Received: 5 February 2018 | Revised: 7 June 2018 | Accepted: 13 June 2018

DOI: 10.1002/da.22806

RESEARCH ARTICLE

WILEY ADAA ANXIETY AND DEPRESSION ASSOCIATION OF AMERICA

# Glucocorticoid administration restores salience network activity in patients with spider phobia

Leila Maria Soravia[1] | Simon Schwab[1,2] | Nico Weber[1] | Masahito Nakataki[1,3] | Roland Wiest[4] | Werner Strik[1] | Markus Heinrichs[5,6] | Dominique de Quervain[7] | Andrea Federspiel[1]

[1]Division of Systems Neuroscience of Psychopathology, University Hospital of Psychiatry, University of Bern, Bern, Switzerland

[2]Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, United Kingdom

[3]Department of Psychiatry, University of Tokushima, Tokushima, Japan

[4]Department of Diagnostic and Interventional Neuroradiology, Inselspital, University Hospital of Bern, Bern, Switzerland

[5]Laboratory for Biological and Personality Psychology, Department of Psychology, University of Freiburg, Freiburg, Germany

[6]Freiburg Brain Imaging Center, University Medical Center, University of Freiburg, Freiburg, Germany

[7]Division of Cognitive Neuroscience, Department of Psychology, University of Basel, Basel, Switzerland

**Correspondence**
Leila Maria Soravia, University Hospital of Psychiatry, University of Bern, Bolligenstrasse 111, 3000 Bern-60, Switzerland.
Email: leila.soravia@upd.unibe.ch

**Funding information**
Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: 32003B_124947; Universität Bern, Grant/Award Number: 520.10

**Background:** Glucocorticoids reduce phobic fear in patients with anxiety disorders. Although the neurobiology of anxiety disorders is not fully understood, convergent structural and functional neuroimaging studies have identified abnormalities in various brain regions, including those in the salience network (SN) and default mode network (DMN). Here, we examine the effects of glucocorticoid administration on SN and DMN activity during the processing of phobic stimuli.

**Methods:** We use functional magnetic resonance imaging to record brain activity in 24 female patients with spider phobia who were administered either 20 mg of cortisol or placebo while viewing pictures of spiders. Fourteen healthy female participants were tested with the same task but without substance administration. Independent component analysis (ICA) performed during stimulus encoding identified the SN and DMN as exhibiting synchronized activation in diverse brain regions; thus, we examined the effects of cortisol on these networks. Furthermore, participants had to rate their level of fear at various time points.

**Results:** Glucocorticoids reduced phobic fear in patients with spider phobia. The ICA performed during stimulus encoding revealed that activity in the SN and DMN was reduced in placebo-treated patients versus healthy controls. Brain activity in the SN, but not the DMN, was altered in cortisol- versus placebo-treated patients to a level that was similar to that observed in healthy controls.

**Conclusions:** Activity in both the SN and DMN was reduced in patients with spider phobia. Cortisol administration altered the SN activity to a level that was comparable to that found in healthy controls. This alteration in SN activity might reflect the fear-reducing effects of glucocorticoids in phobia.

KEYWORDS
default mode network, fear, fMRI, glucocorticoids, phobia, salience network

## 1 | INTRODUCTION

Specific phobias are the most common anxiety disorders and are characterized by persistent and excessive fear, cued by the anticipation or presence of a specific object or situation (American Psychiatric Association [APA], 1994). The confrontation or even anticipation of the phobic stimulus almost invariably provokes the retrieval of stimulus-associated fear memories, which results in a fear response (Cuthbert et al., 2003; de Quervain & Margraf, 2008). The retrieval of past phobic experiences and the subsequent fear response support the (re)consolidation of the fear memories and ultimately reinforce the fear memory trace (Sara, 2000). Thus, the retrieval and (re)consolidation of fearful memories play crucial roles in the symptomatology and maintenance of phobic disorders.

When exposed to stress, the body releases glucocorticoid hormones, and many studies have demonstrated that glucocorticoids modulate memory processes. While the release of glucocorticoids enhances memory consolidation (Abercrombie, Kalin, Thurow, Rosenkranz, & Davidson, 2003; Buchanan & Lovallo, 2001), it also inhibits the retrieval of previously acquired information (for reviews, see de Quervain, Aerni, Schelling, & Roozendaal, 2009; Wolf, 2009). Previously, several clinical studies investigated whether

 © 2018 Wiley Periodicals, Inc. 

glucocorticoids exert fear-reducing effects, including the inhibition of fear memory retrieval, in patients with anxiety disorders. The results of such studies showed that glucocorticoid administration leads to a reduction of phobic fear in patients suffering from social phobia, spider phobia (Soravia et al., 2006; Soravia et al., 2014), and a fear of heights (for review, see de Quervain, Schwabe, & Roozendaal, 2017; de Quervain et al., 2011). Additionally, it has been recently reported that patients with spider phobia exhibit aberrant functional connectivity of the amygdala when they are exposed to phobia-related stimuli, while cortisol administration can alleviate this fear-specific neural connectivity (Nakataki et al., 2017). However, it remains unclear whether cortisol exerts its fear-reducing effects solely by modulating discrete connectivity networks involving the amygdala or whether cortisol also modulates the large-scale brain networks that are involved in fear-processing.

Large-scale brain networks have been gaining increased attention, especially in studies evaluating the cognitive and affective impairments that are present in patients with psychiatric disorders (e.g., schizophrenia, depression, anxiety; Arnold Anteraper et al., 2014; McFadden, Tregellas, Shott, & Frank, 2014; Palaniyappan & Liddle, 2012; Peterson, Thome, Frewen, & Lanius, 2014; Uddin et al., 2013). Examining brain networks during the resting state or during a stimulus-related task allows researchers to identify differences in the crosstalk that occurs among brain regions, which may underlie abnormal task response-related activation (Whitfield-Gabrieli & Ford, 2012). There are two networks of special interest in the context of anxiety disorders, namely the default mode network (DMN) and salience network (SN). The DMN is associated with stimulus-independent thought, self-reflection, interoception, and episodic memory and includes the posterior cingulate (cuneus, precuneus), medial prefrontal, medial temporal, and inferior parietal cortices (Andrews-Hanna, Reidler, Sepulcre, Poulin, & Buckner, 2010; Gusnard, Akbudak, Shulman, & Raichle, 2001; Whitfield-Gabrieli & Ford, 2012). The SN is essential for identifying the relevance of internal and external stimuli in order to guide behavior, which primarily involves the anterior cingulate cortex (ACC) and insula (Menon, 2011; Menon & Uddin, 2010). Moreover, several studies have reported altered resting-state functional brain connectivity within the SN and DMN in patients with social anxiety disorder (Pannekoek et al., 2013a; Pannekoek et al., 2013b; Peterson et al., 2014). While most studies investigating large-scale brain networks used a task-free resting-state functional connectivity approach, a few studies have assessed network activity during task performance (e.g., Fair et al., 2007; Garrity et al., 2007; McMenamin, Langeslag, Sirbu, Padmala, & Pessoa, 2014; Meda, Stevens, Folley, Calhoun, & Pearlson, 2009); this task-related approach is likely more relevant to everyday behavior, and is therefore essential for improving our understanding of psychopathology (McFadden et al., 2014). As such, additional studies examining task-related activity in the SN and DMN in patients with phobias are needed to establish how these networks are affected by phobias and whether the administration of various substances, including glucocorticoids, can alter the activity in these networks and reduce fear.

