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Influence of the stress hormone cortisol on fear conditioning in humans: Evidence for sex differences in the response of the prefrontal cortex

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The stress hormone cortisol is known to influence declarative memory and associative learning. In animals, stress has often been reported to have opposing effects on memory and learning in males and females. In humans, the effects of cortisol have mainly been studied at the behavioral level. The aim of the present experiment was to characterize the effects of a single cortisol dose (30 mg) on the hemodynamic correlates of fear conditioning. In a double-blind group comparison study subjects (17 females and 17 males) received 30 mg cortisol or placebo orally before participating in a discriminative fear conditioning paradigm. Results revealed that cortisol impaired electrodermal signs of learning (the first interval response) in males, while no conditioned SCRs emerged for the females independent of treatment. fMRI results showed that cortisol reduced activity for the CS+ > CS− comparison in the anterior cingulate, the lateral orbitofrontal cortex and the medial prefrontal cortex in males. Opposite findings (increase in these regions under cortisol) were detected in females. In addition, cortisol reduced the habituation in the CS+ > CS− contrast in the dorsolateral prefrontal cortex independent of sex. Finally, cortisol also modified the response to the electric shock (the UCS) by enhancing the activity of the anterior as well as the posterior cingulate. In sum, these findings demonstrate that in humans cortisol mostly influences prefrontal brain activation during fear conditioning and that these effects appear to be modulated by sex.

Introduction

Exposure to stress influences cognition in animals and humans. It has been demonstrated that stress-induced activation of the hypothalamus–pituitary–adrenal (HPA) axis and the subsequent release of glucocorticoids (GCs; corticosterone in rats, cortisol in humans) is amongst other factors such as epinephrine or corticotropin-releasing hormone (CRH) responsible for this effect. Various studies have investigated the effects of stress hormones on hippocampal mediated declarative or explicit memory. Animal as well as human studies have frequently observed that stress or cortisol treatment impairs delayed memory retrieval, while enhancing memory consolidation (for recent reviews, see Het et al., 2005; Lupien et al., 2005; Roozendaal, 2002; Wolf, 2003). Animal studies have demonstrated that the amygdala interacts with the hippocampus in mediating both of these effects (Roozendaal, 2002). Negative effects on retrieval are associated with reduced regional cerebral blood flow in the medial temporal lobe as demonstrated in a PET study (de Quervain et al., 2003; Roozendaal, 2002). In line with this observation, a resting state FDG PET study observed reduced hippocampal glucose uptake after cortisol treatment (de Leon et al., 1997). Recent studies in humans have suggested that cortisol especially enhances the consolidation of emotional memory, which suggests a specific effect of the hormone on amygdala function (Buchanan and Lovallo, 2001; Cahill et al., 2003). In support of this hypothesis, a positive correlation between cortisol levels and amygdala activity has been observed in a PET study with depressed patients (Drevets et al., 2002).

In rodents, multiple experiments have investigated the effects of stress on associative learning. Acute stress for example has been found to enhance delay as well as trace eye-blink conditioning in male rats, yet impair it in female rats (Shors, 2004). Similarly striking sex differences have also been observed for spatial memory tasks, even though here male rats show a stress-induced impairment, while female rats show a stress-induced enhancement (Conrad et al., 2004; Luine, 2002). With respect to the amygdala, mediated forms of memory enhanced fear conditioning as well as enhanced avoidance learning have been observed after acute or chronic stress as well as pharmacological GC treatment (Bohus and

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Lissak, 1968; Conrad et al., 1999; Corodimas et al., 1994; Flood et al., 1978; Hui et al., 2004; Zorawski and Killcross, 2002). These results have been interpreted as indicating enhanced amygdala but reduced hippocampal functioning in times of high cortisol levels (Sapolsky, 2003). In humans, observations of an association between endogenous cortisol levels and galvanic skin responses in fear conditioning paradigms have only recently been reported for the first time. Interestingly, both studies reported that basal (Zorawski et al., 2005) or stress-induced (Jackson et al., 2006) cortisol levels were associated with changes in conditioning in males but not in females, again pinpointing towards substantial sex differences.

In humans and primates, a large number of glucocorticoid receptors are present in the prefrontal cortex, which suggests that these regions are major targets of cortisol action in humans (Lupien and Lepage, 2001). In line with these observations, several studies reported that working memory, which is mediated by prefrontal regions, is impaired after stress or cortisol treatment (Elzinga and Roelofs, 2005; Lupien et al., 1999; Lyons et al., 2000; Wolf et al., 2001a,b). Vice versa, the anterior cingulate gyrus has been implicated in the modulation of the hypothalamus–pituitary–adrenal axis in rodents and humans (Diorio et al., 1993; Wolf et al., 2002). This region and other prefrontal regions inhibit amygdala and HPA responses to stress (Amat et al., 2005). Hariri et al. (2003) further report an inverse correlation of prefrontal cortex and amygdala activation in reaction to fear-related stimuli. A recent perfusion fMRI study reported that activity in the right PFC and the anterior cingulate was associated with stress-induced changes in cortisol secretion (Wang et al., 2005). These correlative fMRI findings indicate together with experimental lesion work in animals (e.g., Diorio et al., 1993) the involvement of prefrontal brain regions in the regulation of the (HPA) stress response in humans.

