cortisol group, 14 patients in the placebo group, and 18 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). The study was registered (ClinicalTrials.gov, NCT01574014).

Power and sample size

We estimated the required sample size for a given effect in the correlation between decoding accuracies in a specific brain region and subjective fear. For a desired statistical power of 80% to detect a correlation of $r = 0.40$, with a two-sided alpha level of 0.05, a total sample site of $N = 46$ was required. There is no canonical approach to calculate power for voxel-wise statistical brain maps, but we applied FDR correction to the images to control false positives (Genovese *et al.*, 2002).

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 (STAI; Spielberger *et al.*, 1983), 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 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 S1B). All participants rated their subjective fear after each trial on an analogue scale between 1 (no fear) and 4 (maximum fear).

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, $3.6 \times 3.6 \times 3$ 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, $1 \times 1 \times 1$ mm^3; TR, 7.92 ms; TE, 2.48 ms). Pre-processing is illustrated in Supplementary Figure S1C. We performed standard pre-processing using SPM8 (http://www.fil.ion.ucl.ac.uk/spm) and normalized to MNI space ($2 \times 2 \times 2$ mm^3), except that data were not smoothed due to subsequent pattern analysis.

MVPA and statistical analysis

We used whole-brain multivoxel pattern analysis (MVPA) with a classifier to investigate individual stimulus decoding on the subject level. The resulting classification accuracy maps represent brain areas that classified phobic content versus the three other categories based on the BOLD response from multiple voxels. A major benefit of MVPA is increased power and sensitivity to detect cognitive states (Haynes and Rees, 2006; Norman *et al.*, 2006), compared to the standard mass-univariate approach.

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 the best practice for the subsequent MVPA (Mumford *et al.*, 2012). We also included six movement parameters and their first derivatives in the model. 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). The searchlight involved a cube of $3 \times 3 \times 3$ voxels (6 mm $\times$ 6 mm $\times$ 6 mm). Classification was performed between 20 spider pictures and 20 randomly drawn 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 a different randomly drawn set of 20 other pictures, and a whole-brain mean accuracy map was created. The resulting mean accuracy map for each participant was directly related to the decoding of spider pictures and could be interpreted as the average percentage of correct classification of spider pictures versus that for the three other categories based on the multivariate BOLD pattern. We related this measure to the decoding of phobic stimulus material. For group inference, we smoothed the individual accuracy maps (FWHM = 8 mm) to

account for individual regional decoding differences. We used voxel-wise two-way 3 × 2 analysis of variance (ANOVA) of the three groups and gender to investigate which brain areas systematically differed among the groups in terms of the decoding accuracy of phobic stimuli (Figure 2). This analysis also yielded brain areas that systematically varied by gender or by a group × gender interaction which we inspected to address the gender imbalance in the groups. There was no gender effect and interaction after correction. The group map was corrected for multiple comparisons using a false discovery rate (FDR) of $P = 0.01$ (Genovese *et al.*, 2002). From the five brain areas with significant group effects (Supplementary Table S1), we inspected the three largest clusters with the strongest decoding: the right and left anterior insula and the middle cingulate cortex.

While the previously described classical group-level analysis demonstrates decoding differences between groups, it cannot exploit the high resolution information that MVPA provides (since a large smoothing kernel needs to be applied), also considering that the interindividual differences within the patient group, between cortisol and placebo, are smaller. Therefore we performed a group-level analysis according to (Pereira and Botvinick, 2011). We FDR corrected ($P = 0.05$) the individual decoding maps on the subject-level, applied a small smoothing (FWHM = 2 mm), and summed the binary significance maps, resulting in a frequency map of number of subjects that showed a significant searchlight pattern at a given voxel (Figure 3).

