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Stress differentially affects fear conditioning in men and women☆

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Summary Stress and fear conditioning processes are both important vulnerability factors in the development of psychiatric disorders. In behavioral studies considerable sex differences in fear learning have been observed after increases of the stress hormone cortisol. But neuroimaging experiments, which give insights into the neurobiological correlates of stress × sex interactions in fear conditioning, are lacking so far. In the current functional magnetic resonance imaging (fMRI) study, we tested whether a psychosocial stressor (Trier Social Stress Test) compared to a control condition influenced subsequent fear conditioning in 48 men and 48 women taking oral contraceptives (OCs). One of two pictures of a geometrical figure was always paired (conditioned stimulus, CS+) or never paired (CS−) with an electrical stimulation (unconditioned stimulus). BOLD responses as well as skin conductance responses were assessed. *Sex-independently*, stress enhanced the CS+/CS− differentiation in the hippocampus in early acquisition but attenuated conditioned responses in the medial frontal cortex in late acquisition. In early acquisition, stress reduced the CS+/CS− differentiation in the nucleus accumbens in men, but enhanced it in OC women. In late acquisition, the same pattern (reduction in men, enhancement in OC women) was found in the amygdala as well as in the anterior cingulate. Thus, psychosocial stress impaired the neuronal correlates of fear learning and expression in men, but facilitated them in OC women. A sex-specific modulation of fear conditioning after stress might contribute to the divergent prevalence of men and women in developing psychiatric disorders.

☆ This study was carried out at the Bender Institute of Neuroimaging, Justus Liebig University Giessen.

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1. Introduction

Stress hormones strongly act on emotional and cognitive processes and cause vivid remembrance of emotionally arousing events (Wolf, 2008). In the case of traumatic experiences, this can occasionally result in excessive fear and anxiety such as in posttraumatic stress disorder (PTSD). Fear conditioning is an emotional learning process critically contributing to the development of PTSD and other psychiatric disorders (e.g. phobias; Bonne et al., 2004). These disorders occur to a much higher degree in women (Kessler et al., 2005). However, the underlying neurobiological mechanisms of stress, which potentially influence fear conditioning in men and women differently, remain insufficiently understood. A better comprehension of this crucial stress-related and sex-dependent fear circuit might ultimately lead to improved treatments.

An environmental threat triggers the stress response activating the sympathetic nervous system (SNS) as well as the hypothalamus–pituitary–adrenal (HPA) axis. The SNS stimulates the adrenal glands to release (nor)epinephrine, which can be indirectly measured via salivary alpha-amylase (sAA; Nater and Rohleder, 2009). Activation of the HPA axis leads to a release of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone, and glucocorticoids such as cortisol, the major stress hormones in humans. Cortisol readily enters the brain and modulates cortical as well as subcortical structures involved in learning and memory, e.g. the amygdala, the hippocampus or the prefrontal cortex (for reviews: Wolf, 2009; Joels et al., 2011). Such learning and memory processes can be investigated using fear conditioning designs. Typically, conditioned responses (CRs) are found in the amygdala, anterior cingulate, hippocampus, and medial prefrontal cortex (e.g. Büchel et al., 1998; LeDoux, 2000; Mechias et al., 2010). Besides, the formation of relations between conditioned (CS) and unconditioned stimuli (UCS) was associated with activation of the nucleus accumbens (Klucken et al., 2009). A prolonged activation of this fear circuit along with the release of stress hormones during initial association is proposed to be related to the development of pathologic fears (for a review: Rodrigues et al., 2009).

A few psychophysiological studies in humans provided evidence that stress hormones affect fear conditioning in men and women differently, e.g. using psychosocial stress (Jackson et al., 2006; Zorawski et al., 2006) or correlational approaches (Zorawski et al., 2006). In these experiments, stress hormones enhanced CRs in males, but reduced them in females or did not exhibit any significant effect in females. Neuroimaging studies from our groups used a pharmacological administration of 30 mg hydrocortisone (cortisol) prior to fear conditioning (Stark et al., 2006; Merz et al., 2010, 2012b; Tabbert et al., 2010): A reversed picture emerged with cortisol attenuating CRs in the fear circuit (including the amygdala, anterior cingulate, hippocampus, and medial prefrontal cortex) in men and in free-cycling women, but elevating CRs at the neuronal level in women taking oral contraceptives (OCs). The contribution of OCs on fear conditioning processes is especially interesting in terms of their common usage, but no studies are available on their possible impact on mental health.

Taken together, stress effects on fear conditioning were tested so far in humans at the electrodermal level only. To translate neuroimaging findings with pharmacological cortisol concentrations (Stark et al., 2006; Merz et al., 2010, 2012b; Tabbert et al., 2010) to physiological stress-induced cortisol concentrations, we used a psychosocial stressor prior to differential fear conditioning. Thus, we mirrored real-life stress with its concurrent activation of the SNS (assessed indirectly by measurement of sAA) and the HPA axis (as indexed by salivary cortisol). We were particularly interested in men and OC women, because they exhibited the most contrasting fear learning pattern in previous pharmacological cortisol studies (Stark et al., 2006; Merz et al., 2012b). All participants were instructed to pay close attention to any regularities between CS and UCS to ensure complete contingency awareness developing very early in the experiment. Accordingly, we expected fear learning related activation (in the amygdala, hippocampus, and nucleus accumbens) during early acquisition and fear expression and regulation related activation during late acquisition (in the amygdala, anterior cingulate, and medial prefrontal cortex; cf. Sotres-Bayon and Quirk, 2010). Based on our previous pharmacological neuroimaging studies (Stark et al., 2006; Merz et al., 2010, 2012b; Tabbert et al., 2010), we predicted that psychosocial stress leads to reduced CRs in men, but heightened CRs in OC women at the electrodermal level as well as in the respective brain regions involved in fear conditioning.

