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Cortisol enhances neural differentiation during fear acquisition and extinction in contingency aware young women

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ABSTRACT

Previously, we observed cortisol induced enhancement of neural fear acquisition in women. Yet, less is known about cortisol effects on neural fear extinction. Via differential fear conditioning, we explored cortisol effects on acquisition and extinction. Twenty contingency aware women taking monophasic oral contraceptives were included; 10 received placebo, 10 cortisol before conditioning. Group differences emerged in anterior cingulate cortex (ACC), hippocampus, and – as trend – in insula and thalamus during acquisition and in hippocampus, thalamus, and – as trend – in amygdala, insula, and ACC during extinction. During acquisition group differences were due to higher responses to the CS+ than to the CS− in the cortisol group. Notably, during extinction, group differences were due to higher responses to the CS− than to the CS+ in this group. Thus, cortisol induced a fear acquisition and extinction specific enhanced neural differentiation.

1. Introduction

Stress, stress-associated glucocorticoid (GC; cortisol in humans) release from the adrenal cortex, and exogenous GC administration have been demonstrated to influence affective learning and memory (e.g. Bangasser & Shors, 2010; de Kloet, Oitzl, & Joëls, 1999; Joëls, 2010; Sandi, 1998; Sandi & Pinelo-Nava, 2007; Wolf, 2009). Thus, many models of the pathogenesis of affective and anxiety disorders have incorporated stress as well changes in cortisol release and cortisol levels as vulnerability factors (Korte, 2001; Mineka & Zinbarg, 2006; Wolf, 2008).

During the last decade, increasing efforts were made in the attempt to identify the neural structures and processes responsible for these effects (Bangasser & Shors, 2010; van Stegeren, 2009). Altogether, animal and human research point to the amygdala, the hippocampus, and the prefrontal cortex as potential candidate regions, as these regions are rich in mineralocorticoid (MRs) and glucocorticoid receptors (GRs) that bind circulating GC and thus are potentially modulated by stress related hormonal responses (Bangasser & Shors, 2010; Rodrigues, LeDoux, & Sapolsky, 2009; van Stegeren, 2009; Wolf, 2008).

In order to study potential effects of stress and stress hormones on affective learning, classical conditioning is a promising and thoroughly validated approach that allows the exploration of effects on the acquisition as well as the extinction of fear. Animal studies provided first evidence for an influence of stress and stress hormones on fear acquisition via conditioning (Bohus & Lissák, 1968; Brinks, Berger, Gass, de Kloet, & Oitzl, 2009; Rodrigues et al., 2009; Wolf, 2008). However, to date the number of human studies on this important topic is still very limited. Interestingly, most of the conducted studies reported sex differences (e.g. Jackson, Payne, Nadel, & Jacobs, 2006; Stark et al., 2006; Wolf, 2008). Three studies showed a positive correlation between basal cortisol concentrations and fear acquisition or a facilitating effect of psychosocial stress on conditioned responses in male subjects (Jackson et al., 2006; Zorawski, Blanding, Kuhn, & LaBar, 2006; Zorawski, Cook, Kuhn, & LaBar, 2005). Yet, contrasting findings have also been reported, e.g. impaired eyeblink conditioning after psychosocial stress in men and women (Wolf, Minnebusch, & Daum, 2009)

Abbreviations: ACC, anterior cingulate cortex; CS, conditioned stimulus; CS+, conditioned stimulus predicting the electrical stimulation; CS−, conditioned stimulus predicting the absence of the electrical stimulation; FIR, first interval response; fMRI, functional magnetic resonance imaging; FWE, family-wise error; GC, glucocorticoid; GR, glucocorticoid receptor; HPA, hypothalamus–pituitary–adrenal; mPFC, medial prefrontal cortex; MR, mineralocorticoid receptor; OC, oral contraceptives; OFC, orbitofrontal cortex; ROI, region of interest; SCR, skin conductance response; SIR, second interval response; UCR, unconditioned response; UCS, unconditioned stimulus.

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and impaired electrodermal conditioning in men after exogenous cortisol treatment (Stark et al., 2006).

Similarly to fear acquisition, acute GC activity has been shown to influence extinction learning in animals, predominantly enhancing effects, but less is known about GC effects on extinction in humans (Barrett & Gonzalez-Lima, 2004; Yang, Chao, & Lu, 2006; for overviews see e.g. Bentz, Michael, de Quervain, & Wilhelm, 2010; Rodrigues et al., 2009; Wolf, 2008). Clinical studies found an attenuation of post-traumatic stress disorder and phobia symptoms in humans after GC treatment presumably via an impairment in traumatic memory retrieval and a facilitated extinction, however, no sex specific effects occurred (Aerni et al., 2004; de Quervain & Margraf, 2008; Schelling, Roozendaal, & de Quervain, 2004; Soravia et al., 2006). Yet, one study investigating electrodermal fear conditioning reported diverging stress effects in males and females during early extinction (Jackson et al., 2006).

Despite the merits of classical fear conditioning paradigms in studying stress and GC effects on the neural activations underlying fear acquisition in humans, only few imaging studies have been conducted so far. Further, to our best knowledge, no fMRI study has directly investigated GC effects on neural activations during extinction in healthy humans. In two previous fMRI studies, we observed impaired conditioned neural differentiation in men after cortisol as compared to placebo intake in prefrontal and subcortical structures as well as the insula, whereas women exhibited the opposite pattern of results (Merz et al., 2010; Stark et al., 2006). Yet, cortisol enhanced unconditioned responses (UCRs) in the anterior and posterior cingulate cortex, irrespective of sex (Stark et al., 2006; but see Merz et al., 2010). Thus, to complement and extend the knowledge about GC effects on neural activation during fear conditioning, we conducted a differential fear conditioning experiment with an acquisition and an adjacent extinction session in a sample of young healthy women. Thus, we investigated the acquisition of extinction, i.e., the initial learning that the UCS no longer follows the CS+, not extinction consolidation and recall or retrieval (Quirk & Mueller, 2008). Prior to the conditioning procedure, half of the participants received an oral dose of hydrocortisone, whereas the other half received placebo.