We correlated individual decoding to anxiety scores, and correlated individual decoding between regions using non-parametric Spearman's correlation (Figure 4A and B). We used a structural equation model (SEM) to test causal relationships between cortisol concentration level, neuronal decoding, and subjective fear (Figure 4C). Baseline variables and anxiety self-ratings were analysed using Kruskal-Wallis and Wilcoxon rank-sum tests. Cortisol levels were investigated using a 2 x 3 repeated measures ANOVA (group cortisol vs. placebo, and 3 time points). MVPA was performed with the "Searchmight" toolbox (Pereira and Botvinick, 2011). Statistical analysis was performed in R (v. 3.2.3); SEM was conducted with "lavaan" (v. 0.5-22). A P-value of < 0.05 was considered statistically significant. The analysis pipeline is illustrated in Supplementary Figure S1C.

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.

Results

Demographics, baseline and endocrine measures

The three groups did not significantly differ in age (Kruskal-Wallis test, $\chi^2 = 0.17$, df = 2, $P = .92$), gender ($\chi^2 = 4.44$, df = 2, $P = .11$; Table 1), or body mass index (Kruskal-Wallis test, $\chi^2 = 0.81$, df = 2, $P = .67$). The cortisol and placebo groups did not differ regarding spider phobia symptoms assessed by FSQ and SPQ before the experiment (Wilcoxon rank-sum test; FSQ: W = 95, $P = .87$; SPQ: W = 110.5, $P = .35$; Table 1). The patients had significantly

higher scores in spider phobia symptoms compared to healthy controls, who had no fear (Kruskal-Wallis test; FSQ: $\chi^2 = 30.0$, df = 2, $P < .0001$; SPQ: $\chi^2 = 30.4$, df = 2, $P < .0001$; Table 1). The cortisol group had 5.4 times higher cortisol levels at the beginning of the fMRI experiment, and 5.5 times higher levels at the end of the experiment compared to their baseline, while the placebo group showed no increase over time (Table 1). This was confirmed by a significant interaction group × time (repeated measures ANOVA; $F_{2,50} = 14.8$, $P < .0001$).

Subjective fear

During the fMRI task, patients (cortisol and placebo group) exhibited 2.6 times higher subjective fear in response to spider pictures compared to controls (Kruskal-Wallis rank sum test, $\chi^2 = 23.7$, df = 2, $P < .0001$), Figure 1A. Patients with cortisol treatment had a 12% decrease in subjective fear while looking at spider pictures compared to patients with placebo (Wilcoxon rank sum test, W = 43, $P = .021$), Figure 1A. We found a 2.6 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 ($\chi^2 = 23.1$, df = 2, $P < .0001$), Figure 1B. Patients with cortisol had a subjective fear reduction of 14% compared to the placebo group (W = 35, $P = .006$), Figure 1B. The three groups did not differ with respect to subjective fear while looking at the negative pictures ($\chi^2 = 2.7$, df = 2, $P = .26$), Figure 1C.

Neuronal decoding

We tested the null hypothesis of no difference in mean decoding accuracies for spider images among the three groups and found five areas that demonstrated significant differences in group means (Supplementary Table S1). The three largest areas were the middle cingulate cortex (MCC), left anterior insula, and right anterior insula (Figure 2A). These areas showed consistently higher local decoding for spider images in the patient groups (median 61%–63%) compared to healthy controls (51%–52%), see Figure 2B. We did not find a significant difference in decoding between the cortisol and placebo group, however, maps of decoding accuracies were smoothed across local values (as in Figure 2), a standard procedure in fMRI group inference, to average across individual functional differences, but MVPA is designed to investigate these. Therefore, we created a frequency map to show significant voxels (of at least three subjects, or more) in local decoding. Decoding in the placebo and cortisol group revealed distinct areas in the anterior insula and MCC in the cortisol and placebo individuals (Figure 3). Especially the MCC shows more prominent pattern activation in individuals of the placebo group compared to cortisol.