2. Materials and methods

2.1. Participants

We recruited 105 persons to ensure a total sample size of 96 participants (48 men). Two women were excluded because they did not develop contingency awareness (see Section 2.4), two women and two men because of excessive head movements, one man canceled the scanning session, one woman fell asleep during the task, and one woman was left-handed, which was an a priori exclusion criterion (assessed by the Edinburgh Inventory of Handedness; Oldfield, 1971). Further exclusion criteria covered standard fMRI exclusion criteria, somatic diseases, in particular endocrine diseases, history of psychiatric or neurological treatment, and regular medication usage except OCs. Women were required to have been taking their birth control pill (only monophasic preparations with an ethinylestradiol and a gestagenic component) for at least the last three months; we tested them during pill intake. Inclusion criteria comprised age between 18 and 35 and body mass index (BMI) between 18 and 28 kg/m^2.

All participants had normal or corrected vision. They received a detailed explanation of the general procedure; the conditioning schedule was not explained until the end. All participants gave written informed consent and received 10 Euros per hour for their attendance. All procedures were in accordance with the Declaration of Helsinki and approved by the university's local ethical review board.

2.2. Stress protocol, negative affect, salivary cortisol, and alpha-amylase

Men and women were randomly assigned to the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) or a non-stressful

control condition (Placebo-TSST; Het et al., 2009) resulting in 24 observations in each cell. The TSST was composed of a short preparation time (5 min), a free speech (5 min), and a subsequent challenging arithmetic task (serial subtraction of the number 17 from 2043; 5 min) in front of a very reserved acting panel (one man and one woman). The free speech and the arithmetic task were recorded and the participant could see this videotape during his/her performance at a large screen behind the committee. The Placebo-TSST also consisted of an oral presentation and an arithmetic task but lacked the stress-inducing components of the TSST (nobody else was present in the room during performance, no recording took place, and the task was less demanding; cf. Het et al., 2009).

We assessed the physiological stress response by measuring salivary cortisol as well as sAA, an indirect marker for noradrenergic activation (Nater and Rohleder, 2009). Individual sessions were scheduled between 2 and 5 p.m. to guarantee low and relatively stable endogenous cortisol concentrations. We instructed all participants to refrain from smoking, food intake, and drinking anything but water for at least 2 h before the start of the experiment. After arrival, they were given a resting phase of 45 min, in which participants filled out questionnaires on demographic variables as well as written informed consent concerning the stress protocol and the fMRI procedure. Further, they were informed about the course of the experiment (stress, saliva sampling, SCR measurement, application of electrical stimulation, fMRI) with the possibility to ask questions. After that, subjects were prepared for scanning and scanner-compatible glasses were prepared when needed. Then, the first saliva sample was taken (baseline) by means of Salivette collection devices (Sarstedt, Nümbrecht, Germany). After that, participants attended either the stress or the control condition after having received detailed instructions on the respective condition. Shortly afterward (second sample, +20 min), 10 min later (third sample, +30 min), and after fear conditioning (fourth sample, +60 min), they provided further saliva samples.

All samples were stored at −20 °C until assayed. A commercial available enzyme immunoassay (IBL International, Hamburg, Germany) was used to measure free cortisol concentrations. For analysis of sAA, a quantitative enzyme kinetic method was used as described in detail elsewhere (Rohleder and Nater, 2009). Intra-assay coefficients of variations were below 5% for cortisol and below 10% for sAA with an inter-assay coefficient of variation below 8% (cortisol), respectively 10% (sAA).

To gain a measure of negative affect, which might be also influenced by stress, participants completed the German version of the positive and negative affect scale (PANAS; Watson et al., 1988) parallel to each saliva sample. The PANAS consists of 20 adjectives, half of them measuring negative affect (e.g. upset) on a five point scale (ranging from 1: "very slightly or not at all" to 5: "extremely"). The ten negative items were averaged to a negative affect score for each of the four times of measurement.

We conducted analyses of variance (ANOVA) separately for cortisol, sAA, and negative affect including the repeated measurement factor time (first vs. second vs. third vs. fourth time of measurement) as well as the between subjects factors stress (stress vs. control) and sex (men vs. women). Statistical analyses were performed in IBM SPSS Statistics for Windows 21.0 with Greenhouse–Geisser correction and the statistical significance level was set to $\alpha = .05$.

2.3. Fear conditioning

Fear conditioning began 25 min after the TSST or the Placebo-TSST was finished. Two pictures of geometric figures (a rhomb and a square) served as CS+ and CS− (cf. Stark et al., 2006). Both figures had identical luminance, were gray-colored, and were presented against a black background for 8 s. Through a mirror mounted on the head coil, participants viewed the stimuli projected onto a screen at the end of the scanner (visual field = 18°) using an LCD projector (EPSON EMP-7250).