Concerning neural structures, we focused on brain regions which are crucially involved in the acquisition and the extinction of fear (e.g. Sehlmeyer et al., 2009) and potentially influenced by GC treatment (de Quervain, Aerni, Schelling, & Roozendaal, 2009; Merz et al., 2010; Rodrigues et al., 2009; Stark et al., 2006; van Stegeren, 2009). The underlying assumption is that cortisol may directly affect these structures altering fear learning processes (cf. Bangasser & Shors, 2010). Based on findings on human fear conditioning, the amygdala, the anterior cingulate cortex (ACC), the insula, the orbitofrontal cortex (OFC), and to a less specifical extend, the thalamus were chosen as regions of interest (ROI) for the acquisition (Büchel & Dolan, 2000; Knight, Cheng, Smith, Stein, & Helmstetter, 2004; Knight, Smith, Stein, & Helmstetter, 1999; LeDoux, 2000; Rolls, 1999; Sehlmeyer et al., 2009; Tabbert, Stark, Kirsch, & Vaitl, 2005; Öhman, 2005). Moreover, the amygdala, the hippocampus, and the medial prefrontal cortex (mPFC) including the ACC seem to play a crucial role during different phases of extinction and thus were selected as ROI for the extinction phase (Knight, Smith, Cheng, Stein, & Helmstetter, 2004; Phelps, Delgado, Nearing, & LeDoux, 2004; Quirk & Mueller, 2008). As with other forms of affective learning (see above), GCs might act in the different subcortical and cortical brain regions influencing fear conditioning (e.g. de Quervain et al., 2009; Rodrigues et al., 2009).

In our female sample, we expected cortisol to facilitate learning during acquisition (cf. Stark et al., 2006). Concerning extinction, facilitating effects of cortisol have been reported previously in animal studies (Rodrigues et al., 2009; Wolf, 2008). However, as we administered cortisol already prior to acquisition, other than in relevant previous studies, and due to little knowledge from human studies, analyses in this phase were explorative. Finally, we also expected enhancing effects of cortisol on UCRs on the neural level (cf. Merz et al., 2010; Stark et al., 2006).

2. Materials and methods

2.1. Subjects

A total of 20 female subjects taking oral contraceptives (placebo group: $n = 10$; cortisol group: $n = 10$) was included in the presented study, which was approved by the ethics committee of the German Psychological Society. The women were required to have been taking their birth control pill (only monophasic preparations including an ethinylestradiol component) at least during the last three months and were tested during the "on phase" of pill intake. All participants were university students who had been recruited via announcements at bulletin boards at the campus. None of them was taking regular medication except oral contraceptives (OC) or had a history of any psychiatric or neurological treatment. Exclusion criteria were somatic and in particular endocrine diseases, which can have an impact on hormonal concentrations (e.g. acute asthma, hypo- or hyperthyroidism). All participants were right-handed as assessed by the Edinburgh Inventory of Handedness (Oldfield, 1971) and had normal or corrected-to-normal vision. Inclusion criteria were age between 19 and 35 and a body mass index (BMI = kg/m2) between 18 and 26.

All subjects were instructed to refrain from any caffeine and food intake, as well as from smoking two hours before the experiment. Five women of the placebo group and eight women of the cortisol were non-smokers. Smoking behavior was not assessed in three placebo women. The remaining four women (two of each group) were smokers. At the beginning, participants received a detailed explanation of the procedure in general (the conditioning schedule was of course not explained until the experiment was finished). Written informed consent was obtained. The cover story concealing the conditioning procedure was the investigation of the impact of cortisol and several distractors (including an electrical stimulation) on memory performance. After finishing the experiment, participants were debriefed about the real purpose of the study and received 25 Euros for their participation.

The experiment is part of a larger study investigating the effects of cortisol on fear acquisition and extinction with respect to contingency awareness and sex differences. Neural activation during conditioning can be modified by contingency awareness (potentially in interaction with stress hormones), which refers to the explicit knowledge of the CS/UCS relationship (e.g. Klucken et al., 2009; Knight, Waters, & Bandettini, 2009; Tabbert, Stark, Kirsch, & Vaitl, 2006; Tabbert et al., 2010; Öhman, 2005). Due to subject selection procedures, distribution of male and free cycling female subjects was unequal between the placebo and the cortisol group (male subjects: placebo: $n = 4$; cortisol: $n = 11$; free cycling women: placebo: $n = 6$; cortisol: $n = 10$). This prevented proper testing of sex differences or differences due to hormonal status. The current manuscript thus only reports the findings of female participants taking OCs who learned the CS/UCS contingencies during the experiment (learned aware group).

Data of the 10 learned aware females who received placebo are also part of a previous publication (Tabbert et al., 2010) investigating the effects of contingency awareness (and the way it is achieved) on fear acquisition. However, this group has not been analyzed separately or in any other study (i.e., addressing cortisol or hormonal effects). The data of the 10 cortisol women have not been analyzed or published elsewhere.

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2.2. Conditioned visual stimuli

Two simple geometric figures (a square and a rhombus) served as CS+ and CS−. A triangle served as distractor stimulus (non-CS) occurring only half as often as the CS−. The three stimuli had identical luminescence, were gray in color, and presented with a duration of 8 s. Visual stimulation inside the scanner was realized with an LCD projector (model EPSON EMP-7250), which projected pictures onto a screen at the end of the scanner (visual field = 18°). A mirror mounted to the head coil allowed the subjects to look at the screen.

2.3. Unconditioned stimulus (UCS)

A custom-made impulse-generator (833 Hz) provided transcutaneous electrical stimulation to the middle of the left shin through two Ag/AgCl electrodes (1 mm^2 surface each), which was applied as the UCS. It was triggered via an optic fiber cable.

Stimulus intensity was set for each participant individually, using a gradually increasing rating procedure to attain an "unpleasant but not painful" level of sensation. The electrical stimulation was applied for 100 ms co-terminating with the CS+ during the acquisition procedure, the duration as well as the onset of the UCS was set by a computer program.

2.4. Two-back task

Due to the research questions of the larger study, the two-back task was included to prevent subjects from detecting the relationship between CS and UCS in a group not reported here (unaware group). Numbers ranging from 1 to 5 were presented sequentially on the screen for 1 s, interspersed in the presentation of the geometric figures. After each number, participants had to indicate whether it was the same or a different number as the number before the last one by pressing one of two buttons (for details, please see Merz et al., 2010 and Tabbert et al., 2010).

Performance on the two-back task (percentage of correct responses) with possible differences between the two groups was tested as a control condition for acquisition and extinction separately with SPSS for Windows (Release 17.0, SPSS Inc. Illinois) via *t*-tests with the between subjects factor group (placebo versus cortisol).

2.5. Conditioning procedure

The conditioning procedure was adapted from previous studies in our laboratory (Stark et al., 2006; Tabbert et al., 2005, 2006) and included an acquisition and an extinction learning phase (cf. Merz et al., 2010). There were 20 trials of CS+ as well as CS− and 10 trials of non-CS presentations throughout the acquisition phase. During extinction, 11 trials of CS+ and CS− were presented and five trials of non-CS. Inter-trial intervals between the numbers and the geometrical figures ranged from 5 to 7.5 s (random jitter between 0 and 2.5 s). Correspondingly, the inter-trial intervals between the CS ranged from 11 to 16 s. The onset of the UCS presentation started 7.9 s after CS+ onset and co-terminated with CS+ offset (delay conditioning; 100% reinforcement). Non-UCS was defined as the UCS omission after the CS− in a time window corresponding to UCS application after the CS+ (i.e., 7.9 s after CS− onset). The CS− and the non-CS were never paired with the UCS and no further CS+ pairing occurred in the extinction phase. The conditioning procedure started with a CS+ for half of the subjects and a CS− for the other half and either square or rhombus served as CS+ or CS−. A triangle always served as the non-CS.