We tested the hypothesis of whether decoding was associated with experienced fear during the fMRI experiment. The strongest relationship was with the MCC explaining 38% of the variance in subjective fear (Figure 4A). The decoding in the two other areas explained 31% (insula l.), and 28% (insula r.) of the variance in subjective fear, rated after the experiment (Spearman's rank correlation; MCC, $\rho = .62$, $P < .0001$; insula l., $\rho = .56$, $P < .0001$; insula r., $\rho = .54$, $P = .0002$). The relationship was even stronger with subjective fear rated during the session (Spearman's rank correlation; MCC, $\rho = .78$, $P < .0001$, insula l., $\rho = .75$, $P < .0001$; insula r., $\rho = .73$, $P = .0002$). We examined the consistency of decoding between in the MCC, left and right insula, as they seem functionally highly connected, for both patient groups. We conducted a pairwise correlation of the three areas across patients

(Figure 4B). Decoding in all the three areas was significantly correlated in both group (= .73 or higher, all $P <$ 0.005; Spearman's rank correlation). The cortisol group had a lower correlation in decoding between the right insula and the MCC (= .73) compared to placebo (= .93), however, this difference in correlation coefficient missed significance level ($z = 1.71$, $P = .09$, Fischer's z; two-tailed).

We performed structural equation modelling (SEM) and path analysis for the patient data (Figure 4C). We found in the placebo group, subjective fear was positively influenced by the right anterior insula (path coefficient: 0.22), and the MCC (path coefficient: 0.13); this model explained 12% of the variance in subjective fear, with indirect effects from the left insula. In the cortisol group, fear was not influenced by the right insula (path coefficient: 0.08), and the mmc (path coefficient: -0.10), explaining only 1% of the variance in fear. Both models had no significant deviation from the data (cortisol: $\chi^2 = 3.48$, df = 2, $P = .32$; placebo: $\chi^2 = 4.95$, df = 2, $P = .08$).

Discussion

In this study, we found that the acute administration of glucocorticoids in patients with spider phobia resulted in a reduction of subjective fear during viewing of phobic images. This is in line with the findings of previous studies that demonstrated that the acute administration of glucocorticoids reduces phobic fear in patients with anxiety disorders (Aerni *et al.*, 2004; Soravia *et al.*, 2006). This fear reduction was accompanied by a change in circuity of limbic system, in particular, the MCC showed fewer in individual decoding in patients with cortisol. Decoding of phobic images in the MCC and anterior insula was highly correlated with subjective fear across individuals, with the MCC demonstrating the highest correlation. The MCC and the anterior insula are well-known key players in the processing of emotions and part of the fear network (Greco and Liberzon, 2016), and it was shown that exposure therapy reduces activity in these regions (Hauner *et al.*, 2012). We show now, that the bilateral anterior insula and especially the MCC appear to be particularly important target areas for the reorganization of neural fear processing after glucocorticoid administration.

The amygdala is a key region in phobia and emotional memory, for example, with dynamic functional connectivity to the hippocampus during emotional arousal (Fastenrath *et al.*, 2014). We could not find involvement of the amygdala in our results. A possible reason might be that participants were not engaged with real spiders, which in turn did not result in sufficient activation of the amygdala. On the other hand, signal dropouts close to this region may have occurred which is not unusual. Imaging the amygdala is problematic (Boubela *et al.*, 2015); large inter-individual variance in BOLD signal may be present in this particular brain region.

We created a causal model in which cortisol level reduces MCC decoding of spider images (in the cortisol group), which in turn influences subjective fear. This model explained 12% of subjective fear in the placebo, with positive influences of fear by the right anterior insula and the MMC. In the cortisol group, the data show a reduced influence on fear by the right anterior insula and an inhibitory (negative) influence by the MCC; also the link from the left anterior insula to the MCC has a reduced strength in the cortisol group. This result suggests that even though decoding in the MCC drives subjective fear, cortisol administration may influence and change the whole network of anterior insula and MCC. The key role of the MCC in the processing of emotional stimuli can be

further highlighted: fear is mainly associated with activity in the anterior MCC, a part of the MCC that receives amygdala input (Phan *et al.*, 2002; Vogt, 2005). The cingulate cortex is not uniformly involved in emotions, and it is striking that we identified the MCC as a particular key region, as no other cingulate cortex region has as strong and direct amygdala input, or as significant a role in fear (Vogt, 2005). 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).