A custom-made impulse-generator (833 Hz) provided transcutaneous electrical stimulation (UCS; 100 ms) through two Ag/AgCl electrodes (1 mm^2 surface each) fixed to the middle of the left shin. Intensity was set individually using a gradually increasing rating procedure to be "unpleasant but not painful". The UCS started 7.9 s after CS+ onset (100% reinforcement). The CS− was never paired with the UCS. The UCS omission 7.9 s after CS− onset was defined as the non-UCS.

The experiment consisted of 42 trials (21 CS+ and 21 CS−), starting with a CS+ for half of the participants, with a CS− for the other half. Allocation of the two stimuli as CS+ was counterbalanced between participants. The first two trials (always a CS+ and CS−) were discarded from all analyses, because learning could not yet have occurred and to avoid orienting responses due to initial stimulus presentation. Each participant received a pseudo-randomized stimulus order with the following restrictions: no more than two consecutive presentations of the same CS and an equal quantity of CS+ and CS− trials within ten trials (five each). Inter-trial intervals (ITI) between two CS were randomly jittered (ITI duration: 9.75–14.25 s).

An early and a late phase of fear acquisition could be investigated reflecting the gradual development of fear learning and expression (cf. Schiller et al., 2008). Early and late phases were defined as the first (3rd to 22nd trial; 10 CS+ and 10 CS−) and the second halves (23rd to 42nd trial; 10 CS+ and 10 CS−) of the experiment.

No prior habituation phase was conducted to ensure the investigation of stress effects on fear acquisition during the cortisol peak.

2.4. Contingency awareness

Before fear conditioning, we instructed participants to attend to both geometrical figures and to watch out for regularities (cf. Schiller et al., 2008) in order to reduce differences in dependent variables due to contingency awareness (Tabbert et al., 2011). Immediately after conditioning, participants rated the contingencies between UCS and CS+ as well as CS−. Next to the picture of the respective CS, the question read always: "Please estimate how often the electrical stimulation succeeded the following geometrical figure"; with the answer to be chosen between "always", "sometimes", "never", or "I don't know". We classified participants as (at least partially) contingency aware if they stated higher probabilities for the UCS

occurrence after the CS+ than after the CS−. We also handed a forced choice questionnaire, in which one of the two CS had to be chosen as the stimulus preceding electrical stimulation.

Combinations for CS+ and CS− contingencies encompassed: always-never ($n = 89$); always-sometimes ($n = 1$); always-I don't know ($n = 2$); sometimes-never ($n = 3$); sometimes-I don't know ($n = 1$); sometimes-sometimes ($n = 2$). These last two participants also marked the wrong geometrical figure as CS+ in the forced choice questionnaire, consequently, they were excluded from the entire sample because of lacking contingency awareness. All of the other participants marked the right geometrical figure as CS+, thus, we declared the remaining sample ($n = 96$) was contingency aware.

Further, participants had to indicate after how many electrical stimulations they had noticed a relationship between UCS and the geometrical figures. Importantly, these subjective estimates revealed that all participants in the final sample discovered the CS−UCS contingency in early acquisition. This fact, together with the explicit instruction to watch out for a contingency between CS and UCS led us to analyze the CRs separately for early and late acquisition as before (e.g. Schiller et al., 2008); early acquisition mainly reflects contingency and fear learning, whereas late acquisition rather reflects fear expression.

2.5. Skin conductance responses (SCRs)

SCRs were sampled with an in-house built optical fiber SCR coupler concurrently with fMRI scans using Ag/AgCl electrodes filled with isotonic (0.05 M NaCl) electrolyte medium attached hypothenar at the left hand. Raw SCR data were low pass filtered with a cutoff frequency of 10 Hz. We defined SCRs in three analysis windows (cf. Prokasy and Ebel, 1967): the maximum amplitude 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. Data were transformed with the natural logarithm to attain a normal distribution. Electrodermal data of five participants had to be discarded because of technical issues or the complete absence of SCRs toward the CS as well as the UCS.

Statistical comparisons of SCRs were conducted in SPSS via ANOVA with the between subjects factors stress and sex. Separately for early and late acquisition, mean differential conditioned SCRs (CS+ minus CS− for the FIR and the SIR) were entered as dependent variables.

2.6. Image acquisition and analyses

Brain images were acquired using a 1.5 Twhole-body tomograph (Siemens Symphony with a quantum gradient system) with a standard head coil. Structural image acquisition comprised 160 T1-weighted sagittal images (MPRAGE, 1 mm slice thickness). For functional imaging, 348 volumes were registered using a T2*-weighted gradient echoplanar 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 pixel × 64 pixel). The first three volumes were discarded due to an incomplete steady state of magnetization. The axial slices were oriented parallel to the orbitofrontal cortex−bone transition to minimize susceptibility artifacts in prefrontal areas. A gradient echo field map sequence was measured before the functional run to get information for unwarping B_0 distortions.

All imaging data were analyzed using Statistical Parametric Mapping (SPM8; Wellcome Department of Cognitive Neurology, London, UK, 2009) implemented in MatLab R2007b (Mathworks Inc., Sherborn, MA). We included the following preprocessing steps: unwarping and realignment (2nd degree b-spline interpolation to the first volume), slice time correction (reference slice: 13), co-registration of functional data to each participant's anatomical image, segmentation into gray and white matter, normalization to the standard space of the Montreal Neurological Institute (MNI) brain, and spatial smoothing (isotropic 3D Gaussian filter; FWHM: 9 mm).