Thus, several structures involved in emotional learning are influenced by cortisol and stress. Fear conditioning is a well-established method to study emotional associative learning processes. Lesion studies as well as functional imaging studies with humans identified the amygdala and prefrontal structures (e.g., anterior cingulate and orbitofrontal cortex) as crucial for the acquisition and expression of fear. Human patients with amygdala damage showed impaired fear conditioning when measuring skin conductance responses and startle response (Bechara et al., 1995; LaBar et al., 1995; Weike et al., 2005). An increasing number of functional fear conditioning studies confirmed the involvement of these structures in healthy humans but also considered a more widespread network including the sensory and insular cortex, the hypothalamus, the thalamus, and the hippocampus (LaBar et al., 1998; Tabbert et al., 2005; Büchel et al., 1999; Knight et al., 1999, 2004a,b; Fischer et al., 2000; Bradley et al., 2003). A variety of stimuli (e.g., human faces or colored lights) and conditioning paradigms (e.g., differential vs. simple conditioning, delay vs. trace conditioning) have thereby been employed, altering the constellation of the structures involved. Regarding the influence of stress-related substances on emotional learning, previous human neuroimaging studies have investigated the influence of the modulation of the adrenergic system on emotional declarative memory formation (Strange and Dolan, 2004; van Stegeren et al., 2005). However, to the best of our knowledge, no human imaging study has so far investigated the effects of the stress hormone cortisol on fear conditioning. The aim of the present experiment was therefore to investigate the effects of cortisol on the patterns of cerebral activation in healthy young subjects exposed to a fear conditioning paradigm. Given the substantial animal literature on sex differences (see above), we were additionally interested in characterizing potential differences between female and male participants.

In detail, we applied a differential conditioning paradigm in which two former neutral visual stimuli served as conditioned stimuli and an electric shock as an unconditioned stimulus (UCS). One of the conditioned stimuli (CS+) always announced the UCS, while the other conditioned stimulus (CS−) was never followed by the UCS. We measured central blood oxygenation level-dependent (BOLD) responses via functional magnetic resonance imaging, as well as autonomic electrodermal responses. We hypothesized that cortisol treatment would affect the learning process at least regarding the following two aspects: first, cortisol can enlarge or diminish the response differences to CS+ and CS−. Secondly, treatment can affect the habituation rates to CS+ and CS−. Büchel et al. (1998) demonstrated for amygdala responses that habituation slopes are a useful measure of conditioning effects. We also examined the cortisol effect on the responses towards the UCS. If a treatment effect on learning indeed occurs, it might well be possible that reactions to the UCS are modulated, as the functional value of associative learning is to prepare the organism to effectively deal with an UCS as Domjan (2005) recently pointed out.

Method

Subjects

A total of 34 subjects (17 female) participated in the study, which was approved by the ethics committee of the German Psychological Society. Corresponding to the experimental design, we divided the sample into four groups: female placebo group (n = 9), female cortisol group (n = 8), male placebo group (n = 8), and male cortisol group (n = 9). The mean age of the entire sample was 24.2 years (SD = 7.5) with no significant differences between the four groups with respect to age. Most of the participants were university students, who had been recruited via announcements at bulletin boards at the campus. Subjects were informed about the procedure in principle (the conditioning schedule was of course not explained until the experiment was finished). All subjects were right-handed as assessed by the Edinburgh Inventory of Handedness (Oldfield, 1971).They signed an informed consent, which also stated that they could terminate the experiment at any time. None of them was taking regular medication or had a history of any psychiatric or neurological treatment.

Previous fMRI studies have observed menstrual cycle related changes in the BOLD response during cognitive (e.g., Fernandez et al., 2003) or emotional (e.g., Protopopescu et al., 2005) processing. In order to avoid an increased variance in our female group – secondary to gonadal steroid induced changes – we tested women using oral contraceptives only. This group constitutes the majority of German university students. All subjects were tested during the pill intake phase (day1–day 21), not during the off phase prior to withdrawal bleeding. Oral contraceptives contain low doses of potent synthetic estrogens and progestins, which suppress the endogenous production of estradiol and progesterone.

Subjects received a payment of 20 Euros for their participation.

Treatment and hormone measurement

The study was designed as a double-blind placebo-controlled group comparison. Eight females and nine males received 30 mg of cortisol (30 mg hydrocortisone; Hoechst) given orally 15 min before the conditioning, the others received visually identical placebos (tablettose and magnesium, Dr. Kade). This cortisol dose has been used in previous behavioral studies by one of the authors (OTW) and has been found to reliably influence declarative memory (e.g., Kuhlmann et al., 2005; Wolf et al., 2001a).