The aim of the present double-blind, placebo-controlled, randomized study is to examine the anxiolytic effects of glucocorticoid administration on fear-relevant network activity, such as that in the SN and DMN, across fear-inducing task conditions in patients with spider phobia. Specifically, patients with spider phobia received 20 mg of cortisol or placebo 1 hr before performing a picture task that provoked phobic fear during functional magnetic resonance imaging (fMRI). Patients with spider phobia were further compared to a healthy control group that did not receive any substances. The sample in this study employing network analyses was used previously in a dynamic causal modeling study (Nakataki et al., 2017). We hypothesized that the blood oxygen level-dependent response in the SN for phobic pictures would differ between patients and healthy control participants. Based on the known behavioral effects of cortisol, we expected that the reduction of subjective fear following glucocorticoid administration in patients with spider phobia would be related to changes in the blood oxygen level-dependent response in the SN during the processing of phobic stimuli. As the results of studies evaluating the DMN activity changes, that occur in patients with anxiety disorders, are inconsistent (Pannekoek et al., 2013b; Whitfield-Gabrieli & Ford, 2012), we did not have a specific hypothesis regarding the effects of glucocorticoids on the DMN.

## 2 | METHODS

### 2.1 | Participants

This study consisted of the following three participant groups: patients with spider phobia who received either 20 mg of cortisol or placebo in a double-blind and randomized manner, and healthy participants who did not receive any medication, as described in detail in our previous study (Nakataki et al., 2017). After the fMRI investigation, all patients were offered the opportunity to attend exposure-based group therapy, in which we investigated the effectiveness of the combination of cortisol treatment and cognitive behavioral therapy for spider phobia (Soravia et al., 2014). All participants were recruited via advertisement.

The patients' diagnoses were based on the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (APA, 1994), using a computer-based structured clinical interview (Essau, Wittchen, & Pfister, 1999; Wittchen & Pfister, 1997) that is based on the Composite International Diagnostic Interview (Rubio-Stipec & Bravo, 1991). The German version (Rinck et al., 2002) of the Spider Phobia Questionnaire (Watts & Sharrock, 1984) and Fear of Spiders Questionnaire (Szymanski & O'Donohue, 1995) were also used to assess the participants' fear of spiders.

Exclusion criteria for all participants included a history of head injury, acute or chronic medical conditions, a recent history of systemic or oral glucocorticoid therapy, psychiatric disorders (other than spider phobia for patients with spider phobia), receiving psychotropic drug treatment, smoking >15 cigarettes per day, neurological diseases, use of hormonal contraceptives, current drug or alcohol abuse, or any contraindication to magnetic resonance imaging (metallic objects, pregnancy).

After participants received a complete description of the study, 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 (Rickham, 1964) and the Swiss agency for the authorization and supervision of therapeutic products (Swissmedic, Bern, Switzerland).

Eighteen patients were assigned respectively to the cortisol group (11 women) and placebo group (15 women), and 27 healthy control participants (17 women) were included after the assessment of inclusion criteria. Two patients and three healthy control participants were excluded due to missing data, and one patient was excluded because of ineffective elevation in the salivary cortisol levels in response to cortisol administration. The final sample consisted of 17 patients in the cortisol group (10 women), 17 patients in the placebo group (14 women), and 24 healthy control participants (14 women). According to our previous study, we used only the data from female participants in the analyses to avoid an imbalanced sex distribution across groups (Nakataki et al., 2017). However, additional analyses including the whole sample (male and female participants) are provided in the Supporting Information.

## 2.2 | Experimental procedure and measurements

The procedures for this study were described in detail in our previous study (Nakataki et al., 2017); therefore, they are described in brief here. The experiments were conducted at the Department of Diagnostic and Interventional Neuroradiology, University Hospital of Bern, Switzerland, between 14:00 and 17:00. Since sex differences in the effects of cortisol on fear extinction are known to exist (Andreano & Cahill, 2006) and the responsiveness to exposure therapy is affected by endogenous estradiol and hormonal contraceptive use (Graham, Li, Black, & Öst, 2018), we only included female participants with a natural menstrual cycle. All measurements were performed in the luteal phase of the menstrual cycle; the luteal phase was defined as the second half of the menstrual cycle, which was determined based on reports provided by the women. Patients and control participants underwent the same experimental procedures, except for the diagnostic interview, substance administration, and collection of saliva samples (only in the patient group). Saliva samples were collected to monitor and document the effectiveness of the glucocorticoid administration in patients. Upon arrival, participants were informed of the procedures, given instructions regarding the fMRI picture task, and asked to complete a short exercise on the computer to familiarize themselves with the ratings. Furthermore, all participants were asked to complete the State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970) and to rate their actual subjective anxiety, physical discomfort, and avoidance behavior on a visual analog scale (VAS) ranging from 0 (no symptoms) to 100 (maximal symptoms). Additionally, the first saliva sample was collected using a Salivette (Sarstedt Inc., Rommelsdorf, Germany). After the oral administration of 20 mg of hydrocortisone (two tablets, each containing 10 mg of hydrocortisone; Hydrocortone®, MSD, Merck Sharp & Dohme-Chibret AG, Switzerland) or placebo (Galepharm, Zürich, Switzerland), the patients rested for approximately 30 min. Additional saliva samples were collected 60 and 120 min after drug administration to confirm an elevation in their cortisol levels. After the fMRI session, the participants retrospectively rated their fear levels on a VAS from 0 to 100 while observing the spider pictures in the scanner and were asked to complete again the STAI and VAS questionnaires. We also inquired about any side effects that they might have experienced owing to the glucocorticoid or placebo administration. The saliva samples were stored at −20°C until the biochemical analyses. The free salivary cortisol concentrations were analyzed using a commercially available chemiluminescence immunoassay (cortisol: CLIA; IBL-Hamburg, Germany).