The statistical model for each participant included the following experimental conditions: CS+ (early and late), CS− (early and late), UCS, and non-UCS. An additional regressor was introduced containing the first two geometrical figures. All regressors were modeled by a stick function convolved with the canonical hemodynamic response function in the general linear model, without specifically modeling the durations of the different events (i.e. event-related design). The six movement parameters from the realignment step constituted covariates in the model. A high pass filter (time constant = 128 s) was implemented by using cosine functions in the design matrix.

The individual contrasts were analyzed in random effects group analyses and focused on the contrasts CS+ minus CS− separately for early and late acquisition. ANOVA was conducted with the group factors stress and sex in the full factorial model implemented in SPM8. In particular, we were interested in the interaction between stress and sex as well as the main effect of stress. For all statistical analyses, we used region of interest (ROI) analyses including the following ROI: amygdala, anterior cingulate gyrus, hippocampus, medial frontal cortex (MFC), and nucleus accumbens. We tested all ROI separately for the left and the right hemisphere except the anterior cingulate gyrus and the MFC. Thus, we tested a separate hypothesis for each ROI. The required masks for these analyses were maximum probability masks with the probability threshold set to 0.25 taken from the Harvard-Oxford Cortical and Subcortical Structural Atlases provided by the Harvard Center for Morphometric Analysis (http://www.cma.mgh.harvard.edu/fsl_atlas.html). Peak voxels were labeled using the SPM anatomy toolbox (Eickhoff et al., 2005), which permits the mapping of peak voxels to subregions of the ROI. However, results of this labeling procedure are included only for the sake of completeness and have to be interpreted with great caution because of the limited spatial resolution of data obtained with a 1.5 T magnetic field strength. The intensity threshold was set to $\alpha \leq .05$ uncorrected, the minimal cluster size was 5 voxels, and the significance threshold was set to $\alpha \leq .05$ on voxel-level, family-wise error (FWE) corrected (using the small volume correction options of SPM8). Besides, we also conducted exploratory whole brain analyses (intensity

threshold: $\alpha \leq .05$ FWE-corrected; $k = 10$ voxels; significance threshold: $\alpha \leq .05$ on voxel-level, FWE-corrected).

3. Results

3.1. Sample description

Age of the final sample ranged from 19 to 33 years ($M = 23.22$, $SD = 2.87$) and BMI from 18 to 28 kg/m^2 ($M = 22.32$, $SD = 2.22$). ANOVA with the between subjects factors stress and sex did not reveal any significant main or interaction effects concerning BMI (all $F_{(1;92)} < 3.56$; $p > .062$) or age (all $F_{(1;92)} < 2.05$; $p > .15$) with the exception that men overall ($M = 24.33$, $SD = 3.16$) were slightly older than women ($M = 22.10$, $SD = 2.02$; $F_{(1;92)} = 17.06$; $p < .001$).

3.2. Stress induction

Salivary cortisol increased in the stress compared to the control condition over time in men and women differently (main effects: time [$F_{(2.28;209.98)} = 33.73$; $p < .001$], stress [$F_{(1;92)} = 20.88$; $p < .001$], sex [$F_{(1;92)} = 23.16$; $p < .001$]; interactions: time x stress [$F_{(2.28;209.98)} = 51.53$; $p < .001$], time $\times$ sex [$F_{(2.28;209.98)} = 19.55$; $p < .001$], stress $\times$ sex [$F_{(1;92)} = 7.08$; $p = .009$], time $\times$ stress $\times$ sex [$F_{(2.28;209.98)} = 16.67$; $p < .001$]; Fig. 1A). In men, post hoc t-tests indicated that cortisol concentrations were significantly higher in the stress in comparison to the control group in the second, third, and fourth sample (all $T_{(46)} > 2.97$; $p \leq .002$; no differences at baseline [$T_{(46)} = 1.09$; $p = .28$]). In women, salivary cortisol increased after stress compared to the control condition in the third and fourth sample (both $T_{(46)} > 3.26$; all $p \leq .005$; no differences at baseline and in the second sample [both $T_{(46)} < 1.11$; $p > .27$]). In addition, the stressor led to significantly higher cortisol increases (compared to baseline) in stressed men compared to stressed women in the second, third, and fourth sample (all $T_{(46)} > 3.00$; $p \leq .005$). No significant sex differences were found in the control group (all $T_{(46)} < 2.00$; $p > .051$).

For sAA, the stressor elicited higher levels in the stress compared to the control condition over time (main effect: time [$F_{(2.65;241.36)} = 7.32$; $p < .001$]; interaction: time - $\times$ stress [$F_{(2.65;241.36)} = 4.38$; $p = .007$]; Fig. 1B); no other main (all $F_{(1,91)} < 0.67$; $p > .41$) or interaction effects were found (all $F_{(2.65;241.36)} < 1.30$; $p > .27$). Post hoc t-tests in both sexes combined revealed that sAA concentrations compared to baseline were higher in the stress compared to the control group immediately after stress ($T_{(94)} = 3.17$; $p = .002$) and before fear conditioning ($T_{(94)} = 2.75$; $p = .007$), but not after fear conditioning ($T_{(93)} = 1.47$; $p = .15$).