All experiments took place between 12 p.m. and 2 p.m. Saliva was collected using Salivette collection devices (Sarstedt, Nümbrecht, Germany). Samples were taken before treatment, 15 min (immediately before the fMRI run), and 40 min (immediately after the fMRI run) after treatment. Free cortisol was measured using an immunoassay (IBL, Hamburg, Germany). Inter-assay and intra-assay variations were < 15%.

Conditioned visual stimuli

Two pictures of simple geometrical figures, a rhomb and a square, served as CS+ and CS− in a differential conditioning paradigm. Both stimuli were grey in color and had identical luminance. For visual stimulation inside the scanner, an LCD projector (model EPSON EMP-7250) was used, which projected pictures onto a screen at the end of the scanner (visual field = 18°). Pictures were presented for 8 s and viewed by means of a mirror mounted to the head coil.

Unconditioned stimulus

A custom-made impulse-generator (833 Hz) provided transcutaneous electrical stimulation to the left shin through two silver/silverchlorid electrodes (0.5 cm^2 surface each), that was applied as unconditioned stimulus (UCS). Stimulus intensity was set for each subject individually to an "unpleasant, but not painful" level using a gradually increasing rating procedure. Each electrical stimulus was applied for 100 ms. The onset and the duration of the electrical stimulation were set by a computer program, and the generator inside the scanning chamber was triggered via a fibre optic cable.

Conditioning procedure

There were 30 trials of CS+ and 30 trials of CS− presentation throughout the acquisition procedure. Inter-trial intervals ranged from 8 to 12 s. The onset of the UCS-presentation was delayed 7.9 s after CS+ onset and terminated with CS+ offset (delay conditioning). For each participant, a different stimulus order was used in pseudo randomized order comprising the following restrictions: no more than two consecutive presentations of the CS and an equal quantity of CS+ and CS− trials in the first and the second half of the experiment. The acquisition procedure started with a CS+ for one half of the subjects, with a CS− for the other half and either the rhomb or the square served as CS+. The experiment contained only an acquisition, not an extinction phase. The onsets of the stimulus events (CS+, CS−, UCS) were randomly varied with respect to the fMRI scans (random jitter between 0 and 2500 ms).

Skin conductance response

Skin conductance responses (SCRs) were sampled simultaneously with MR 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 (Prokasy and Ebel, 1967): the maximal response within a time window of 1 to 5 s after the CS onset was counted as a first interval response (FIR), within the time window of 5 to 8.5 s as a second interval response (SIR) and within the time window of 8.5 to 13 s as the unconditioned response (UCR). Conditioning was assumed to have been successful when the CS+ provoked larger magnitudes than the CS− in the FIR and SIR. Statistical comparisons were performed via analysis of variance in a 2 (stimulus-type) × 2 (treatment) × 2 (sex) × 30 (trial) factorial design within the general linear model (GLM) as it is implemented in SPSS for Windows (Release 12.0, SPSS Inc., Illinois). Stimulus-type (CS+ vs. CS−; UCS vs. UCS omission) and trial (1 to 30) were introduced as repeated measurement factors and treatment (cortisol vs. placebo) and sex as between subjects factors, respectively. Greenhouse–Geisser correction was applied when sphericity assumption was not met.

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. For functional imaging, a total of 535 volumes was registered using a T_2^*-weighted gradient echo-planar imaging sequence (EPI) 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 5 volumes were discarded due to an incomplete steady state of magnetization. The orientation of the axial slices was parallel to the AC–PC line. Data were analyzed using Statistical Parametric Mapping (SPM2, Wellcome Department of Cognitive Neurology, London, UK; 2002) implemented in MatLab 6.5 (Mathworks Inc., Sherborn, MA). Origin coordinates were adjusted to the anterior commissure (AC). Realignment (b-spline interpolation), slice time correction and normalization to the standard space of the Montreal Neurological Institute brain (MNI-EPI-template) were performed. Smoothing was executed with an isotropic three-dimensional Gaussian filter with a full width at half maximum (FWHM) of 9 mm. The experimental conditions were CS+, CS−, UCS, and NoUCS. NoUCS was defined as the UCS omission after the CS− in a time window corresponding to UCS application after the CS+. These conditions were modeled by a stick function convolved with a hemodynamic response function (hrf) in the GLM, without specifically modeling 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. 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 region of interest (ROI) analyses to enhance the statistical power concerning the structures we were especially interested in: the amygdala, the anterior and posterior cingulate cortex, the hippocampus/parahippocampus, the hypothalamus, the insula, the lateral and ventromedial orbitofrontal cortex, the dorsolateral and medial prefrontal cortex, and the thalamus. Since we were also interested in the temporal course of the BOLD

responses towards CS+, CS− we added two regressors in our statistical design, which modeled linear temporal decrease of activation. Thus, the regressors of interest were CS+, CS−, UCS, NoUCS, CS+ by time, and CS− by time. Statistical analyses were done in a random effects design and focused on the contrasts CS+ > CS−, UCS > NoUCS, and CS+ by time > CS− by time. In a first step, we analyzed these contrasts for the whole sample; then analyses of variance were conducted with the group factors sex (females vs. males) and treatment (cortisol vs. placebo). This was achieved by introducing four groups (male cortisol, male placebo, female cortisol, female placebo) in the one-way ANOVA implemented in SPM2. Choosing appropriate contrasts, the respective main and interaction effects could be tested. Appropriate post hoc tests were done for significant main effects and interactions.