## 2.3 | fMRI task

The fMRI task was an adapted version of the picture task published by Rasch et al. (2009). It contained 80 photographs of four emotional categories (negative, neutral, animal [positive], and spider [phobic]), partly selected from the International Affective Picture System (Lang, Bradley, & Cuthbert, 2005). To investigate the neuronal correlates of the specific effects of cortisol on phobic fear processing, we aggregated the negative, neutral, and positive/animal pictures into one non-phobic category, which we compared with the phobic condition (spider). Each participant rated every picture immediately after presentation in accordance with its emotional valence on a scale ranging from 1 (no fear) to 4 (maximum fear) during fMRI. The pictures were presented for 5 s, followed by a fixation cross for 1 s, and then the rating, which had a maximum duration of 3 s. Each block (picture, fixation, rating) lasted 9 s and was separated from the next block by an interval that was randomly jittered from 3 to 5 seconds. The stimulus presentation and response registration were performed using E-Prime 2.0 (Psychology Software Tools Inc., Pittsburgh, PA).

## 2.4 | MRI data acquisition

The fMRI experiment was conducted at the Department of Diagnostic and Interventional Neuroradiology, University Hospital of Bern, on a 3-T Siemens Trio unit (Erlangen, Germany). Both functional and structural MRI data were acquired. Details of all sequence parameters are provided in the Supporting Information.

## 2.5 | MRI data analysis

The functional and structural MRI data were preprocessed and analyzed using the Statistical Parametric Mapping, version 8, software (Wellcome Department of Imaging Neuroscience, University of London; https://www.fil.ion.ucl.ac.uk/spm/). Details of all preprocessing steps are provided in the Supporting Information.

## 2.6 | Statistical procedures

### 2.6.1 | First-Level statistics (subject level)

Each participant's individual activity was extracted from the time course of the independent component analysis (ICA) performed for the DMN and SN, respectively. In order to associate the activity that occurred during the phobic and non-phobic conditions within these networks, we created a design matrix that coded for the time points related to the stimulus encoding of the phobic and non-phobic

pictures. In this study, the exact time points corresponding to these stimuli were considered, without any hemodynamic modulation. Moreover, the activity of these two predictors (phobic vs. non-phobic) was further subdivided to account for the emotional valence (four levels: 1 = no fear to 4 = maximum fear). Therefore, for each participant, a total of eight activity values (extracted from the ICA activity time course) were available for further analysis and were used in the second-level statistics as datasets.

#### 2.6.2 | Second-Level statistics (result-generating statistics)

To assess possible differences at the group level, three-way analysis of variances (ANOVA) with subsequent two-sample *t*-tests was performed for the following pairs: cortisol versus healthy controls, placebo versus healthy controls, and cortisol versus placebo. Furthermore, we divided the group level *t*-testing depending on the category, phobic versus non-phobic.

#### 2.6.3 | Statistical analysis of behavioral data

Group differences in demographics, clinical characteristics, and baseline salivary cortisol levels (before substance administration) were analyzed with an ANOVA and unpaired *t*-tests. The effects of cortisol administration on salivary cortisol concentrations, VAS ratings, and the extracted beta values were analyzed with two-way repeated measures ANOVA, using treatment or group as the between-subjects factor and the time point as the within-subjects factor. As the salivary cortisol data did not follow the normal distribution, cortisol data were log-transformed. A Greenhouse–Geisser correction was used to correct violations of sphericity. Subsequent unpaired *t*-tests were used to analyze the treatment effects at specific time points. All tests were two-tailed, and statistical significance was set at $P < 0.05$.

### 2.7 | Hormone analysis

Free cortisol concentrations in saliva were analyzed using commercially available chemiluminescence immunoassays (cortisol: CLIA; IBL-Hamburg, Germany). The inter- and intra-assay coefficients of variation were <10%. The samples of all participants were analyzed in the same run to reduce error variance caused by imprecision in the intra-assay.

## 3 | RESULTS

The cortisol and behavioral data has been described in detail in our previous study and is briefly recapitulated here (Nakataki et al., 2017).

### 3.1 | Behavioral and cortisol measurements

The three groups (patients: cortisol and placebo; healthy controls) did not differ in terms of their demographic characteristics (age, sex, body mass index) and baseline measurements on the day of the experiment, but patients differed from controls with regard to the presence of spider-phobic symptoms. The salivary cortisol levels were increased in the patients who received 20 mg of cortisol, as demonstrated by a significant time × group interaction effect ($F[1.4, 30.2] = 44.37$, $P < 0.001$). Additional post hoc comparisons showed a significant increase in salivary cortisol at 60 and 120 min after substance administration in the cortisol group compared with the placebo group (Table 1).

During the presentation of spider pictures in the scanner and after the scanning session when participants were asked to rate their perceived fear in response to the spider pictures, the subjective fear ratings of patients with spider phobia who received cortisol were significantly lower than the ratings of the placebo group but still significantly higher than those of the control group (Table 1). No significant effects of cortisol were identified for the fear ratings of non-phobic pictures or for the ratings of phobia-unrelated state anxiety before and after the scanning session (Table 1).

### 3.2 | Activity in the SN

The ICA we performed during stimulus encoding identified the SN via synchronous activity in the following brain regions: ACC, bilateral insula, bilateral parietal lobe, and bilateral Brodmann areas (BAs) 9 and 10 (Figure 1a). Moreover, we identified activity in the bilateral mediodorsal thalamic nuclei.

A three-way ANOVA, with the factors of category, group, and emotional valence, was conducted to reveal differences in SN activity. We identified a significant main effect of group ($F[2, 189] = 10.15$, $P < 0.001$) but no significant main effects of category ($F[1, 189] = 0.85$, $P = 0.3574$) or emotional valence ($F[3, 189] = 0.54$, $P = 0.6522$). No significant interaction effects were observed for the SN activity (category × group: $F[2, 189] = 0.8$, $P = 0.451$; category × emotional valence: $F[3, 189] = 0.61$, $P = 0.6079$; group × emotional valence: $F[6, 189] = 0.5$, $P = 0.8043$). To further explore and isolate the origin of the observed main group effect we performed additional subsequent post hoc test (Tukey). These revealed significant mean differences (*MD*) and Standard Error (*SE*) for "Cortisol" versus "Placebo" $MD = -0.0346$, $SE = 0.013$, $P < 0.001$; "Placebo" versus "Healthy Controls" $MD = 0.1049$, $SE = 0.014$, $P < 0.01$. We observed no significant *MD* for "Cortisol" versus "Healthy Controls" $MD = 0.0302$, $SE = 0.012$, $P$ = not significant (n.s.). Subsequent two-sample *t*-tests comparing the activity to phobic and non-phobic stimuli between the groups revealed significantly higher brain activity in the SN for both stimulus types in the placebo group than in the cortisol group (phobic: $t[63] = -3.15$, $P = 0.003$; non-phobic: $t[72] = -2.90$, $P = 0.005$) and healthy control group (phobic: $t[59] = -3.06$, $P = 0.004$; non-phobic: $t[78] = -2.44$, $P = 0.02$; Figure 2). However, the SN activity in patients treated with cortisol did not differ significantly from that of healthy control participants for either stimulus type (phobic: $t[54] = 0.28$, $P = 0.77$; non-phobic: $t[72] = 1.21$, $P = 0.23$).