Negative affect was affected over the time of the experiment and as a function of stress (main effects time [$F_{(2.77,254.95)} = 15.43$; $p < .001$]; stress [$F_{(2.77,254.95)} = 4.75$; $p = .032$]; interaction: time $\times$ stress [$F_{(2.77,254.95)} = 27.01$; $p < .001$]), but no interaction with sex occurred (all $F_{(2.77,254.95)} < 1.33$; $p > .26$). Post hoc t-tests in both sexes combined revealed that negative affect was rated significantly higher only immediately after stress ($M = 1.66$; $SD = 0.07$) compared to the control condition ($M = 1.20$; $SD = 0.03$; $T_{(94)} = 6.06$; $p < .001$), but not at baseline or before or after fear conditioning (all $T_{(94)} < 0.99$; $p > .32$).

3.3. Differential skin conductance responses (SCRs)

Differential CRs (i.e. main effect CS-type) were found in the entire group in the FIR (early acquisition: $F_{(1;87)} = 80.44$; $p < .001$; late acquisition: $F_{(1;87)} = 27.49$; $p < .001$; Fig. 2A) and SIR (early acquisition: $F_{(1;87)} = 50.30$; $p < .001$; late acquisition: $F_{(1;87)} = 28.33$; $p < .001$; Fig. 2B). Additionally, stress differentially modulated fear CRs in men and women in the SIR during early acquisition (interaction: stress $\times$ sex [$F_{(1;87)} = 4.73$; $p = .032$]; Fig. 2B). Post hoc t-tests indicated that the stressor compared to the control condition significantly reduced conditioned SCRs in men ($T_{(43)} = 2.75$; $p = .009$), more precisely the response to the CS+ ($T_{(43)} = 2.10$; $p = .040$), but not to the CS− ($T_{(43)} = 0.56$; $p = .58$); no differences emerged in women (all $T_{(44)} < 1.02$; $p > .31$). No other main or interaction effects were observed (all $F_{(1,87)} < 2.44$; $p > .12$).

Since OC women displayed lowered cortisol responses compared to men (see Section 3.1), it could be argued that the stress $\times$ sex interaction results from different cortisol concentrations. To exclude this possibility, we included the covariate area under the curve with respect to ground (AUC_G; cf. Prüssner et al., 2003) as a measure of total cortisol output in the ANOVA. After adjusting the results for differential cortisol responses in men and OC women, the stress x sex interaction effect ($F_{(1;86)} = 3.06$; $p = .084$) and the stress effect in men ($F_{(1;42)} = 4.16$; $p = .048$) in early acquisition only dropped slightly.

To gain more insight into the stress x sex interaction in early acquisition of the SIR, we correlated stress-induced cortisol increases (third sample minus baseline; cf. Kirschbaum et al., 1995) with mean differential conditioned SCRs in men and women separately. In contrast to the AUC_G, cortisol increase is independent of baseline concentrations and a change over time is pronounced. No significant associations emerged in women ($r = -.14$; $p = .37$), but in men, a significant negative association was found ($r = -.34$; $p = .021$), which did not reach statistical significance in the control or stress group separately (both $r < -.15$; $p > .48$).

3.4. Differential neuronal activation

In early acquisition, the contrast CS+ minus CS− revealed a significant stress x sex interaction in the right nucleus accumbens ($p_{corr} = .036$; Table 1A, Fig. 3A): Stress impaired the activation of the nucleus accumbens in men but facilitated it in women. Independent of sex, stress compared to the control condition increased fear learning bilaterally in the hippocampus (cornu ammonis; main effect: stress; left: $p_{corr} = .029$; right: $p_{corr} = .027$; Table 1A; Fig. 4A). After inclusion of the covariate AUC_G, the stress effect in the right hippocampus only remained significant ($x = 33$; $y = -34$; $z = -5$; $T_{max} = 3.26$; $p_{corr} = .040$).

To have a closer look at the interaction, a correlation between stress-induced cortisol increases and differential neuronal activation in the nucleus accumbens was conducted in men and women separately. Cortisol increases were significantly negatively correlated with the contrast CS+ minus CS− in all men ($x = 12$; $y = 17$; $z = -8$; $T_{max} = 3.39$; $p_{corr} = .013$). This effect decreased to a statistical trend, when the control ($x = 6$; $y = 8$; $z = -5$; $T_{max} = 2.70$; $p_{corr} = .078$)