For the explorative whole brain analyses, the significance threshold was set to $\alpha = 0.05$ on voxel-level, corrected for multiple testing, and a minimum cluster size of 5 was required. Region of interest (ROI) analyses were performed using small volume correction options of SPM2 ($\alpha < 0.05$). The required masks for these analyses were designed using the software program MARINA (Walter, 2002).

Results

Cortisol levels

Cortisol levels of the four groups did not differ at baseline. In response to cortisol administration, all subjects showed pronounced increases of 90 nmol/l and larger. Some subjects displayed extremely high cortisol levels 15 min after cortisol intake (larger than 1000 nmol/l), which most likely reflects some micro residue of the uncoated tablet in the mouth of the participants. In the placebo groups, none of the participants showed elevated cortisol levels. In fact a moderate decrease typical for the circadian cortisol rhythm was detected. ANOVA with the repeated measurement factor time and the grouping factors treatment and sex revealed a significant main effect of time ($F(2,44) = 33.67$; $P < 0.001$; $\varepsilon = 0.67$), a main effect of treatment ($F(1,22) = 127.00$; $P < 0.001$), and a significant time by treatment interaction ($F(2,44) = 34.13$; $P < 0.001$; $\varepsilon = 0.67$). For the factor sex neither a main effect nor any interactions were observed.

Cortisol levels are displayed in Table 1.

Table 1
Mean (SE) cortisol levels before the cortisol (30 mg) or placebo administration, 15 min after administration, and 40 min after administration

Sex	Before treatment	15 min after treatment (before fear conditioning)	40 min after treatment (after fear conditioning)
Placebo			
Males	11.00 (2.60)	10.08 (2.54)	8.31 (1.94)
Females	8.68 (0.74)	7.49 (0.51)	6.02 (0.40)
Cortisol			
Males	7.26 (1.98)	301.28 (73.91)	150.53 (19.64)
Females	7.04 (0.71)	321.20 (63.90)	162.87 (47.53)

Unrealistic high levels of cortisol (larger than 1000 nmol/l) probably reflecting some microresidue of the cortisol tablet in the mouth of the participants were excluded from the descriptive statistics in this table.

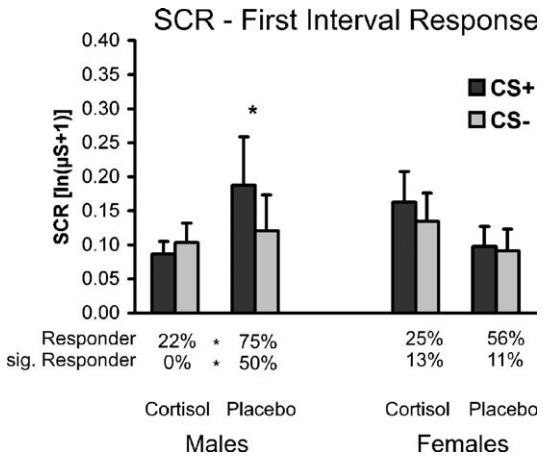

Fig. 1. Mean (SE) logarithmic skin conductance responses (SCR) to CS+ and CS− for the first interval responses (FIR) separately for male and female participants in the cortisol and placebo condition respectively. The percentages of responders (defined by greater FIR and SIR towards CS+ in contrast to CS−) and significant responders (significant differences between FIR or SIR towards CS+ and CS− on an individual level) are depicted below the bars. *$P < 0.05$.

Skin conductance responses

For the first interval responses (FIR), males under placebo showed a stronger response to the CS+ than to the CS−. No such effect was observed in women. Cortisol abolished the enhanced FIR in males while tending to increase it in women (Fig. 1). In detail, analysis of variance (stimulus-type × treatment × sex × trial) resulted in a main effect of stimulus-type ($F(1,30) = 5.29$; $P < 0.05$), in a main effect trial ($F(29,870) = 9.87$; $P < 0.001$; $\varepsilon = 0.20$), and in a three-way interaction of stimulus-type × treatment × sex ($F(1,30) = 8.29$; $P < 0.01$) for the FIR. The trial effect resulted from a decrease of FIRs over time. Subsequent analyses of the stimulus-type × treatment × sex effect revealed that this interaction can be traced back to the fact that the males in the placebo group displayed greater FIR towards CS+ than CS− ($F(1,7) = 8.32$; $P < 0.05$) while females showed increased responses to CS+ in contrast to CS− in the cortisol group, however without achieving statistical significance.

For the second interval responses (SIR), a main effect of stimulus-type ($F(1,30) = 5.90$; $P < 0.05$) and a main effect trial ($F(29,870) = 2.0$; $P < 0.05$; $\varepsilon = 0.30$) occurred. SIRs were greater towards the CS+ compared to the CS−. All the SIRs decreased with time.