For each participant, a pseudo-randomized stimulus order was used comprising the following restrictions: no more than two consecutive presentations of the same CS, no more than three consecutive identical numbers, an equal distribution for any number before or after CS+ trials to avoid conditioning to any of the numbers, and an equal quantity of CS+ and CS− trials within the first and the second half of the experiment (10 each).

2.6. Contingency awareness

Contingency awareness was assessed via a short recognition questionnaire immediately following the acquisition session and an additional questionnaire and interview after the extinction session (for details please see Merz et al., 2010; Tabbert et al., 2010). Only subjects who recognized the correct relationship between the CS and UCS without prior instruction were included in the present study.

2.7. Experimental treatment and salivary cortisol analyses

This study was conducted as a double-blind, randomized and placebo-controlled experiment. Ten women received three 10 mg tablets of cortisol (30 mg hydrocortisone; Hoechst) 45 min before the start of the functional scans, 10 women received visually identical placebos (tablettose and magnesium; cf. Merz et al., 2010). Each experiment started between 14.00 and 17.00 h to control for the circadian cortisol rhythm with its different occupation of MRs and GRs (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; Lupien et al., 2002).

Saliva samples for the analysis of free cortisol were collected by use of glass tubes. Samples were taken directly before (baseline), 25 min after (before the fMRI run and before acquisition), and 90 min after the intake (after the fMRI run and after extinction; for details of storage and analysis please see Merz et al., 2010).

Statistical analyses were conducted in SPSS for Windows via analyses of variance (ANOVA) with the repeated measurement factor time and the between subjects factor treatment (cortisol versus placebo). In case of violation of the sphericity assumption, Greenhouse–Geisser correction was applied. After the experiment, participants were asked to give a treatment guess with the possible answers "placebo", "hydrocortisone" or "no idea". Fisher's exact test, which included the answers "placebo" and "cortisol" only, was performed in SPSS for Windows to check if subjects were somehow aware of their treatment.

2.8. Skin conductance responses

Skin conductance responses (SCRs) were sampled simultaneously with fMRI scans using Ag/AgCl electrodes filled with isotonic (0.05 M NaCl) electrolyte medium, placed hypothenar at the non-dominant hand. SCRs were defined in three analysis windows (cf. Prokasy & Ebel, 1967): the maximum response within a window of 1–5 s after the CS onset was counted as the first interval response (FIR), within the time window of 5–8.5 s as the second interval response (SIR), and within the time window of 8.5–13 s as the unconditioned response (UCR). Conditioned responses were defined as larger response magnitudes in reaction to the CS+ than to the CS− in the FIR and SIR.

The data were transformed with the natural logarithm in order to render the distribution more towards normal and to account for individual differences. Statistical comparisons were performed via ANOVA in a 2 (CS-type: CS+ and CS− for the FIR and SIR; UCS and non-UCS for the UCR) × 20 (trial) (extinction: 10) factorial design within the general linear model as it is implemented in SPSS for Windows. Treatment (cortisol versus placebo) was introduced as between subjects factor. Greenhouse–Geisser correction was applied when sphericity assumption was not met. Post hoc ANOVA were performed for significant interactions. Electrodermal data of

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one woman in the placebo group had to be discarded because of a technical problem.

2.9. Magnetic resonance imaging

Brain images were acquired using a 1.5 T whole-body tomograph (Siemens Symphony with a quantum gradient system) with a standard head coil. Structural image acquisition consisted of 160 T1-weighted sagittal images (magnetization-prepared, rapid acquisition gradient echo sequence, 1 mm slice thickness). For functional imaging, a total of 750 volumes (480 for the acquisition and 270 for the extinction phase) were registered using a T2*-weighted gradient echo-planar imaging sequence with 25 slices covering the whole brain (slice thickness = 5 mm; 1 mm gap; descending slice order; TA = 100 ms; TE = 55 ms; TR = 2.5 s; flip angle = 90°; field of view = 192 mm × 192 mm; matrix size = 64 × 64). The first three volumes were discarded due to an incomplete steady state of magnetization. The orientation of the axial slices was parallel to the orbitofrontal cortex–bone transition in order to minimize susceptibility artefacts in prefrontal areas. A gradient echo field map sequence was measured before the functional run to get information for unwarping B_0 distortions.

Data were analyzed using Statistical Parametric Mapping (for preprocessing and first level analyses: SPM5, Wellcome Department of Cognitive Neurology, London, UK; 2005; for group analyses: SPM8, Wellcome Department of Cognitive Neurology, London, UK; 2009) implemented in MatLab R2007b (Mathworks Inc., Sherborn, MA). Realignment (2nd degree b-spline interpolation to the first image) and unwarping, slice time correction (reference slice: 13), co-registration of functional data to each participant's anatomical image, segmentation into gray and white matter, and normalization to the standard space of the Montreal Neurological Institute (MNI) brain were performed. Spatial smoothing was executed with an isotropic three-dimensional Gaussian filter with a full width at half maximum of 9 mm to allow for corrected statistical inference.

The acquisition and extinction were integrated as separate sessions in one model, including the following experimental conditions: CS+, CS−, non-CS, UCS, non-UCS, target, and non-target (excluding UCS and non-UCS for the extinction). An additional regressor was introduced containing the first two numbers and the first two geometrical figures of the extinction (cf. Phelps et al., 2004). All regressors were modelled by a stick function convolved with the canonical hemodynamic response function in the general linear model, without specifically modelling the durations of the different events. The six movement parameters of the rigid body transformation applied by the realignment procedure were introduced as covariates in the model, separately for the acquisition and extinction phase. The voxel-based time series were filtered with a high pass filter (time constant = 128 s).

For the statistical analyses, we used explorative whole brain as well as ROI analyses to enhance the statistical power. The following structures were included as ROI for the CS analyses: the amygdala, the ACC, the hippocampus, the insula, the OFC (lateral and medial), the mPFC, and the thalamus. For UCS analyses, the amygdala, the anterior and posterior cingulate cortex, and the insula were included as ROI. The required masks for these analyses were designed using the software-program MARINA (Walter, 2002). Regressors of interest were CS+ and CS− during acquisition and extinction as well as UCS and non-UCS during acquisition. Statistical analyses were done in a random effects design and focused on the contrasts CS+ minus CS− and CS− minus CS+ during acquisition and extinction and UCS minus non-UCS during acquisition. We investigated general conditioning and extinction effects (CS+ versus CS−; acquisition: UCS minus non-UCS) in the entire sample via one-sample *t*-tests and additionally compared differential responses (CS+ versus CS−; acquisition: UCS minus non-UCS) between the placebo and the cortisol group via two-sample *t*-tests. Post hoc one-sample *t*-tests in the single treatment groups were done in structures showing a group effect to further clarify the underlying response patterns.