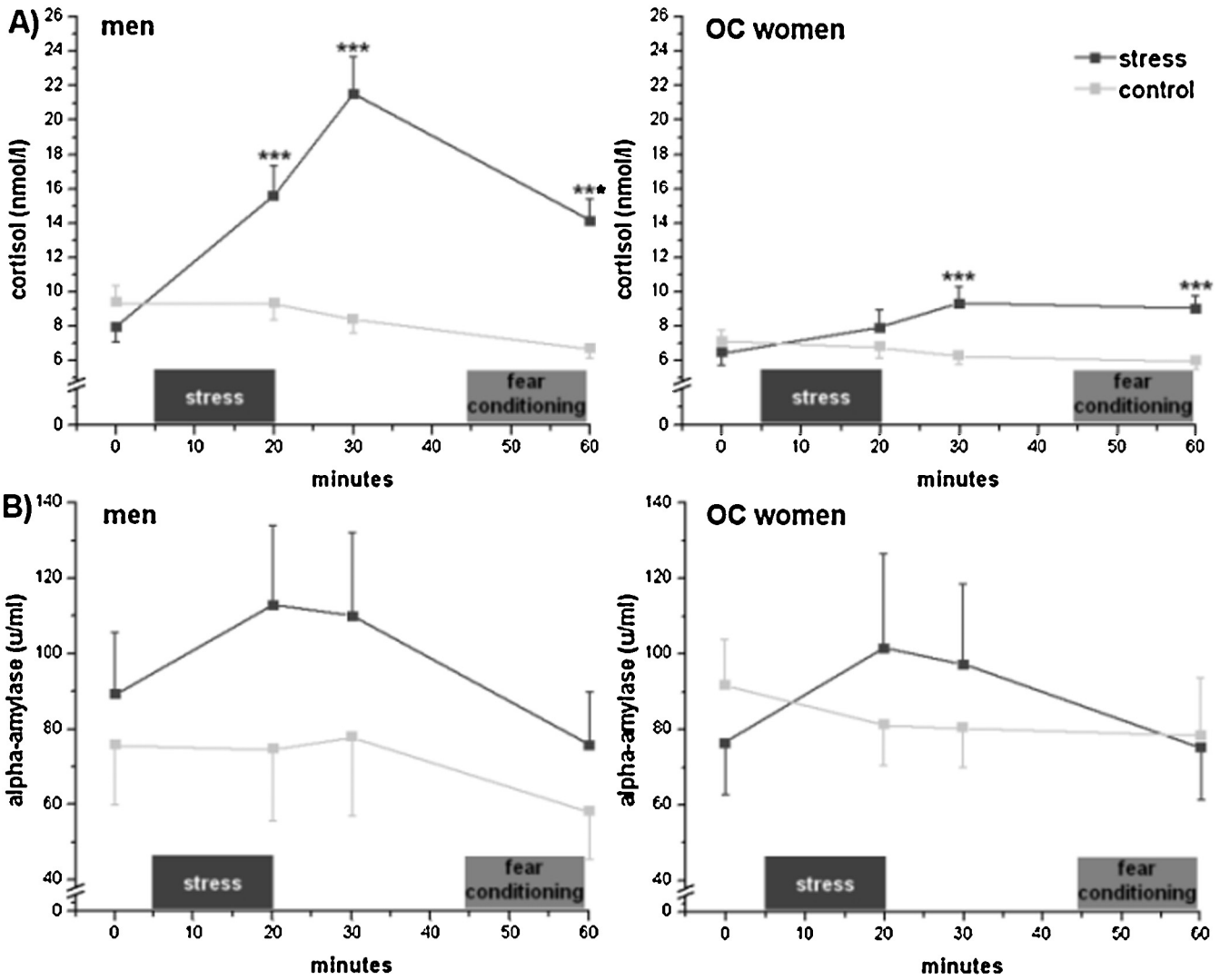

Figure 1 (A) Cortisol responses in the stress or the control condition are displayed for men (left) and OC women (right). (B) Concentrations of the enzyme alpha-amylase are shown for men (left) and OC women (right). Error bars display standard errors of the mean. Both stress indicators revealed that the psychosocial stressor evoked a stress response in both men and OC women. ***$p \leq .005$. During each of the four times of measurement, cortisol, sAA, and negative affect were assessed. Between psychosocial stress and the fear conditioning protocol, all relevant electrodes were attached. Fear conditioning consisted of pairings between a picture of a geometrical figure (CS+) with an electrical stimulation as UCS, whereas another picture was not paired (CS−). During the course of fear conditioning, BOLD responses and SCRs were measured in parallel.

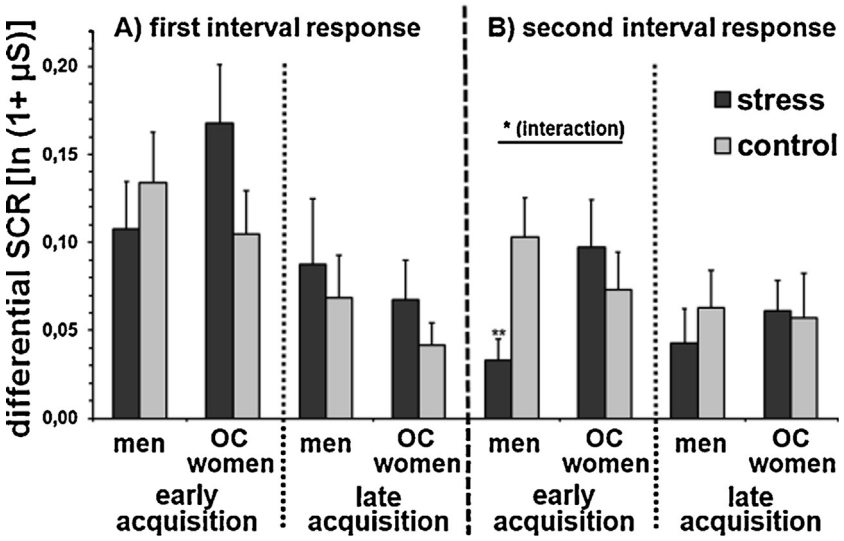

Figure 2 Mean differential (CS+ minus CS−) skin conductance responses (SCRs; transformed with the natural logarithm) for the first (A) and second interval response (B) are shown separately for early and late acquisition in men and OC women. Error bars are standard errors of the mean. Significant conditioned responses were found in men and OC women in early and late acquisition as well as both experimental conditions. Additionally, in early acquisition of the second interval response, a significant stress × sex interaction occurred. *$p < .05$; **$p < .01$ (compared to control men).

and the stressed male group ($x = 12$; $y = 14$; $z = -8$; $T_{max} = 2.79$; $p_{corr} = .065$) were considered separately. In women, no significant correlations between cortisol increases and differential neuronal activation in the right nucleus accumbens were found.