As expected the application of the UCS as compared to its omission elicited greater responses indicated by a main effect stimulus-type ($F(1,30) = 57.82$; $P < 0.001$) in the analysis of variance (stimulus-type × treatment × sex × trial) of UCR. A main effect of trial ($F(29,870) = 9.54$; $P < 0.001$; $\varepsilon = 0.31$) and an interaction stimulus-type × trial ($F(29,870) = 4.05$; $P < 0.001$; $\varepsilon = 0.29$) also emerged. No other main effects or interactions were observed.

Due to the unexpected outcome, that the placebo females did not show conditioning, we additionally analyzed the SCR data on an individual level once using a weak conditioning criterion and once a stricter criterion. We defined subjects as conditioning "responders", when the FIR and SIR responses towards CS+ were greater than the FIR and SIR responses towards CS−. If significant

differences (paired t-test, $P < 0.05$) between either FIR or SIR responses emerged, we called a subject "significant responder". The percentages of responders and significant responders are depicted in the lower part of Fig. 1. Interestingly, contrary to the impression gained from mean SCR responses, more females in the placebo group than in the cortisol group were responders (yet not significant responders). This mirrors the results of the males: with more responders in the placebo than in the cortisol group. Cortisol and placebo groups were compared with respect to differences in response frequencies by Fishers Exact Test. These analyses were done independently for females and males and separately for both response definitions (response and significant response). Only in males the difference between the cortisol and the placebo group reached statistical significance (response: $P = 0.05$; significant response: $P = 0.03$).

We further tested whether the lack of conditioning effects in the female placebo group could be traced back to single subjects, but even when omitting the non responders of the placebo female group, the responses towards CS+ and CS− did not significantly differ. The hemodynamic results did also not change substantially when analyzing this reduced sample.

Hemodynamic responses

CS+ > CS−

For the whole group the CS+ resulted in stronger hemodynamic responses than the CS− in the anterior cingulate (bilateral), the right hippocampus, the hypothalamus, the left lateral orbitofrontal cortex, the left thalamus and bilaterally in the ventromedial orbitofrontal cortex (see Table 2).

The analyses of variance revealed a main effect sex in the right insula and in the right ventromedial orbitofrontal cortex, with enhanced differentiation of CS+ and CS− for the males (Table 2). The hemodynamic responses were higher to CS+ and lower to CS− in males in comparison to females in these structures.

A significant interaction between treatment and sex was observed in the right anterior cingulate, the right lateral orbitofrontal cortex, and the left medial prefrontal cortex (Table 2, Fig. 2). In all three regions cortisol reduced the response to the CS+ compared to CS− in males. In contrast, females reacted stronger to the CS+ compared to CS− when they had received cortisol. Almost all post hoc t-tests of the contrast estimates (exception in the right lateral orbitofrontal cortex: female cortisol group vs. female placebo group) resulted in significant differences (all $P < 0.05$).

Table 2
Significant activations for the contrast CS+ > CS− for the entire sample and the significant results of the analysis of variance *treatment × sex*

Brain structure	Side	x	y	z	z_{max}	Cluster	P_{corr}
CS+ > CS−: entire sample							
Anterior cingulate	Left	0	15	21	3.71	246	0.015
Anterior cingulate	Right	3	15	24	3.41	34	0.032
Hippocampus	Right	24	−36	6	3.52	59	0.036
Hypothalamus		6	0	−18	4.12	176	0.002
Lateral orbitofrontal cortex	Left	−18	18	−24	3.55	210	0.037
Thalamus	Left	−6	−30	6	3.21	62	0.042
Ventromedial orbitofrontal cortex	Left	0	27	−24	4.06	226	0.005
Ventromedial orbitofrontal cortex	Right	6	24	−18	4.38	190	0.002
CS+ > CS−: main effect sex							
Insula	Right	33	12	12	3.89	7	0.010
Ventromedial orbitofrontal cortex	Right	21	33	−15	3.96	9	0.008
CS+ > CS−: interaction treatment × sex							
Anterior cingulate	Right	12	48	15	3.48	9	0.028
Lateral orbitofrontal cortex	Right	48	27	−21	3.93	42	0.013
Medial prefrontal cortex	Left	−6	42	51	3.54	10	0.047

The threshold was $P < 0.05$ (small volume correction according to SPM2). All coordinates (x, y, z) are given in MNI space.

CS+ by time > CS− by time

Analyzing all subjects (ignoring the group factors), a more pronounced decrease in activation of the left insula was observed in reaction to the CS+ in comparison to the CS− ($x = −33$, $y = −18$, $z = 15$, $z_{max} = 4.01$, $P_{corr} < 0.01$). The analyses of variance revealed a main effect of treatment in the right dorsolateral prefrontal cortex: for both groups (placebo and cortisol), the reactions to CS+ and CS− decreased over time, however, the decrease to CS+ was less pronounced in the cortisol group ($x = 30$, $y = 54$, $z = 30$, $z_{max} = 3.98$, $P_{corr} = 0.04$). Thus, cortisol seems to have delayed habituation to the CS+.