### 3.3 | Activity in the DMN

Our ICA during stimulus encoding identified the DMN via synchronized activity in the ACC, posterior cingulate cortex, and bilateral parietal lobe (Figure 1b).

A three-way ANOVA, with the factors of category, group, and emotional valence, was conducted to reveal differences in DMN

**TABLE 1** Demographics and descriptive statistics of the behavioral data

| Group | Cortisol | Placebo | Healthy control |
|---|---|---|---|
| Number of female participants | 10 | 14 | 14 |
| Age (years) | 31.3 ± 11.2 | 26.6 ± 11.2 | 27.5 ± 5.8 |
| BMI | 22.7 ± 4.9 | 22.8 ± 3.7 | 22.1 ± 2.4 |
| FSQ | 75.3 ± 24.4*** | 77.9 ± 11.7*** | 14.0 ± 15.4 |
| Pre-scan | | | |
| STAI-state | 41.2 ± 13.9 | 35.6 ± 8.3 | 31.7 ± 3.9 |
| Mid-scan, picture task[a] | | | |
| VAS fear: phobic pictures | 2.9 ± 0.6***, ### | 3.3 ± 0.5*** | 1.9 ± 0.8 |
| VAS fear: negative pictures | 1.8 ± 0.7 | 1.7 ± 0.5 | 1.9 ± 0.4 |
| VAS fear: neutral pictures | 1.1 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.1 |
| VAS fear: animal pictures | 1.2 ± 0.2 | 1.1 ± 0.1 | 1.2 ± 0.2 |
| Post-scan | | | |
| STAI-state | 33.8 ± 10.7 | 36.9 ± 14.1 | 29.0 ± 5.5 |
| VAS fear retrospect[b] | 64.3 ± 19.1*,# | 78.4 ± 11.2* | 29.2 ± 25.8 |
| Cortisol concentration in the saliva (nmol/L)[c] | | | |
| Before administration | 0.91 ± 0.25 | 0.03 ± 0.20 | |
| 60 min after administration | 1.49 ± 0.27### | 0.85 ± 0.22 | |
| 120 min after administration | 1.61 ± 0.39### | 0.67 ± 0.20 | |

BMI: body mass index; FSQ: Fear of Spiders Questionnaire (German version); STAI: State-Trait Anxiety Inventory; VAS: visual analog scale.
[a]During imaging, each picture had to be rated in the scanner on a scale from 1 (no fear) to 4 (maximum fear).
[b]After the scanning session, participants were asked to rate the experienced emotion during the viewing of spider pictures in the scanner retrospectively on a scale from 0 to 100. All data are presented as the mean ± the standard deviation.
[c]Cortisol data were log-transformed.
***$P < 0.001$ compared to the control group; *$P < 0.05$ compared to the control group; ###$P < 0.001$ compared to the placebo group; #$P < 0.05$ compared to the placebo group.
*Note*. This table was previously reported in Nakataki et al. (2017).

activity. This analysis yielded a significant main effect of group (F[2, 189] = 23.46, $P < 0.001$) but no significant main effects of category (F[1, 189] = 0.66, $P = 0.4164$) or emotional valence (F[3, 189] = 0.25, $P = 0.8616$). No significant interaction effects were observed in the DMN (category × group: F[2, 189] = 0.86, $P = 0.4363$; category × emotional valence: F[3, 189] = 0.48, $P = 0.6986$; group × emotional valence: F[6, 189] = 0.43, $P = 0.8552$). To further explore and isolate the origin of the observed main group effect we performed additional subsequent post hoc test (Tukey). These revealed significant *MD* and *SE* for "Cortisol" versus "Healthy Controls" *MD* = 0.1573, *SE* = 0.017, $P < 0.001$; "Placebo" versus "Healthy Controls" *MD* = 0.1502, *SE* = 0.018, $P < 0.001$. We observed no significant *MD* for "Cortisol" versus "Placebo" *MD* = 0.0071, *SE* = 0.016, *P* = n.s. Two-sample *t*-tests comparing the DMN activity to phobic and non-phobic stimuli between the groups showed that the activity in the placebo group did not differ significantly from the cortisol group for either stimulus type (phobic: $t[63] = -0.33$, $P = 0.74$; non-phobic: $t[72] = 0.82$, $P = 0.41$); however, significantly lower DMN activity was observed in the two patient groups than in the healthy control group for both stimulus types (cortisol vs. healthy controls: phobic: $t[54] = -5.54$, $P = 0.001$; non-phobic: $t[72] = -4.88$, $P = 0.001$; placebo vs. healthy controls: phobic: $t[59] = -5.83$, $P = 0.001$; non-phobic: $t[78] = -5.1$, $P = 0.001$).

## 4 | DISCUSSION

As most studies have investigated large-scale brain networks using task-free resting-state functional connectivity approaches, to our knowledge, ours is the first study to evaluate the anxiolytic effects of glucocorticoid administration on SN and DMN activity using fMRI, while patients with spider phobia were exposed to phobic stimuli. In this study, we investigated the effects of glucocorticoid administration on fear-relevant network activity in patients with spider phobia across fear-inducing task conditions. We found that activity in both the SN and DMN was aberrant in placebo-treated patients with spider phobia compared to healthy controls. Furthermore, our findings revealed that glucocorticoid administration altered SN activity in patients with spider phobia to a level that was comparable to that in healthy controls, while it had no effect on DMN activity. As reported in detail in our previous study, cortisol administration reduced phobic fear in patients with spider phobia but did not affect phobia-unrelated anxiety (Nakataki et al., 2017). The present results are in line with previous findings from studies investigating brain networks using task-free resting-state functional connectivity approaches, which showed that brain activity within the SN and DMN plays a crucial role in anxiety disorders (Menon, 2011; Menon & Uddin, 2010; Pannekoek et al., 2013a; Whitfield-Gabrieli & Ford, 2012). Thus, glucocorticoids