In late acquisition, the stress x sex interaction showed that stress significantly influenced the CS+/CS− differentiation in the left amygdala ($p_{corr} = .025$; superficial complex) and in the anterior cingulate ($p_{corr} = .035$; middle cingulate cortex as indexed by the SPM anatomy toolbox), again in a sex-dependent fashion (Table 1B, Fig. 3B): Whereas stress led to an attenuated neuronal activation in men, stressed women exhibited larger differential responses in these structures. In addition to these sex-dependent effects, sex-independent effects were found in the MFC ($p_{corr} = .043$; rectal gyrus). Here, controls relative to stressed participants exhibited more differential activation (main effect: stress; Table 1B, Fig. 4B). Effects in the left amygdala ($x = -15$; $y = -4$; $z = -20$; $T_{max} = 3.32$; $p_{corr} = .015$) and MFC ($x = -3$; $y = 50$; $z = -23$; $T_{max} = 3.20$; $p_{corr} = .043$) remained significant even when the covariate AUCG was included, whereas the interaction effect in the anterior cingulate decreased to a statistical trend ($x = 0$; $y = -4$; $z = 31$; $T_{max} = 3.32$; $p_{corr} = .073$).

Correlation analyses between stress-induced cortisol increases and the anterior cingulate as well as the left amygdala did not reveal any significant associations, neither in men, nor in women.

Exploratory whole brain analyses did not reveal any significant effect in each sub-analysis. Non-significant results concerning ROI testing can be found in Supplementary Table S1.

Table 1 Localization and statistics of the peak voxels for the interaction stress × sex and the comparison between stress and control condition (contrast CS+ minus CS−) in the first half of the experiment (A) and the second half of the experiment (B).

	Brain structure	x	y	z	T_{max}	p_{corr}
(A) First half						
Stress × sex	R nucleus accumbens	9	8	−11	2.81	.036
Control−stress	No significant activations					
Stress−control	L hippocampus	−30	−37	−2	3.56	.029
	R hippocampus	30	−34	−2	3.57	.027
(B) Second half						
Stress × sex	Anterior cingulate gyrus	−3	−4	31	3.81	.035
	L amygdala	−15	−4	−20	3.32	.025
Control−stress	Medial frontal cortex	0	50	−23	3.44	.043
Stress−control	No significant activations					

The significance threshold was $\alpha \leq .05$ (FWE-corrected; small volume correction). All coordinates (x, y, z) are given in MNI space. L: left, R: right.