UCS > non-UCS

For the whole sample, the UCS as compared to the NoUCS provoked an extended activation cluster in the parietal lobe (supramaginal gyrus, $x = 63$, $y = −24$, $z = 30$, $z_{max} = 7.36$, clustersize = 21821, $P_{corr\ for\ the\ whole\ brain} < 0.001$) and bilaterally in all regions of interest (all $P_{corr} < 0.001$, except left posterior cingulate ($P_{corr} < 0.01$), right posterior cingulate ($P_{corr} < 0.05$), and left ventromedial orbitofrontal cortex ($P_{corr} < 0.01$)).

In the analyses of variance, a main effect of treatment was found in the right anterior ($x = 15$, $y = 42$, $z = 6$, $z_{max} = 3.28$; $P_{corr} < 0.05$) and posterior cingulate ($x = 6$, $y = −39$, $z = 30$, $z_{max} = 3.58$; $P_{corr} < 0.01$). Post hoc analyses showed that these effects were due to enhanced reactions to the UCS in the cortisol compared to the placebo group; these were significant for the posterior cingulate ($P_{corr} < 0.05$).

Additionally, there was a main effect of sex in the left dorsolateral prefrontal cortex ($x = −12$, $y = 39$, $z = 54$, $z_{max} = 4.36$; $P_{corr} < 0.01$), the left medial prefrontal cortex ($x = −9$, $y = 39$, $z = 54$, $z_{max} = 3.69$; $P_{corr} < 0.05$) and the left thalamus ($x = −12$, $y = −21$, $z = 9$, $z_{max} = 3.20$; $P_{corr} < 0.05$). Post hoc analyses revealed that this was due to enhanced reactions of the females to the UCS, but none of the differences revealed statistical significance.

Discussion

The present study was designed to examine the neural correlates of the influence of the stress hormone cortisol on fear conditioning. Concerning the fear conditioning process, we were interested in the effects of cortisol on the learned differentiation between CS+ and CS− and the time course of this differentiation. We further asked whether the hemodynamic responses to the electric shock were altered by cortisol.

Before focusing on the treatment effects, we would like to shortly discuss the general findings of our conditioning procedure.