For the explorative whole brain analyses, the significance threshold was set to $\alpha = .05$ on voxel-level, corrected for multiple testing (family-wise error (FWE) correction), and a minimum cluster size of five voxels (i.e., voxel volume: 135 mm^3). ROI analyses were performed using the small volume correction options of SPM8 ($p \leqslant .05$). Additionally, trends up to a threshold of $p_{corr} \leqslant .10$ are reported for ROI.

3. Results

3.1. Descriptive data and cortisol concentrations

The mean age was 23.2 years (*SD* = 2.5) with no significant differences between the placebo and the cortisol group. The same was true for BMI, with a mean BMI of 21.8 (*SD* = 2.1).

One woman from the cortisol group displayed extremely high cortisol levels (larger than 1000 nmol/l) 25 min after cortisol intake. The concentration most likely reflects some micro hydrocortisone residue of the uncoated tablet in her mouth. Thus, this subject was excluded from hormonal analyses, but remained in all other analyses. Elevated cortisol concentrations were not observed in the placebo group. The ANOVA with the within subjects factor time (baseline, before acquisition, after extinction) and the between subjects factor treatment revealed a significant main effect of time ($F_{(1.4,23.4)} = 16.14$; $p < .001$) and of treatment ($F_{(1,17)} = 42.62$; $p < .001$) and a significant time × treatment interaction ($F_{(1.4,23.4)} = 16.29$; $p < .001$). Post hoc *t*-tests revealed significant differences in cortisol levels after cortisol intake before acquisition ($T_8 = 4.17$; $p < .01$) and after extinction ($T_8 = 7.74$; $p < .001$), with higher values in the cortisol group, but not at baseline ($p > .10$). Thus, treatment was successful at elevating cortisol levels in the cortisol group, while cortisol concentrations in the placebo group remained unchanged (see Table 1).

Fisher's exact test showed that subjects were not able to indicate whether they had received hydrocortisone or placebo ($p > .10$). None of the women in the cortisol group, but one in the placebo group, indicated to have received hydrocortisone. Six cortisol and three placebo women indicated to have taken placebo. The remaining participants had no treatment guess ($n = 10$).

3.2. Two-back task performance

There were no significant group differences in the percentage of correct responses in the two-back task during acquisition ($T = 2.14$; $p = .057$) and extinction ($T = 1.84$; $p = .095$). Percentage of correct responses was 77.00% (SE = 5.91) for the placebo and 90.20% (SE = 1.78) for the cortisol group during acquisition and 82.59%

Table 1
Mean (*SE*) salivary cortisol concentrations (in nmol/l) before the administration of cortisol (30 mg) or placebo as well as 25 min after administration (before acquisition) and 90 min after administration (after extinction).

	Before treatment	25 min after treatment	90 min after treatment
Placebo	5.04 (0.48)	4.48 (0.72)	5.22 (0.72)
Cortisol	4.74 (1.16)	134.86 (31.28)	115.22 (14.20)

One woman from the cortisol group with unrealistically high cortisol concentrations (larger than 1000 nmol/l) was excluded from the statistical hormonal analyses and the descriptive statistics in this table.

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(SE = 6.63) for the placebo and 95.19% (SE = 1.75) for the cortisol group during extinction. Thus, cortisol slightly enhanced performance in the two-back task without reaching the statistical significance threshold.

3.3. Skin conductance responses (SCRs)

During acquisition, the ANOVA with the repeated measurement factors CS-type and trial and the between subjects factor treatment demonstrated a main effect of CS-type for the FIR ($F_{(1,17)} = 6.83$; $p < .05$) and the SIR ($F_{(1,17)} = 8.09$; $p < .05$), indicating successful conditioning (see Fig. 1). For the UCR interval, significant main effects of UCS, indicating higher responses during the UCS than during its omission, and trial, indicating an overall signal decrease, emerged (both $p < .001$). No effects of or interactions with treatment were observed.

During extinction, no conditioning effects were observed in the FIR or SIR. A signal decrease across the extinction phase was reflected in a significant effect of trial in the FIR ($F_{(3.09,52.59)} = 2.83$; $p < .05$). A main effect of treatment revealed elevated SCRs in the cortisol group in the FIR ($F_{(1,17)} = 14.83$; $p < .01$) and the SIR ($F_{(1,17)} = 14.86$; $p < .01$) time window, irrespective of CS-type (see Fig. 1).

3.4. Hemodynamic responses

3.4.1. CS+ versus CS− acquisition

During acquisition, conditioned responses were seen in all selected ROI (all $p_{corr\ ROI} < .05$) except the left hippocampus and the left mPFC. Differential activation in the thalamus was significant even when applying whole brain correction ($T_{max} = 9.01$, $p_{corr\ whole\ brain} = .001$). In addition, significant differential responses emerged from the explorative analyses in the cerebellum ($x = -33$, $y = -54$, $z = -27$, $T_{max} = 7.21$, $p_{corr\ whole\ brain} < .05$) and in the precentral gyrus ($x = -45$, $y = -6$, $z = 48$, $T_{max} = 7.08$, $p_{corr\ whole\ brain} < .05$). No higher responses to the CS− as compared to the CS+ were observed.

The comparison of differential responses (CS+ versus CS−) between the two groups revealed significant left ACC and left hippocampus activation as well as trends in left insula, right ACC, and right thalamus responses (see Table 2 and Fig. 2). Descriptively, the group differences were based on higher responses to the CS+ than to the CS− in the cortisol group and a less pronounced differentiation in the opposite direction (i.e., higher responses to the CS− than to the CS+) in the placebo group (Fig. 2). Follow-up one-sample *t*-tests in these structures revealed significantly enhanced responses to the CS+ compared to the CS− in the cortisol group ($p_{corr\ ROI} < .05$) in all structures except the right ACC where a trend in the same direction was observed ($p_{corr\ ROI} < .10$). No significant or tendential differentiation was revealed in these structures in the placebo group.

Table 2
Group comparisons (cortisol versus placebo) of differential responses (CS+ versus CS−) during fear acquisition and extinction. During acquisition, group differences were based on higher responses to the CS+ compared to the CS− in the cortisol group. During extinction, the cortisol group exhibited higher responses to the CS− than to the CS+, whereas the placebo group showed slightly less pronounced responses in the opposite direction (i.e., relatively higher responses to the CS+).