Decoding of phobic pictures was positively correlated between the insula and MCC in the patients, in particular between the left and right anterior insula, and between the left anterior insula and the MCC, forming a functional network. The left and right anterior insula showed the highest correlations. This is highly consistent as most studies have shown abnormal activation mainly in the amygdala, anterior cingulate cortex, thalamus, and insula (Del Casale *et al.*, 2012). The anterior part of the insula is particularly involved in processing of emotionally salient information as it is heavily connected with limbic structures, the cingulate cortex, the amygdala, and the orbitofrontal cortex. 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, 2009). Recently, a study investigated the effects of CBT on anticipatory anxiety and post-event processing of fearful stimuli in spider phobic patients. Symptom reduction achieved through CBT was associated with reduced cerebral blood flow during post-event processing of fear inducing stimuli in the bilateral insula and part of the anterior cingulate cortex, with the right insula producing more significant clusters (Soravia et al., 2016). Thus, the acute administration of glucocorticoids as well as successful CBT is able to 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).

Pattern analysis is a method performed on the individual subject level. It is not surprising that results only partly overlap due to local functional decoding differences among individuals, especially in large structures such as the cingulate gyrus. It can thus be challenging to find consistent results in voxel-wise group analyses and this issue is generally addressed by applying a smoothing kernel. However, this artificially reduces the magnitude and spatial resolution of the decoding accuracies. Therefore, we found it important to show neural decoding on the subject level by showing a voxel-wise frequency maps of individual with significant patterns, accounting for individual variation in decoding. Rapid developments in fMRI such as improved data quality and advanced analysis tools now opens a new field of building a science of individual differences from fMRI (Dubois and Adolphs, 2016) and personalized investigation of brain function.

Some limitations of this study merit discussion. First, we present data from a small sample (N = 46), but this is not uncommon in a neuroimaging study. 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. Accordingly, we carefully investigated movement and excluded subjects that were affected. It is not surprising that some phobic patients moved their head while confronted with spider images. Interestingly, however, non-phobic healthy controls did not exhibit less head

movements. Small sample sizes may also 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 strong voxel-vise corrections (FDR 0.01 and 0.05 on the subject-level) and non-parametric tests in most of our 3rd-level analyses, which is regarded as safe (Eklund *et al.*, 2016). Non-parametric test are suitable for small samples, do not make the assumption of normality, and are generally more conservative in showing significant effects. Another limitation is the gender imbalance, even though, the gender deviation did not significantly differ among the three groups. We addressed this issue by systematically investigating gender and gender × group interactions in neural decoding. We found that decoding varied with gender in a smaller area of the MCC that the one we reported, but this effect does not survive a proper *p*-value threshold and could be purely noise. The gender imbalance is not an analysis problem, rather a design issue influenced by the random assignment to the groups and the exclusions of some of the subjects due to head movements.

In this study, we elucidate how glucocorticoid treatment could interact with brain regions and reduce fear. 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, 2011; Soravia *et al.*, 2014). The efficacy of cognitive-behavioural therapy (CBT) for anxiety disorders such as specific phobia has been demonstrated in various studies (Hofmann and Smits, 2008; Zlomke and Davis, 2008), but more than one-third of patients do not respond to this treatment (Cuthbert, 2002; Heimberg, 2002; Hofmann and Smits, 2008). Similarly, stress-induced cortisol elevation can reduce negative affect after stress (Het *et al.*, 2012). Therefore, glucocorticoid can potentially be beneficial to patients not responding to CBT or to boost psychotherapy outcome. Cortisol might be a promising approach for all disorders where the retrieval and reconsolidation of memories play a key role, such as posttraumatic stress disorder, anxiety disorders, and substance abuse disorders (Holz *et al.*, 2014; Drexler *et al.*, 2015).