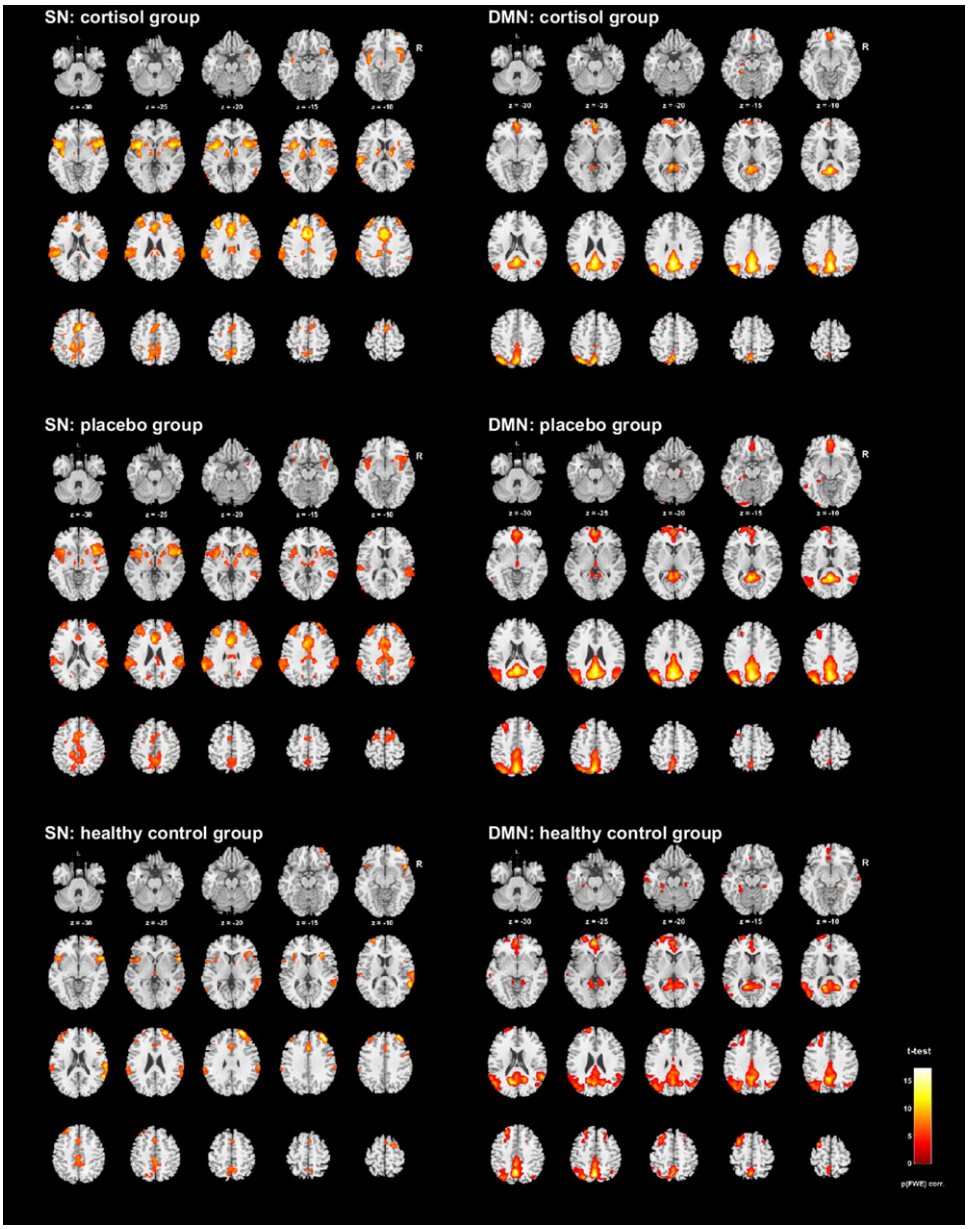


**FIGURE 1** Active brain regions of the (a) salience network (SN) and (b) default mode network (DMN) during encoding within the three experimental groups: (1) cortisol group, (2) placebo group, (3) healthy control group. Axial view with *t*-test values in hot colors (white, yellow, red)

seem to modulate both subjective fear and SN activity, while exerting no effects on DMN activity, in patients with spider phobia.

The ACC is an essential part of the SN, and we identified activity within this region in all of our participants (Menon & Uddin, 2010; Shang et al., 2014). A meta-analyses performed by Shang et al. highlighted the importance of the ACC in emotion regulation, affectional expression, and physiological reactions. The authors suggested that alterations in the ACC and medial prefrontal cortex underlie the occurrence of anxiety disorders, because emotional processing cannot be modulated through the conscious executive control over emotional stimulus and cannot inhibit the fear that appears during periods of anxiety.

Another key brain structure in the SN that is involved in the perception and processing of fear is the anterior insula. A study in patients with spider phobia showed that activity in the ACC is correlated and overlaps with activity in the anterior insula during phobic reactions (Caseras et al., 2013). The authors suggested that the concurrent activity within these two brain structures might be associated with the integration of perceived stimuli characteristics and bodily responses that lead to fear. The results indicate a lateralization effect in the insula, with the right anterior insula being particularly involved during emotional value processing and interoceptive perception and the left insula primarily responding to the emotional value of external stimuli. This is consistent with the discovery of increased activation in specific brain regions, including the ACC and insula, in participants with spider phobia during the anticipation of phobic-relevant stimuli (Straube, Mentzel, & Miltner, 2007). Thus, our findings add to the existing evidence supporting the strong involvement of the bilateral insula in the processing of phobic, and therefore highly emotional, stimuli.