Brain structure	Side	*x*	*y*	*z*	*Tmax*	p_{corr}
Acquisition						
Anterior cingulate cortex	Left	−3	18	18	4.82	.023
Hippocampus	Left	−15	−27	−9	4.51	.027
Insula	*Left*	*−30*	*−9*	*18*	*4.48*	*.052*
Anterior cingulate cortex	*Right*	*3*	*15*	*21*	*3.93*	*.086*
Thalamus	*Right*	*12*	*−21*	*6*	*3.61*	*.074*
Extinction						
Hippocampus	Right	42	−18	−12	4.50	.032
Thalamus	Right	18	−18	0	4.01	.045
Amygdala	*Left*	*−27*	*−3*	*−15*	*3.31*	*.054*
Amygdala	*Right*	*30*	*−9*	*−12*	*3.28*	*.065*
Anterior cingulate cortex	*Right*	*9*	*42*	*0*	*4.31*	*.055*
Insula	*Right*	*42*	*−15*	*−9*	*4.41*	*.066*

The threshold was $p_{corr} < .05$ (FWE-corrected according to SPM8; small volume correction). Additionally, trends are reported in italic letters up to a threshold of $p_{corr} < .1$. All coordinates (*x*, *y*, *z*) are given in MNI space.

To test for general conditioning effects, separate one-sample-*t*-test for the two groups were extended also to ROI not showing treatment effects. For the placebo group, significantly higher responses to the CS+ as compared to the CS− emerged in the right amygdala and thalamus as well as trends in the same direction in the left amygdala and thalamus and the right insula and lateral OFC (cf. Supplementary Table 1). For the cortisol group, significant differential responses (CS+ minus CS−) were observed in the bilateral mPFC, insula, lateral OFC, thalamus as well as in the left ACC, amygdala, and hippocampus, and in the right temporal cortex (cf. Supplementary Table 1). Further, a trend emerged in the right ACC. No group showed higher responses to the CS− than to the CS+.

3.4.2. CS+ versus CS− extinction

During extinction, no significant differential responses (CS+ minus CS− and CS− minus CS+) were found (all $p_{corr\ ROI} > .05$) in the entire group.

Two-sample *t*-tests revealed significant group differences in differential responses (CS+ versus CS−) in the right hippocampus and the right thalamus as well as trends in the bilateral amygdala, the right ACC, and the right insula (see Table 2 and Fig. 3). Descriptively, the group differences were based on higher responses to

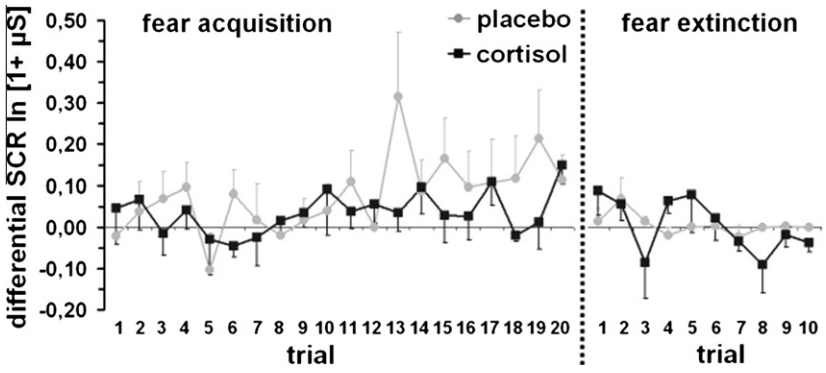

Fig. 1. Differential SCRs (CS+ minus CS−) for the placebo and cortisol group in the SIR for fear acquisition and fear extinction. Error bars are standard errors of the mean.

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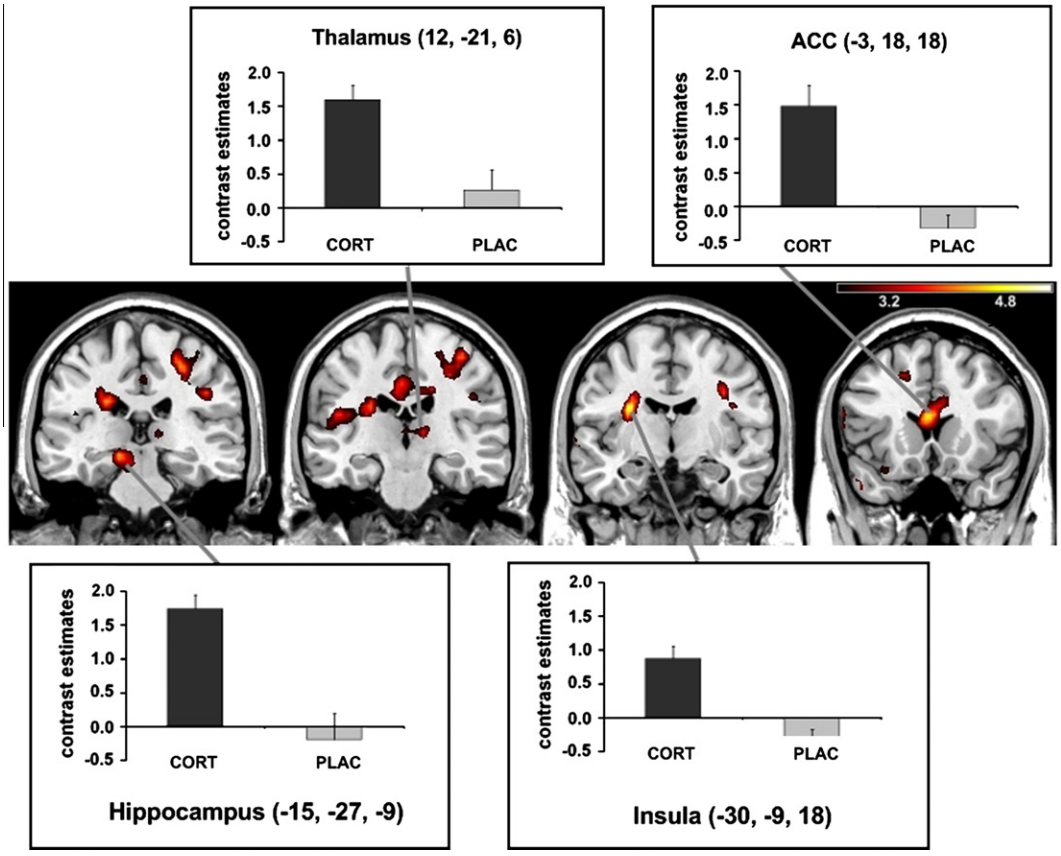

Fig. 2. Group differences (cortisol minus placebo) in neural activation for the contrast CS+ minus CS− during fear acquisition (CORT = cortisol group; PLAC = placebo group). Bar graphs depict the group means of peak voxel activation in the respective structure (ACC = anterior cingulate cortex) for this contrast (CS+ minus CS−), error bars are standard errors of the mean. Color bars indicate *T*-values of the group contrast. For more specific statistical information please see Table 2.

the CS− than to the CS+ in the cortisol group and a less pronounced differentiation in the opposite direction (i.e., higher responses to the CS+ than to the CS−) in the placebo group. Follow-up one-sample *t*-tests in those structures showed significantly enhanced responses of the bilateral amygdala and the right hippocampus to the CS− as compared to the CS+ in the cortisol group as well as a trend in the same direction in the right insula. In the placebo group, no significant or tendential differentiation between CS+ and CS− in either direction emerged.