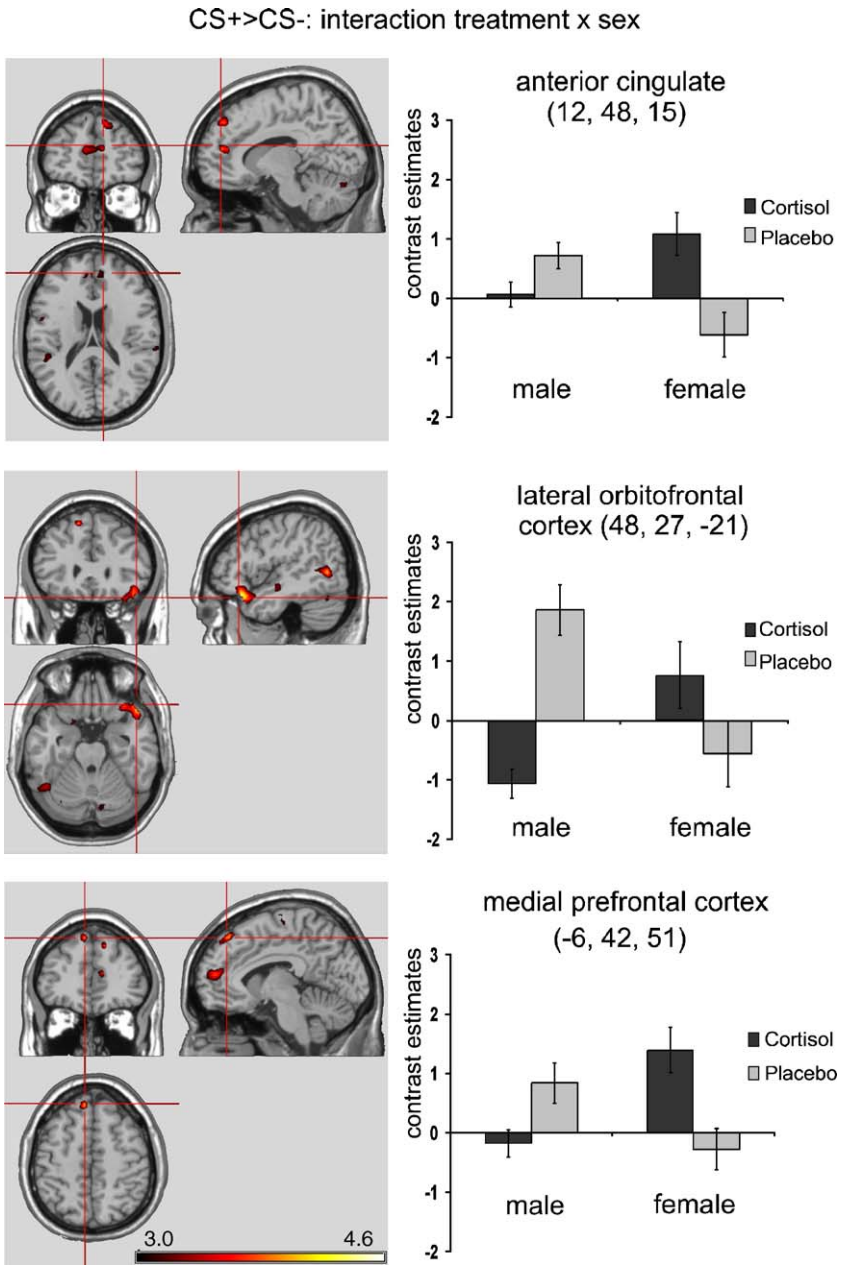

Fig. 2. Mean (SE) of the contrast CS+ > CS− for males and females of the placebo and cortisol groups in the anterior cingulate, lateral orbitofrontal cortex and the medial prefrontal cortex.

Comparing the responses to CS+ and CS−, we observed that CS+ resulted in greater activations in the anterior cingulate, the thalamus, the orbitofrontal cortex, the hippocampus and the hypothalamus. The activations in these structures are well in line with other imaging studies (anterior cingulate: Büchel et al., 1998; LaBar et al., 1998; Knight et al., 2004b; thalamus: Knight et al., 2004b; orbitofrontal cortex: Kringelbach and Rolls, 2004; Tabbert et al., 2005; hippocampus: Knight et al., 2004a; hypothalamus: Fischer et al., 2000).

Although the amygdala is a key structure of the neural fear network (Davis and Whalen, 2001; LeDoux, 2000), we did not observe differences in amygdala activation to CS+ in comparison to CS−. Other fMRI studies, however, also failed to find amygdala activation during fear acquisition (Knight et al., 1999, 2004b). Individual differences in the laterality of the amygdala activation and its habituation rates may be responsible for not detecting amygdala involvement (e.g., Furmark et al., 1997; Cheng et al., 2003). In a previous study, we observed greater amygdala responses to the CS+ only when analyzing the second part of the acquisition period (Tabbert et al., 2005).

The most striking outcome with regard to the effects of cortisol treatment on fear conditioning in our study was that cortisol influenced associative learning differentially for males and females. This was the case at the central level (regional hemodynamic responses) as well as to a certain extent at the peripheral autonomic level (skin conductance). Concerning skin

conductance responses, males displayed conditioning signs under placebo. This was observed in the group analysis as well as in the individual responder analyses. Yet, these effects were almost completely blocked by the cortisol treatment. In contrast, females under placebo did not show strong signs of learning. Here, the group statistics showed no conditioning effects and on the individual level only about half of the females showed signs of conditioning even when using a rather weak criterion for conditioning. Under cortisol, the group statistics revealed a weak tendency for more increased conditioning effects compared to the placebo group, however, the individual responder analysis with the weak criterion points towards more reduced conditioning under cortisol similar to that observed for the males. Thus, cortisol had impairing effects on males but no clear-cut effects on females.

Sex-dependent effects of cortisol were also observed for regional blood flow in the brain, particularly in the prefrontal cortex: males under placebo showed greater hemodynamic responses to the CS+ than to the CS− in the anterior cingulate, the lateral orbitofrontal cortex, and the medial prefrontal cortex. This effect was abolished in the cortisol group. Different effects emerged for the female participants: only when they had received cortisol they displayed greater hemodynamic responses to CS+ than CS−. These partly similar results regarding SCRs and prefrontal responses may well be explained by functional connections among these structures and autonomic correlates of conditioning. All three structures, as well as SCR, have often been found to be involved in emotional processing, especially in fear conditioning (Phan et al., 2002; Büchel et al., 1998). There are numerous findings, which suggest that the orbitofrontal cortex together with the amygdala is responsible for the evaluation of the reinforcement properties of a stimulus (for overview, see Kringelbach and Rolls, 2004). Further, Critchley et al. (2000) were able to demonstrate that variations in SCR responses were directly correlated with the neural activity measured by BOLD response in the medial prefrontal cortex.

Animal and human neuroanatomical studies have described the existence of a large number of glucocorticoid receptors in the PFC and impaired PFC function after GC treatment (see for review Lupien and Lepage, 2001). Previous behavioral studies in humans reported impaired prefrontal mediated working memory performance after stress or cortisol treatment (Elzinga and Roelofs, 2005; Lupien et al., 1999; Wolf et al., 2001a,b). The present study was able to show that associative emotional learning, which involves parts of the PFC (e.g., orbitofrontal cortex, Rolls, 1999) is impaired by cortisol at least in males. This indicates that specific functions of the PFC are hampered by GCs. This supports the notion that the PFC is a main target of GC action in the human brain (Lupien and Lepage, 2001).