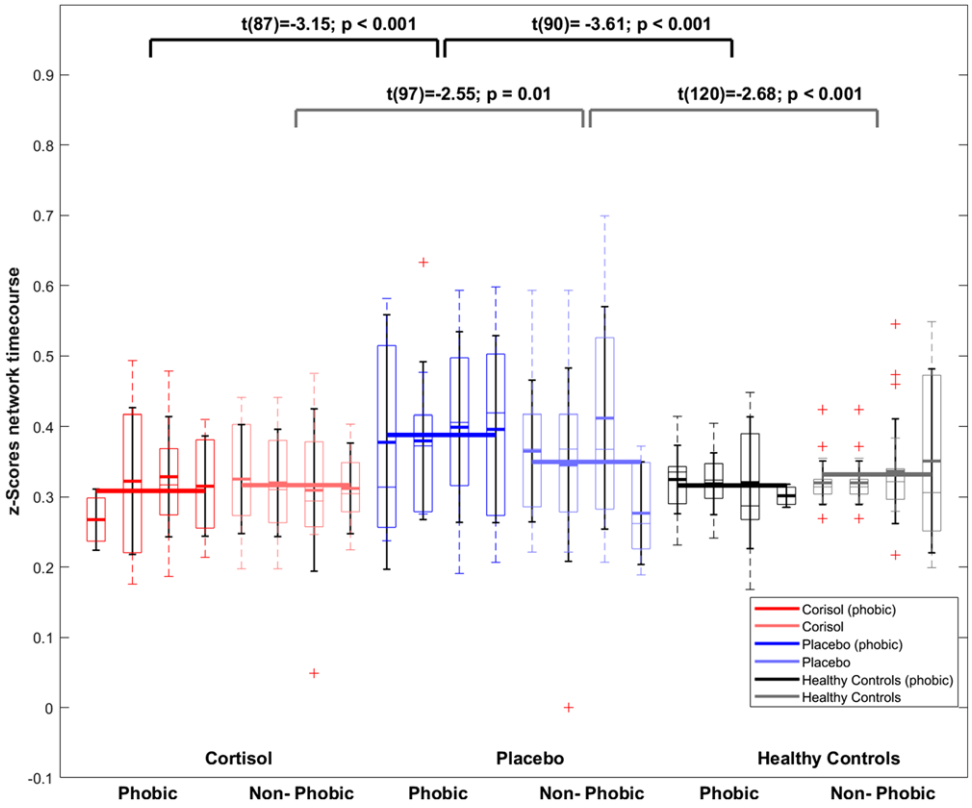


**FIGURE 2** Salience network (SN) activity as a function of the response to stimuli (phobic vs. non-phobic). Colors reflect the different groups (red: cortisol, blue: placebo, grey: healthy controls). The brightness gradation of the colors (dark and light) distinguishes between the categories (phobic pictures and non-phobic pictures). The four bars within the two categories represent the mean of the ratings for the emotional valence of the pictures (1: no fear to 4: maximal fear)

As BA10 is part of the orbitofrontal cortex (Kringelbach, 2005), its activity during stimulus encoding is in accordance with studies showing that the orbitofrontal cortex is involved in sensory integration, representing the affective value of reinforcers, and decision-making and expectation, making it critical for adaptive learning. Additionally, according to Hilbert and colleagues (Hilbert, Evens, Maslowski, Wittchen, & Lueken, 2015), individuals with snake and dental phobias exhibited increased grey matter volumes in the ACC, orbitofrontal cortex, insula, and dorsomedial prefrontal cortex. Therefore, in sum, the abovementioned findings show that various brain regions in the SN, including parts of the prefrontal cortex (ACC, BAs 9 and 10), anterior insula, and bilateral basal ganglia, are active during stimulus encoding and are crucially involved in the processing of emotional stimuli, while activity in regions of the DMN, namely the ACC, posterior cingulate cortex, and bilateral parietal lobe, is associated with stimulus-independent thought and self-reflection.

Here, glucocorticoid administration in patients with spider phobia reduced phobic fear and altered activity in the SN to a level that was comparable to the SN activity of healthy control participants, which may reflect reduced fear processing in patients treated with glucocorticoids (Shang et al., 2014). As the SN and its underlying neuroanatomical structures are key players in the processing of stimuli with emotional value (Menon, 2011), the findings of the present study suggest that glucocorticoids may modify SN activity and thereby the associated fear response. However, the present study cannot identify the causal relationship between these factors, that is, we cannot conclude whether glucocorticoid administration altered SN activity resulting in reduced phobic fear or whether glucocorticoid administration affected other brain structures, such as the amygdala or parahippocampus, thus leading to reduced phobic fear and, consequently, altered SN activity.

Although the DMN activity in the cortisol group was not different from that in the placebo group, we did observe altered DMN activity in both patient groups compared to the control group, suggesting that activity in the DMN is sufficiently sensitive to differentiate between patients with spider phobia and healthy control participants. This finding is in line with the results of a study that examined SN and DMN activity across task conditions in patients with current anorexia nervosa, recovered patients, and healthy controls (McFadden et al., 2014). In that study, reduced SN activity was observed in both the currently ill and recovered patients, while altered DMN activity was observed in currently ill, but not recovered, patients. These results are similar to our findings showing that DMN activity was able to differentiate patients with a current diagnosis of spider phobia from healthy control participants. It was suggested by McFadden et al. (2014) that alterations in the DMN might reflect a state marker that normalizes with recovery, while altered SN activity might be a consequence of the illness or a trait vulnerability that was present before symptom onset. According to a review of functional neuroimaging studies performed on patients with specific phobias, most studies have shown aberrant activation in brain regions involved in emotion generation

and emotion regulation, particularly the amygdala, ACC, thalamus, and insula, which normalizes after successful psychotherapy (Del Casale et al., 2012; Paquette et al., 2003; Soravia et al., 2016a). However, additional studies are needed to investigate the specific network activity in patients with phobia before and after treatment. It is also possible that the different DMN activity between patients and control participants during encoding reflects the patients' deeper emotional involvement during the presentation of phobic stimuli. This possibility concurs with the results of studies showing that the suppression of internally oriented thoughts leads to a more comprehensive and thorough evaluation of a stimulus and its emotional valence and is associated with better concentration, which in turn leads to deeper encoding and increases learning and subsequent retrieval (Soravia et al., 2016b; Whitfield-Gabrieli & Ford, 2012).

This study has some limitations that should be considered. First, our relatively small sample size. Second, because the number of male participants differed among the groups, we excluded the male participants from the analysis to avoid this confound. However, the results of the additional analyses we performed using the whole sample (male and female participants) did not differ significantly from those of our analysis employing only female participants (see Supporting Information). Furthermore, only female participants with a natural menstrual cycle were included in the study and tested within their luteal phase. Therefore, our findings may not be applicable to women during the follicular phase or women who use hormonal contraceptives, limiting the generalizability of the current findings. Furthermore, the luteal phase was defined based on the women's self-reports about their menstrual cycle and was not verified through estradiol measurements. It is important to note that we assessed the SN and DMN during a task and not during rest, as is done traditionally. A further limitation is that we did not include a group of healthy participants who received glucocorticoids. Although we have previously shown that cortisol administration does not affect the fear response in healthy participants (Soravia, de Quervain, & Heinrichs, 2009), the administration of glucocorticoids to healthy control participants may have yielded insight into the effects of stress hormones on brain activity independent of psychopathology.

In conclusion, the present findings show that the administration of glucocorticoids reduced phobic fear in patients with spider phobia and enabled us to link the fear-reducing effects of glucocorticoid administration to a distinct neurophysiological correlate. The results demonstrate a deviant pattern of brain activity in both the SN and DMN in patients with spider phobia compared to healthy controls. However, glucocorticoid administration altered SN activity to a level that was comparable to that of healthy control participants, while it had no effects on DMN activity. These findings support the key role of the SN in emotion processing and its potential link to the behavioral fear response. On the other hand, the DMN was found to be a sensitive network for differentiating patients from healthy control participants. Thus, the SN might function as a sensitive tool to monitor therapy outcome. Additionally, it may enable the development of novel methods of specifically influencing the SN that may eventually lead to fear reduction in patients with spider phobia. As we only focused on two specific networks (DMN and SN) in the current study, it might be interesting to extend these analyses to other networks in the future. Moreover, it might be useful to enlarge the tested population and to include patients with other anxiety disorders.