Conditioning effects were again tested also for the remaining ROI. We did not find significant differential responses or trends in the placebo group during extinction. The cortisol group showed significant higher responses to the CS− than to the CS+ bilaterally in the amygdala and in the right hippocampus. Further, the cortisol group exhibited a trend in the same direction in the right insula and the left medial OFC (cf. Supplementary Table 2).

3.4.3. UCS minus non-UCS

All ROI showed significant unconditioned responses in the entire sample (all $p_{corr\ ROI\ or\ whole\ brain} < .01$). Additional whole brain corrected cluster emerged with peaks in the caudate nucleus and bilateral superior temporal cortex (all $p_{corr\ whole\ brain} < .001$).

The analyses did not reveal significant differences or trends in unconditioned neural responses between the two groups.

4. Discussion

In the present study, we investigated effects of a single oral dose of cortisol on human fear conditioning in OC women who learned the CS/UCS contingencies. For the first time, we addressed cortisol effects on neural activation in healthy humans not only during fear acquisition, but also during extinction learning. As the main finding, we observed an acquisition and extinction specific enhanced neural differentiation in several structures in the cortisol as compared to the placebo group.

4.1. Skin conductance responses

During acquisition, we observed significant conditioned responses in the FIR and the SIR time window, as well as reliable unconditioned responses, but no effects of treatment on learning related or unconditioned responses.

In a previous study, we did not find significant conditioning effects in a sample of contingency aware (learned aware) and unaware women taking OCs during the acquisition phase (Stark et al., 2006). It was speculated that particularly the OC intake might have influenced electrodermal responses. Yet in our present sample, we did observe conditioned SCRs in contingency aware OC women, contradicting this former hypothesis. Thus, the previous lack of conditioned SCRs may be traced back to differences regarding contingency awareness or an interaction of awareness with OC usage.

We did not observe cortisol effects on the acquisition of electrodermal responses in the present study, other than reported previously (Stark et al., 2006). Yet, in our previous study, we observed a sex × cortisol effect on conditioned SCRs. It might not be possible to find this kind of response pattern in a unisex sample. Consistently, the previously observed effect was based on cortisol effects on SCRs in only male, but not female subjects (Stark et al., 2006).

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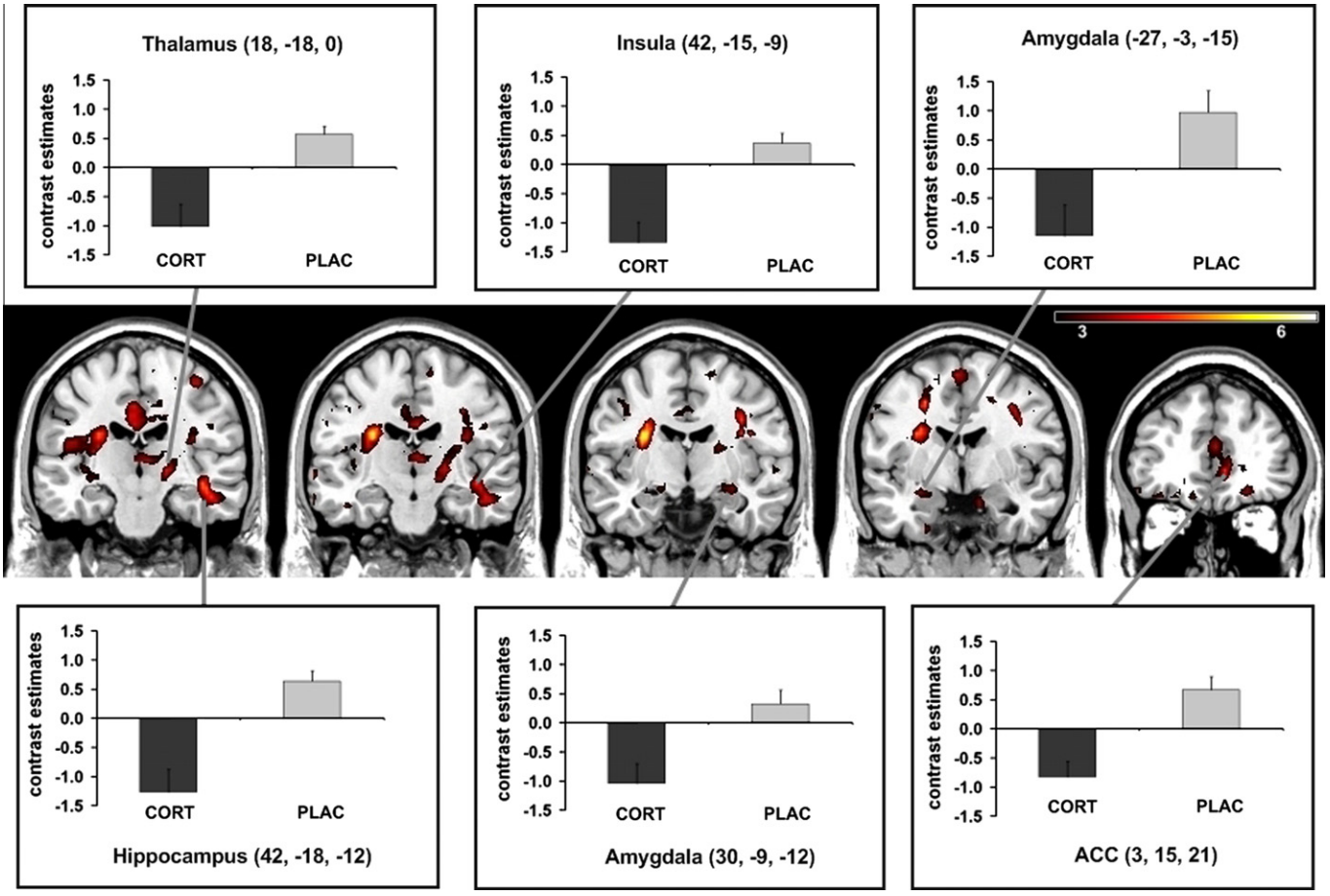

Fig. 3. Group differences (placebo minus cortisol) in neural activation for the contrast CS+ minus CS− during fear extinction learning (CORT = cortisol group; PLAC = placebo group). Bar graphs depict the group means of peak voxel activation in the structures showing significant group differences (ACC = anterior cingulate cortex) for this contrast, error bars are standard errors of the mean. Color bars indicate *T*-values of the group contrast. For more specific statistical information please see Table 2.