In summary, relating changes in neuronal decoding within the limbic system, we have revealed neural mechanisms that provide new insight into the phobic brain.

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Conflict of Interest

The authors declare no conflict of interest.

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Figures

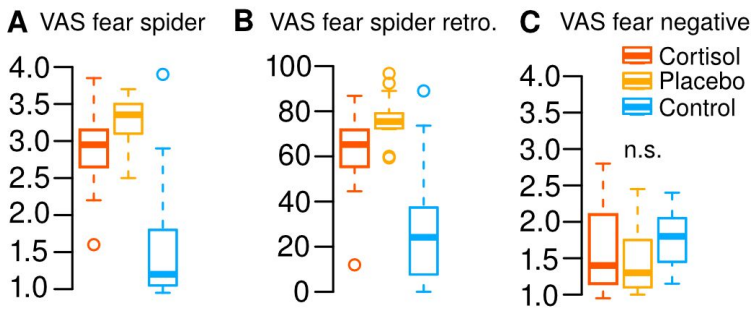

Figure 1. **(A)** The cortisol group exhibits a reduction of 12% in experienced fear towards spider pictures (compared to placebo; W = 43, $P = .021$) in the fMRI session as assessed during the experiment using a visual analogue scale (VAS). **(B)** The cortisol group shows a reduction of 14% in experienced fear from spiders during fMRI as assessed retrospectively after the experiment (W = 35, $P = .006$). Patients had a 2.6 times higher subjective fear compared the controls ($\chi^2 = 23.7$; $\chi^2 = 23.1$; both df = 2, $P < .0001$) in both assessments. **(C)** No significant group differences were found regarding emotional negative pictures.