## ACKNOWLEDGMENTS

We gratefully thank Melanie Fisler, Joëlle Witmer, and Basil Preisig for the excellent research assistance. This work was supported by a grant from the Swiss National Science Foundation to L.S. (32003B_124947) and a grant from the Medical Faculty of the University of Bern to L.S. (520.10).

## DISCLOSURE

The authors report no potential financial conflict of interest. This trial was registered at ClinicalTrials.gov (Nr. NCT01574014).

## ORCID

*Leila Maria Soravia* http://orcid.org/0000-0002-4924-7771

## REFERENCES

Abercrombie, H. C., Kalin, N. H., Thurow, M. E., Rosenkranz, M. A., & Davidson, R. J. (2003). Cortisol variation in humans affects memory for emotionally laden and neutral information. *Behavioral Neuroscience*, *117*(3), 505–516.

American Psychiatric Association (APA). (1994). *Diagnostic and statistical manual of mental disorders* (4th ed.). Washington, DC: Author.

Andreano, J. M., & Cahill, L. (2006). Glucocorticoid release and memory consolidation in men and women. *Psychological Science*, *17*(6), 466–470.

Andrews-Hanna, J. R., Reidler, J. S., Sepulcre, J., Poulin, R., & Buckner, R. L. (2010). Functional-anatomic fractionation of the brain's default network. *Neuron*, *65*(4), 550–562.

Arnold Anteraper, S., Triantafyllou, C., Sawyer, A. T., Hofmann, S. G., Gabrieli, J. D., & Whitfield-Gabrieli, S. (2014). Hyper-connectivity of subcortical resting-state networks in social anxiety disorder. *Brain Connectivity*, *4*(2), 81–90.

Buchanan, T. W., & Lovallo, W. R. (2001). Enhanced memory for emotional material following stress-level cortisol treatment in humans. *Psychoneuroendocrinology*, *26*(3), 307–317.

Caseras, X., Murphy, K., Mataix-Cols, D., López-Solà, M., Soriano-Mas, C., Ortriz, H., … Torrubia, R. (2013). Anatomical and functional overlap within the insula and anterior cingulate cortex during interoception and phobic symptom provocation. *Human Brain Mapping*, *34*(5), 1220–1229.

Cuthbert, B. N., Lang, P. J., Strauss, C., Drobes, D., Patrick, C. J., & Bradley, M. M. (2003). The psychophysiology of anxiety disorder: Fear memory imagery. *Psychophysiology*, *40*(3), 407–422.

de Quervain, D. J. F., Aerni, A., Schelling, G., & Roozendaal, B. (2009). Glucocorticoids and the regulation of memory in health and disease. *Frontiers in Neuroendocrinology*, *30*(3), 358–370. https://doi.org/10.1016/j.yfrne.2009.03.002

de Quervain, D. J.-F., Bentz, D., Michael, T., Bolt, O. C., Wiederhold, B. K., Margraf, J., & Wilhelm, F. H. (2011). Glucocorticoids enhance extinction-based psychotherapy. *Proceedings of the National Academy of Sciences*, *108*(16), 6621–6625.

de Quervain, D. J.-F., & Margraf, J. (2008). Glucocorticoids for the treatment of post-traumatic stress disorder and phobias: A novel therapeutic approach. *European Journal of Pharmacology*, *583*(2-3), 365–371. https://doi.org/10.1016/j.ejphar.2007.11.068

de Quervain, D., Schwabe, L., & Roozendaal, B. (2017). Stress, glucocorticoids and memory: Implications for treating fear-related disorders. *Nature Reviews Neuroscience*, *18*(1), 7–19.

Del Casale, A., Ferracuti, S., Rapinesi, C., Serata, D., Piccirilli, M., Savoja, V., … Girardi, P. (2012). Functional neuroimaging in specific phobia. *Psychiatry Research: Neuroimaging*, *202*(3), 181–197.

Essau, C., Wittchen, H., & Pfister, H. (1999). DIA-X-Interview. *Diagnostica*, *45*(3), 163–164.

Fair, D. A., Schlaggar, B. L., Cohen, A. L., Miezin, F. M., Dosenbach, N. U., Wenger, K. K., … Petersen, S. E. (2007). A method for using blocked and event-related fMRI data to study "resting state" functional connectivity. *Neuroimage*, *35*(1), 396–405.

Garrity, A. G., Pearlson, G. D., McKiernan, K., Lloyd, D., Kiehl, K. A., & Calhoun, V. D. (2007). Aberrant "default mode" functional connectivity in schizophrenia. *American Journal of Psychiatry*, *164*(3), 450–457.

Graham, B. M., Li, S. H., Black, M. J., & Öst, L.-G. (2018). The association between estradiol levels, hormonal contraceptive use, and responsiveness to one-session-treatment for spider phobia in women. *Psychoneuroendocrinology*, *90*, 134–140.

Gusnard, D. A., Akbudak, E., Shulman, G. L., & Raichle, M. E. (2001). Medial prefrontal cortex and self-referential mental activity: Relation to a default mode of brain function. *Proceedings of the National Academy of Sciences*, *98*(7), 4259–4264. https://doi.org/10.1073/pnas.071043098

Hilbert, K., Evens, R., Maslowski, N. I., Wittchen, H.-U., & Lueken, U. (2015). Neurostructural correlates of two subtypes of specific phobia: A voxel-based morphometry study. *Psychiatry Research: Neuroimaging*, *231*(2), 168–175.

Kringelbach, M. L. (2005). The human orbitofrontal cortex: Linking reward to hedonic experience. *Nature Reviews Neuroscience*, *6*(9), 691–702.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2005). *International Affective Picture System (IAPS): Affective ratings of pictures and instruction manual*. Gainesville, FL: University of Florida.

McFadden, K. L., Tregellas, J. R., Shott, M. E., & Frank, G. K. (2014). Reduced salience and default mode network activity in women with anorexia nervosa. *Journal of Psychiatry & Neuroscience: JPN*, *39*(3), 178–188.

McMenamin, B. W., Langeslag, S. J., Sirbu, M., Padmala, S., & Pessoa, L. (2014). Network organization unfolds over time during periods of anxious anticipation. *Journal of Neuroscience*, *34*(34), 11261–11273.

Meda, S. A., Stevens, M. C., Folley, B. S., Calhoun, V. D., & Pearlson, G. D. (2009). Evidence for anomalous network connectivity during working memory encoding in schizophrenia: An ICA based analysis. *PLoS One*, *4*(11), e7911.

Menon, V. (2011). Large-scale brain networks and psychopathology: A unifying triple network model. *Trends in Cognitive Sciences*, *15*(10), 483–506.