There were no significant conditioning effects observed in the FIR of the placebo treated females. This was surprising, as we observed conditioning effects in a group mainly consisting of females (12 of 18 subjects) in a previous study (Tabbert et al., 2005). It is, however, altogether difficult to discuss a lack of significance with a sample that consisted of eight subjects only. Yet, it is important to note that the somewhat unusual responses of the female placebo group are not solely responsible for the observed sex differences, since cortisol treated men and women showed substantially different hemodynamic response patterns (see Fig. 2).

While sex differences in fMRI activation have been reported for emotional memory tasks (e.g., Cahill, 2003), we are not aware of studies reporting sex differences in the BOLD response during fear conditioning. Some animal studies, at least, reported stronger fear conditioning responses in male rats (e.g., Pryce et al., 1999).

The unexpected finding of a missing differentiation between the CS+ and the CS− in the female placebo group is certainly problematic. Nonetheless, this observation does not effect the strong and consistent impairment due to cortisol in the male group. We can, however, not exclude the possibility that the effects observed in women reflect atypical results of the placebo group, rather than a cortisol effect in the treatment group. Thus, our findings call for additional studies with female subjects. Here, oral contraceptive users should be compared to naturally cycling women. We studied oral contraceptive users only in order to guarantee a stable sex steroid milieu in the female subjects, thereby reducing the variance possibly introduced by menstrual cycle associated changes in the BOLD signal during emotional or cognitive processing (see Fernandez et al., 2003; Protopopescu et al., 2005). Future fMRI studies in this field should pay more attention to possible influences of gonadal hormones and should provide information about the use of hormonal contraceptives of their female participants in order to allow a better comparison between different studies.

Animal studies have repeatedly observed that stress can influence conditioning or memory differently in male and female animals (Conrad et al., 2004; Luine, 2002; Shors, 2004). Preliminary evidence for comparable human sex-specific associations have been provided for declarative memory (Wolf et al., 2001b). Moreover, two recent behavioral fear conditioning studies observed associations between basal or stress-induced cortisol levels and galvanic skin responses for their male participants only (Jackson et al., 2006; Zorawski et al., 2005). The present study complements these observations describing sex differences in response to an acute cortisol treatment at the peripheral as well as the central level during fear conditioning.

In our study, we observed that cortisol treatment impaired fear conditioning in males. The two recent psychophysiological studies mentioned above, in contrast, observed positive associations between endogenous cortisol levels and fear conditioning (Jackson et al., 2006; Zorawski et al., 2005). This discrepancy might in part be due to the different paradigms used (e.g., neutral CS in our study, emotional CS in the two previous studies) as well as the different learning phases investigated (acquisition in our study, extinction and consolidation in the previous studies). However, it is also possible that small cortisol elevations facilitate fear conditioning, while large increases (as in our study) impair fear conditioning. The idea of an inverted U-shaped dose response curve is supported by psychophysiological and behavioral animal studies, as well as by human memory studies (see for review Lupien and Lepage, 2001). Finally, while endogenous cortisol levels reflect the activation status of the HPA axis, exogenous cortisol levels actually inhibit the HPA axis (and thus central CRH). Thus, we cannot rule out that indirect effects mediated via the cortisol induced inhibition of central CRH secretion are underlying the observed effects (Croiset et al., 2000).

Instead of influencing the BOLD response amplitudes to a neutral stimulus, it is also possible that fear conditioning modifies the habituation that is usually observed with repeated presentations. We therefore tested whether cortisol influenced the habituation slopes towards CS+ and CS−, and indeed, habituation to CS+ was found to occur slower under cortisol treatment than under placebo in the dorsolateral prefrontal cortex. The dorsolateral

prefrontal cortex has often been found in working memory tasks (for review, e.g., Curtis and D'Esposito, 2004), but also for example during expectancy of emotional stimuli (Ueda et al., 2003) as well as in decision making as an executive function (Bechara and Van Der Linden, 2005). As to which function of the dorsolateral prefrontal cortex is responsible for the differences in the habituation rates cannot be answered by this study.

Cortisol not only influenced associative learning (CS+ CS− discrimination) in interaction with sex but also increased the hemodynamic responses in the anterior and posterior cingulate when subjects received the electronic shock (the UCS). The anterior cingulate is, as mentioned above, an important structure for the experience of emotions and a structure rich in GC receptors. This region has however also been related to an array of other functions: the regulation of emotional but also cognitive processing, error detection and monitoring as well as conflict or competition processing (for review, e.g., Bush et al., 2000). The anterior and the posterior cingulate have both been found to be involved in pain processing (Bromm, 2001, 2004). This indicates that cortisol might modulate the processing of aversive and painful stimuli.

In sum, this study reports for the first time in the human the effects of cortisol treatment on regional brain activation during a fear conditioning paradigm. The induced hormone enhanced as well as decreased activations. These alterations were sex, stimulus, and time-dependent in several prefrontal regions. Yet, we failed to detect changes in the amygdala. In our study, cortisol had different effects on the CS+ > CS− contrast for males and females. This reiterates the need to carefully pay attention to sex differences when investigating emotional processing and emotional memory formation (Cahill, 2003; Jackson et al., 2006; Schienle et al., 2005; Shors, 2004; Zorawski et al., 2005).

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