During extinction, elevated SCRs in the cortisol compared to the placebo women emerged, irrespective of CS-type, however, no main effect of CS-type was observed. In contrast, in one previous study globally reduced responses to the CS in the acquisition phase were observed in contingency unaware women after cortisol as compared to placebo treatment (Merz et al., 2010). Thus, the effects of cortisol on SCRs appear to be modulated by contingency awareness (learned aware versus unaware), learning phase (acquisition versus extinction), or both.

In their study on conditioned SCRs, Jackson and colleagues (2006) reported a stress-induced attenuation of conditioned responses in women during early extinction. Yet, the authors interpreted their data in terms of a reduced acquisition in women, not in terms of an altered extinction. Furthermore, the observed effect was present only during early extinction comprising four trials. This severely restricts comparability to the present study comprising ten extinction trials.

4.2. Hemodynamic responses

4.2.1. General conditioning effects

Concerning general conditioning effects during acquisition, we observed significantly enhanced responses to the CS+ as compared to the CS− in all predefined ROI at least in one hemisphere. We thus demonstrated overall successful conditioning also on the neural level (e.g. LeDoux, 2000; Sehlmeyer et al., 2009).

Unconditioned neural responses were observed, as expected, with no statistically relevant differences between the two treatment groups.

During extinction, we found no statistically meaningful differentiation between CS+ and CS− on the neural or electrodermal level. The lack of differential responses in the amygdala, the hippocampus, and medial prefrontal areas (mPFC, ACC) was unexpected as these structures have been previously related to extinction (e.g. Delgado, Nearing, LeDoux, & Phelps, 2008; Knight, Smith, et al., 2004; Phelps et al., 2004). A possible explanation may lie in experimental characteristics, for example, previous studies used lower pairing rates of CS+ and UCS during acquisition resulting in a prolonged extinction (cf. Phelps et al., 2004). Interestingly, we observed treatment effects in most of these structures, with differential responses in the cortisol group (see below for a detailed discussion).

4.2.2. Cortisol effects on fear acquisition

During acquisition, enhanced differential responses in women receiving cortisol compared to women receiving placebo were revealed in the left ACC and the right hippocampus. Trends were observed in the left insula, the right ACC, and the right thalamus. These group differences were based on a relatively better differentiation of CS+ and CS− in cortisol women, with enhanced responses to the CS+ as compared to the CS−. The frontal cortex and the hippocampus are major targets of GCs, as they possess a high density of GRs and, concerning the hippocampus, MRs (e.g. de Kloet et al., 1999; Lupien & Lepage, 2001; McEwen, de Kloet, & Rostène, 1986). Additionally, frontal cortex areas as the ACC are involved in the regulation of the HPA axis (Amat et al., 2005; Prüssner et al., 2010; Wolf, Convit, de Leon, Caraos, & Qadri, 2002). The hippocampus is assumedly involved in the enhancement of stress effects if

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emotional material is used (de Quervain et al., 2009; Roozendaal, Okuda, de Quervain, & McGaugh, 2006; van Stegeren, 2009; Wolf, 2008; see also Richardson, Strange, & Dolan, 2004). Acute stress increases hippocampal excitability, which is also related to enhanced learning (Weiss, Sametsky, Sasse, Spiess, & Disterhoft, 2005).

The insula has been related to fear conditioning (Büchel, Morris, Dolan, & Friston, 1998; Klucken et al., 2009; Sehlmeyer et al., 2009; Öhman, 2005) and the anticipation and evaluation of future emotional states (Nitschke, Sarinopoulos, Mackiewicz, Schaefer, & Davidson, 2005; Paulus & Stein, 2006; Simmons, Matthews, Stein, & Paulus, 2004). Our finding of cortisol augmenting insula activity in response to threat cues (i.e., CS+) also fits with the finding that GCs enhance the memory consolidation of conditioned taste aversion when administered into the insula post training (Miranda, Quirarte, Rodriguez-Garcia, McGaugh, & Roozendaal, 2008). Consistently, the elicitation of a subjective stressful experience was positively correlated with insula and ventral PFC activation in a previous study measuring cerebral blood flow (Wang et al., 2005). Subjects reporting high levels of stress exhibited prolonged activation in this structure as well as in the ACC. Taken together, these findings demonstrate a role of these structures in the subjective experience of stress.

The thalamus, a structure crucial for the gating of important sensory information, has been related to cortisol induced changes in fear acquisition previously (Merz et al., 2010). This structure has also been ascribed a role in vigilance and sustained attention and showed enhanced activation after stress (Sarter, Givens, & Bruno, 2001; Wang et al., 2005). Further, the thalamus was related to the interplay between attention and arousal (Portas et al., 1998), a further indication of possible modulation mechanisms by activation of the HPA axis and the sympathetic nervous system. Conditioning related neural activity in the thalamus was found in several studies at least as far as visual or acoustic CS are concerned (e.g. Blaxon et al., 1996; Morris, Friston, & Dolan, 1997; Shi & Davis, 2001) alluding to its role in sensory and salience processing. Early animal studies already pointed to the thalamus–amygdala pathway mediating conditioned responses (LeDoux, Sakaguchi, & Reis, 1984).

To sum it up, we observed a facilitation of conditioned responses by cortisol in women, already observed in two independent previous studies with different subject samples (Merz et al., 2010; Stark et al., 2006). While this was the case in the insula, the hippocampus, and the thalamus in a sample consisting of unaware subjects (Merz et al., 2010), in a study comprising aware and unaware subjects likewise, this pattern of results was observed in the ACC, the lateral OFC, and the mPFC (Stark et al., 2006). With respect to the acquisition phase, the present study largely replicates these previous findings with an independent subject sample. Differences concerning the neural structures showing such effects may be related to differences in contingency awareness as well as other sample (e.g. sex and sex hormones) or experimental (e.g. distraction task) features (cf. Tabbert et al., 2010). It is important to note that we found no group differences concerning UCRs on the neural or electrodermal level. Further, the two-back task performance did not reveal a reduced attention of the cortisol group to the distractor, which could have influenced the engagement in the conditioning task (if at all there was a slightly but not significantly enhanced performance in this group). Thus, the observed changes most probably are not due to an overall altered responding to emotional stimuli or the distractor task, but a specific modulation of learning related activation by cortisol.

Other than expected, we did not observe cortisol effects on amygdala activation during acquisition. Instead, we found significant bilateral amygdala activation for the contrast CS+ minus CS− in the entire sample, independent of treatment. One may speculate that potential treatment effects in the amygdala were concealed due to ceiling effects based on high arousal resulting in similar activation of the sympathetic nervous system in both groups. On the other hand, the lack of cortisol effects on amygdala activation during fear acquisition is in line with one previous study from our lab (Stark et al., 2006), with predominantly learned aware subjects. Yet, our previous fear conditioning study in contingency unaware subjects (Merz et al., 2010) showed a trend for enhanced amygdala responses in the placebo as compared to the cortisol group. Thus again, heterogeneous findings could be due to specific characteristics in unaware versus aware fear conditioning (see also Merz et al., 2010). Further, the two previous studies included female and male subjects.