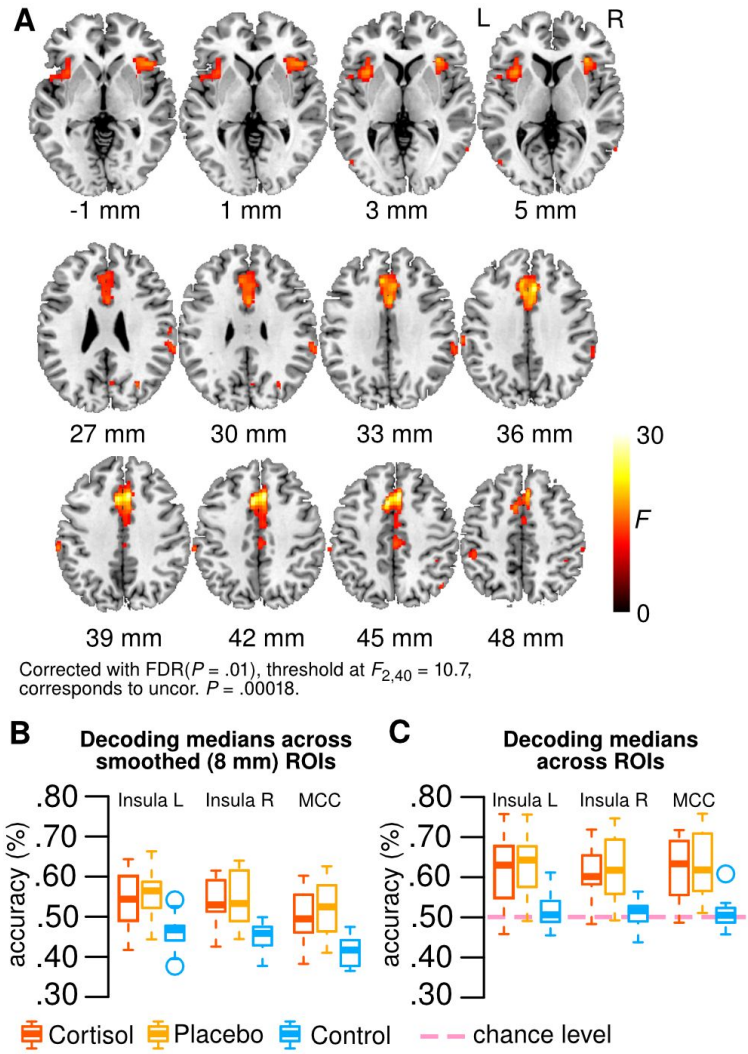

Figure 2. **(A)** Three regions (ROIs), left insula, right insula, and middle cingulate cortex (MCC) show a significant difference in group means (voxel-wise ANOVA, FDR corrected) in decoding (classification accuracy of spider vs. other pictures, such as negative, animal, and neutral). **(B)** Patients (cortisol and placebo) generally demonstrate higher decoding accuracies compared to controls. Due to voxel-wise group inference in (A), accuracies were spatially smoothed (FWHM 8 mm), but extracting unsmoothed local decoding values from the ROIs shows generally higher magnitudes and are closer to actual classification performance (though these are still averaged cross ROIs).

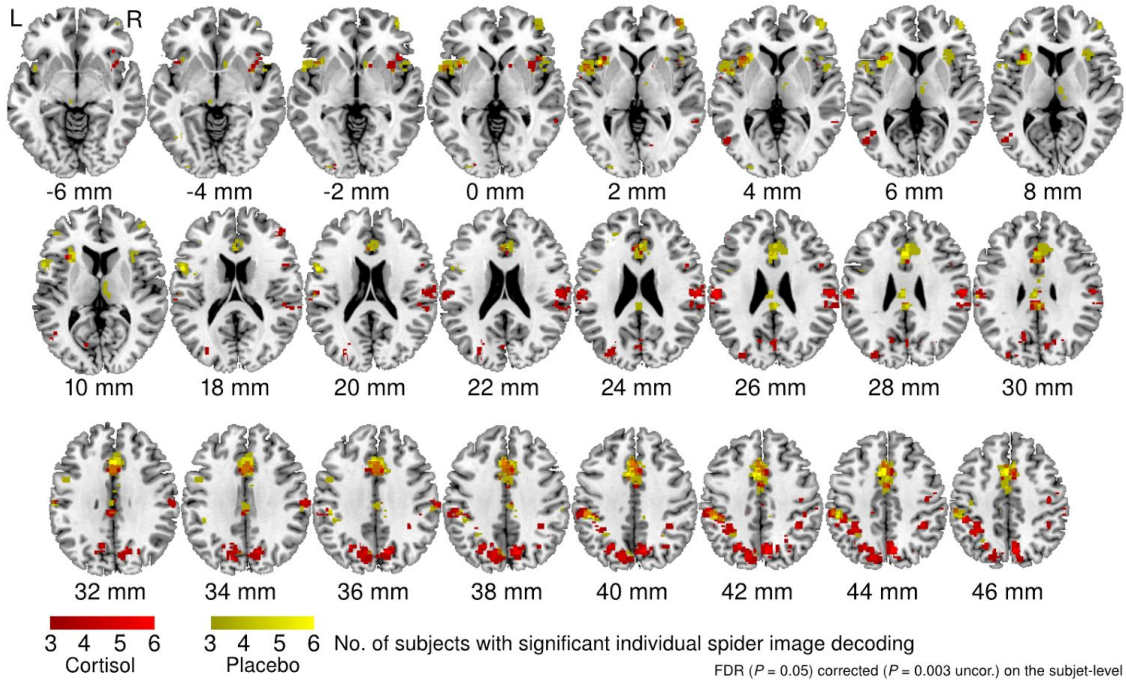

Figure 3. Aggregated individual decoding maps (each FDR 0.05 corrected on the subject-level), voxel represents significant decoding from at least three subjects, or more. Individuals of the placebo group show decoding of spider images most prominently in the MCC and left insula compared to the cortisol group. Individuals with cortisol have higher decoding in parietal and temporal areas.