Menon, V., & Uddin, L. Q. (2010). Saliency, switching, attention and control: A network model of insula function. *Brain Structure and Function*, *214*(5-6), 655–667.

Nakataki, M., Soravia, L. M., Schwab, S., Horn, H., Dierks, T., Strik, W., … Morishima, Y. (2017). Glucocorticoid administration improves aberrant fear-processing networks in spider phobia. *Neuropsychopharmacology*, *42*(2), 485–494.

Palaniyappan, L., & Liddle, P. F. (2012). Does the salience network play a cardinal role in psychosis? An emerging hypothesis of insular dysfunction. *Journal of Psychiatry & Neuroscience: JPN*, *37*(1), 17–27.

Pannekoek, J. N., Veer, I. M., van Tol, M.-J., van der Werff, S. J., Demenescu, L. R., Aleman, A., … van der Wee, N. J. (2013a). Aberrant limbic and salience network resting-state functional connectivity in panic disorder without comorbidity. *Journal of Affective Disorders*, *145*(1), 29–35.

Pannekoek, J. N., Veer, I. M., van Tol, M.-J., van der Werff, S. J., Demenescu, L. R., Aleman, A., … van der Wee, N. J. (2013b). Resting-state functional connectivity abnormalities in limbic and salience networks in social anxiety disorder without comorbidity. *European Neuropsychopharmacology*, *23*(3), 186–195.

Paquette, V., Lévesque, J., Mensour, B., Leroux, J.-M., Beaudoin, G., Bourgouin, P., & Beauregard, M. (2003). "Change the mind and you change the brain": Effects of cognitive-behavioral therapy on the neural correlates of spider phobia. *Neuroimage*, *18*(2), 401–409. https://doi.org/10.1016/s1053-8119(02)00030-7

Peterson, A., Thome, J., Frewen, P., & Lanius, R. A. (2014). Resting-state neuroimaging studies: A new way of identifying differences and similarities among the anxiety disorders? *The Canadian Journal of Psychiatry*, *59*(6), 294–300.

Rasch, B., Spalek, K., Buholzer, S., Luechinger, R., Boesiger, P., Papassotiropoulos, A., & de Quervain, D. J.-F. (2009). A genetic variation of the noradrenergic system is related to differential amygdala activation during encoding of emotional memories. *Proceedings of the National Academy of Sciences*, *106*(45), 19191–19196. https://doi.org/10.1073/pnas.0907425106

Rickham, P. (1964). Human experimentation. Code of ethics of the World Medical Association. Declaration of Helsinki. *British Medical Journal*, *2*, 177.

Rinck, M., Bundschuh, S., Engler, S., Muller, A., Wissmann, J., Ellwart, T., & Becker, E. (2002). Reliability and validity of German versions of three instruments measuring fear of spiders. *Diagnostica*, *48*(3), 141–149.

Rubio-Stipec, M., & Bravo, M. (1991). The Composite International Diagnostic Interview (CIDI): An epidemiologic instrument suitable for using in conjunction with different diagnostic systems in different cultures. *Acta Psiquiatrica y Psicológica de América Latina*, *37*, 191–204.

Sara, S. J. (2000). Retrieval and reconsolidation: Toward a neurobiology of remembering. *Learning and Memory*, *7*, 73–84.

Shang, J., Fu, Y., Ren, Z., Zhang, T., Du, M., Gong, Q., … Zhang, W. (2014). The common traits of the ACC and PFC in anxiety disorders in the DSM-5: Meta-analysis of voxel-based morphometry studies. *PLoS One*, *9*(3), e93432.

Soravia, L. M., de Quervain, D. J. F., & Heinrichs, M. (2009). Glucocorticoids do not reduce subjective fear in healthy subjects exposed to social stress. *Biological Psychology*, *81*(3), 184–188.

Soravia, L. M., Heinrichs, M., Aerni, A., Maroni, C., Schelling, G., Ehlert, U., … de Quervain, D. J.-F. (2006). Glucocorticoids reduce phobic fear in humans. *Proceedings of the National Academy of Sciences*, *103*(14), 5585–5590.

Soravia, L. M., Heinrichs, M., Winzeler, L., Fisler, M., Schmitt, W., Horn, H., … de Quervain, D. J. F. (2014). Glucocorticoids enhance in vivo exposure-based therapy of spider phobia. *Depression and Anxiety*, *31*(5), 429–435. https://doi.org/10.1002/da.22219

Soravia, L. M., Orosz, A., Schwab, S., Nakataki, M., Wiest, R., & Federspiel, A. (2016a). CBT reduces CBF: Cognitive-behavioral therapy reduces cerebral blood flow in fear-relevant brain regions in spider phobia. *Brain and Behavior*, *6*, e00510.

Soravia, L. M., Witmer, J. S., Schwab, S., Nakataki, M., Dierks, T., Wiest, R., … Jann, K. (2016b). Prestimulus default mode activity influences depth of processing and recognition in an emotional memory task. *Human Brain Mapping*, *37*(3), 924–932.

Spielberger, C. D., Gorsuch, R. C., & Lushene, R. E. (1970). *Manual for the state-trait anxiety inventory*. Palo Alto, CA: Consulting Psychologists Press.

Straube, T., Mentzel, H.-J., & Miltner, W. H. (2007). Waiting for spiders: Brain activation during anticipatory anxiety in spider phobics. *Neuroimage*, *37*(4), 1427–1436.

Szymanski, J., & O'Donohue, W. (1995). Fear of spider questionnaire. *Journal of Behavior Therapy and Experimental Psychiatry*, *26*, 31–34.

Uddin, L. Q., Supekar, K., Lynch, C. J., Khouzam, A., Phillips, J., Feinstein, C., … Menon, V. (2013). Salience network-based classification and prediction of symptom severity in children with autism. *JAMA Psychiatry*, *70*(8), 869–879.

Watts, F. N., & Sharrock, R. (1984). Questionnaire dimensions of spider phobia. *Behavior Research and Therapy*, *22*, 575–580.

Whitfield-Gabrieli, S., & Ford, J. M. (2012). Default mode network activity and connectivity in psychopathology. *Annual Review of Clinical Psychology*, *8*, 49–76.

Wittchen, H. U., & Pfister, H. (1997). *Instruktionsmanual zur Durchführung von DIA-X-Interviews*. Frankfurt, Germany: Swets Test Services.

Wolf, O. T. (2009). Stress and memory in humans: Twelve years of progress? *Brain Research*, *1293*, 142–154.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Soravia LM, Schwab S, Weber N, et al. Glucocorticoid administration restores salience network activity in patients with spider phobia. *Depress Anxiety*. 2018;35:925–934. https://doi.org/10.1002/da.22806