4.2.3. Cortisol effects on fear extinction

During extinction, significant differences between the two treatment groups emerged in the right hippocampus and thalamus, as well as trends in the bilateral amygdala, the right ACC, and the right insula. Remarkably, in contrast to the acquisition phase, these group differences were mainly driven by higher responses to the CS− as compared to the CS+ of the cortisol group in the bilateral amygdala, the hippocampus, and, as trend, the right insula. In the placebo women, no statistically relevant differential responses in either direction were detected in these structures. Descriptively, opposing patterns of results were underlying all group differences during extinction (i.e., CS− minus CS+ in cortisol women and CS+ minus CS− or no differentiation in placebo women; see also Fig. 3).

The hippocampus and the amygdala have previously been shown to be involved in fear extinction, with relatively enhanced responses to the CS− as compared to the CS+ during extinction learning (Knight, Smith, et al., 2004; Phelps et al., 2004). This amygdala activation pattern (i.e., higher responses to the CS− than to the CS+) during extinction has been interpreted as active coding of the predictive value of the CS+ with a learning related response adaption, when new information is available and new relationships between the CS and the UCS have to be established (Knight, Smith, et al., 2004; Phelps et al., 2004). In further support of this interpretation, differential amygdala responses (with relatively higher responses to the CS−) were correlated with extinction success as measured by SCRs in the study by Phelps et al. (2004). Thus, altogether the previous results indicate that the amygdala is important not only for the acquisition but also for the extinction of conditioned fear. Similarly, the hippocampus has also been related to learning of altered stimulus relations during extinction, again with relatively higher responses to the CS−; yet hippocampal activation may reflect more declarative aspects of this process (cf. Knight, Smith, et al., 2004; Tabbert et al., 2010). Both, the amygdala and the hippocampus, show a high density of GRs and MRs (e.g. de Kloet et al., 1999; McEwen et al., 1986) and are critically involved in the augmenting effects of stress and GCs on memory, especially when arousing stimuli are employed, as was the case in the present study (de Quervain et al., 2009; Roozendaal et al., 2006; van Stegeren, 2009; Wolf, 2008; see also Richardson et al., 2004). In line, results from a recent rodent study suggest that amygdaloid GC receptors are involved in facilitating GC effects on fear extinction (Yang et al., 2006).

As described above, the insula is involved in the expectation of aversive events (Nitschke et al., 2005; Paulus & Stein, 2006; Simmons et al., 2004). During extinction, subjects' expectation of the UCS may shift from the CS+, which no longer predicts the aversive stimulus, to the CS−. A changed anticipatory insula activation (cf. Miranda et al., 2008) and subjective stress experience (cf. Wang et al., 2005) in response to the CS− may have been enhanced by cortisol, potentially also influencing responses in other structures. Of course, this interpretation has to remain highly speculative, since we did not assess individual expectations. Consistent with

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this hypothesis, however, the insula, together with the ACC, is recruited during emotional recall or imagery (Phan, Wager, Taylor, & Liberzon, 2002). As mentioned above, the thalamus has been related to cortisol and stress induced changes previously (e.g. Merz et al., 2010; Wang et al., 2005). Its role in sensory gating and vigilance processes (e.g. Sarter et al., 2001) makes it prone to respond to changes in the contingencies, as is the case during extinction learning.

In summary, for the first time, we showed that cortisol induced an enhanced neural differentiation between CS+ and CS− during extinction in a female sample. Interestingly, opposed to acquisition relatively higher responses to the CS− than to the CS+ emerged under cortisol. This pattern has previously been shown to reflect extinction specific learning (Knight, Smith, et al., 2004; Phelps et al., 2004). Thus, cortisol seems to enhance fear extinction in women as has been already suggested for fear acquisition (cf. Rodrigues et al., 2009).

4.3. Limitations

There are some limitations of the present study we would like to address. First, due to the overall frame of this experiment, we were not able to collect data of a sufficiently high number of male participants or free cycling women for both treatment groups and thus analyzed only female subjects taking oral contraceptives. This, of course, reduces the possibility to generalize our findings and to compare present and previous studies on this issue (Merz et al., 2010; Stark et al., 2006).

Due to the relatively small sample sizes, only large to medium effects could be revealed reliably. The absence of statistically relevant findings does not exclude smaller effects that may have remained undetected because of the small sample size.

Similarly to our previous studies (Merz et al., 2010; Stark et al., 2006), we used a constant dose of 30 mg of hydrocortisone, which prevented us from examining effects of different cortisol doses or effects of stress induced cortisol levels (Lupien, Gillin, & Hauger, 1999; cf. Merz et al., 2010). The effects of cortisol have been proposed to follow an inverse U-shaped cortisol dose–response curve (Lupien & McEwen, 1997; Lupien et al., 2007), however, whether this applies in the case of classical conditioning is still topic of a current debate (e.g. Sandi & Pinelo-Nava, 2007).

It has to be noted that in the present study we used a 100% pairing rate between CS+ and UCS, which may not be optimal for the study of extinction learning because learning then may occur too rapidly to be detected reliably (Phelps et al., 2004).

Other than previous studies addressing the effects of acute stress and cortisol on fear extinction, we administered cortisol before fear acquisition instead of before or after extinction (for overviews see de Quervain et al., 2009; Rodrigues et al., 2009). Thus, effects of cortisol on fear extinction cannot be unequivocally disentangled from acquisition related influences or interactions with acquisition effects. It would, however, be difficult to explain the effects observed during extinction simply with prolonged acquisition effects because the two groups showed opposing results. Further, there is only an incomplete overlap of the structures showing treatment effects during acquisition and extinction. Future studies should test the impact of cortisol on extinction more specifically by administering cortisol after acquisition (before extinction).

4.4. Conclusions

The observed neural activation patterns may reflect facilitating effects of cortisol on fear acquisition and extinction learning. An advanced understanding of GC effects on both, the acquisition and the extinction of fear is important for the understanding and treatment of stress related disorders like post-traumatic stress disorder or phobias. Thereby, a facilitated processing during extinction is in line with studies showing beneficial effects of cortisol on symptom severity in such disorders (Aerni et al., 2004; de Quervain & Margraf, 2008; Schelling et al., 2004; Soravia et al., 2006), but more closely links these effects to facilitated extinction instead of reduced retrieval of fear memory (see also Quirk & Mueller, 2008; Rodrigues et al., 2009). Thus, taken together, cortisol may increase the risk of acquiring an anxiety disorder but at the same time, it might be able to facilitate its extinction.

Conflict of interest

All authors declare that they have no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.nlm.2010.08.006.

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