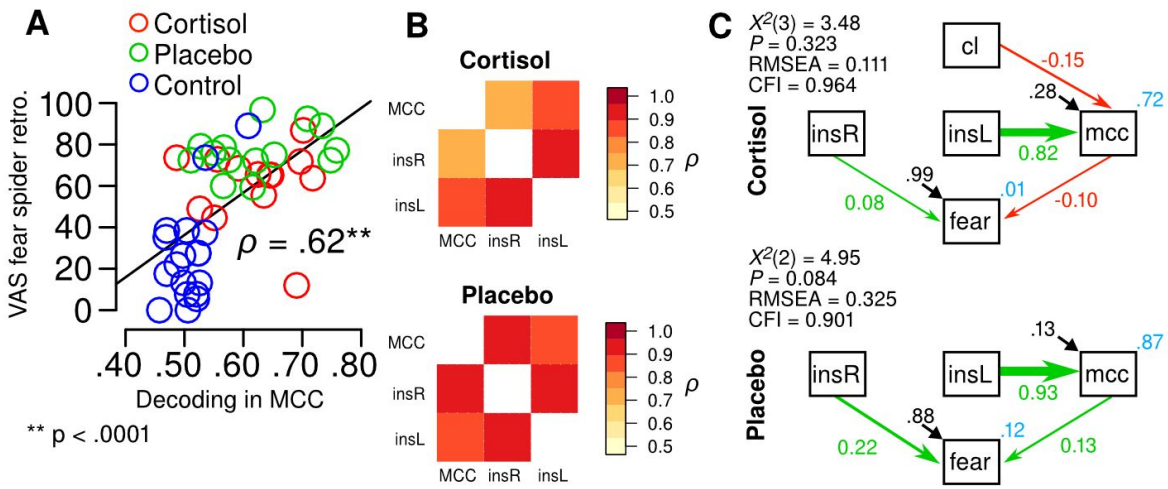

Figure 4. **(A)** Decoding in the middle cingulate cortex (MCC) of spider images positively correlates with subjective fear from spider pictures (Spearman) and explains 38% of variance (R^2) in these scores across all participants (N = 46). **(B)** Correlation matrix (Spearman's) of neural decoding between the three regions medial cingulate cortex (mcc), right insula (insR), and left insula (insL), separate for cortisol (n = 14) and patients group (n = 14), all P < 0.005. **(C)** Structural equation model for the two groups. Path coefficients (negative in red, positive in green) between cortisol concentration level in nmol/l (cl), neural decoding in the three regions, and subjective fear during the experiment. Residual variance is indicated at the upper left of the boxes, R^2 is in blue. In the cortisol group, the right insula and the mcc do not influence fear. In the placebo group, fear is positively influenced by the right insula and the mcc explaining 12% of variance. Both models had no significant deviation from the data (P > 0.05). Measures of overall model fit are shown in the upper left. RMSEA: root mean square error of approximation; CFI: comparative fit index.

Tables

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

	Patients with cortisol (n = 14)	Patients with placebo (n = 14)	Healthy controls (n = 18)
Median age (IQR)	28 (23–39)	29 (21–40)	27 (25–30)
Gender (male/female)	5/9	2/12	9/9
Mean BMI (SD)	22.4 (20.8–24.6)	22.7 (21.3–25.0)	21.9 (20.3–23.9)
Baseline fear from spiders:			
FSQ	77.0 (69.0–92.0)	77.0 (67.8–84.8)	8.0 (0–13.0)
SPQ	22.0 (20.0–27.0)	21.0 (18.3–22.0)	4.0 (3.0–5.8)
Median (IQR) cortisol concentration of saliva (nmol/l):			
baseline before administration	9.2 (8.0–12.2)	7.1 (6.7–12.5)	–
60 min. after administration	49.8 (18.3–56.4)	7.6 (4.9–10.1)	–
120 min. after administration	50.5 (22.8–82.0)	4.6 (3.0–5.8)	–

IQR: Interquartile range
BMI: Body Mass Index
FSQ: Fear of Spider Questionnaire
SPQ: Spider Phobia Questionnaire