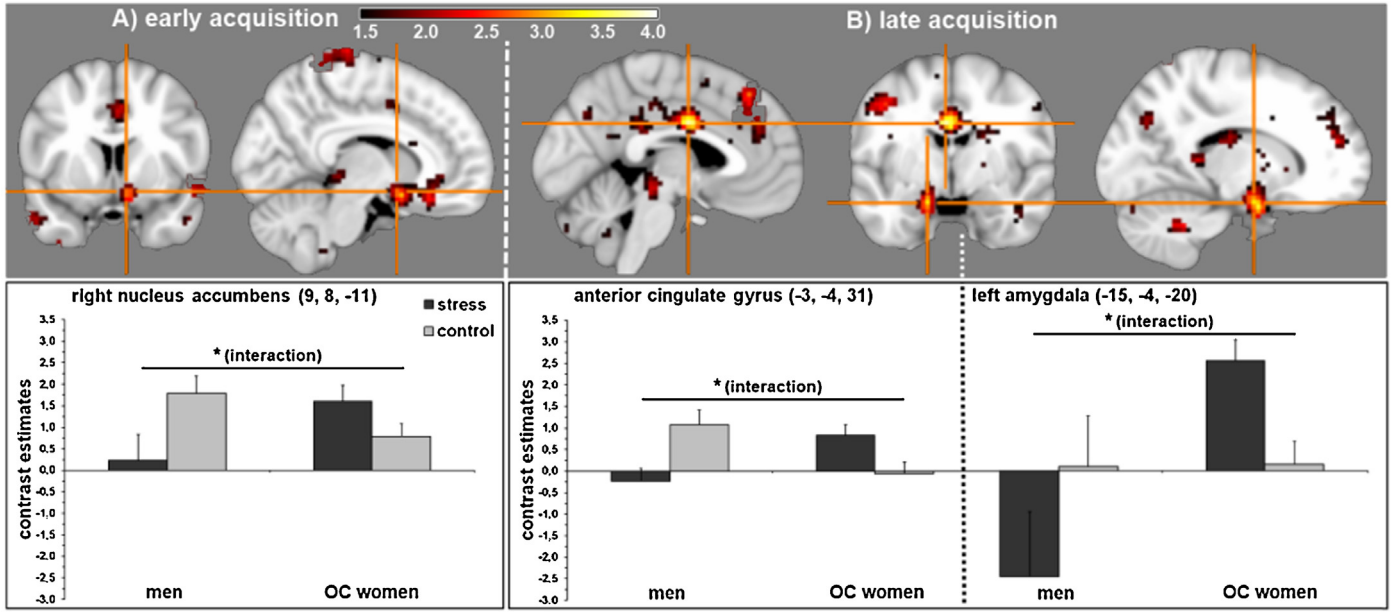

Figure 3 Neuronal activations (contrast CS+ minus CS−) for the stress × sex interaction are shown separately for early (A) and late acquisition (B). The depicted coronal and sagittal slices were selected according to the reported activation in the right nucleus accumbens, the anterior cingulate gyrus, and the left amygdala. For demonstration purposes, data were thresholded with $T \geq 1.5$ (see color bar for exact T values) and displayed on the standard MNI brain template. In the bar graphs mean differential contrast estimates (CS+ minus CS−) are additionally given for the stress and the control group in the respective peak voxel separately for men and OC women. Error bars display standard errors of the mean. Stress significantly attenuated the CS+/CS− differentiation in men, but enhanced it in OC women. $^{*}p_{corr} < .05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

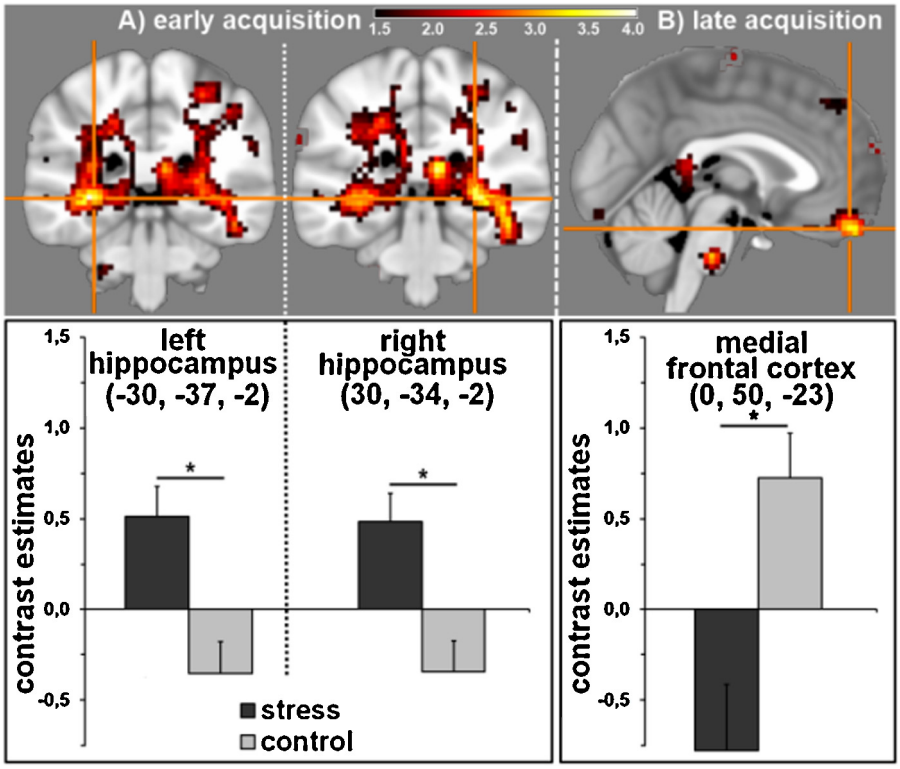

Figure 4 Neuronal activations (contrast CS+ minus CS−) for the main effect stress are shown separately for early (A) and late acquisition (B). The depicted coronal and sagittal slices were selected according to the reported activation in the left and right hippocampus and the medial frontal cortex. For demonstration purposes, data were thresholded with $T \geq 1.5$ (see color bar for exact T values) and displayed on the standard MNI brain template. In the bar graphs, mean differential contrast estimates (CS+ minus CS−) are additionally given for the stress and the control group in the respective peak voxel. Error bars are standard errors of the mean. Stress significantly increased the CS+/CS− differentiation in the hippocampus, but reduced it in the medial frontal cortex. *$p_{corr} < .05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2013.05.015.

4. Discussion

This neuroimaging study investigated acute effects of psychosocial stress on subsequent fear conditioning. Our results indicate that stress differentially influences correlates of fear conditioning in men and women. Most importantly, the stressor impaired fear conditioned responses in the nucleus accumbens, the amygdala, and the anterior cingulate in men, but enhanced the neuronal differentiation in women. Sex-independently, stress heightened the CS+/CS− differentiation in the hippocampus, but attenuated CRs in the MFC.

First of all, our stress induction was successful as indicated by significantly elevated cortisol and sAA concentrations as well as negative affect in men and women in the stress condition. As could be expected, women showed a less pronounced cortisol increase than men, most likely due to the fact that we tested women using OCs. In response to psychosocial stress, OC women show diminished free cortisol concentrations, because OCs enhance cortisol-binding globulin, thus attenuating free cortisol (e.g. Kirschbaum et al., 1999). Nevertheless and in contrast to previous studies (Jackson et al., 2006), cortisol concentrations were substantially higher during fear conditioning in the stress compared to the control group in both men and OC women.

In early acquisition, psychosocial stress attenuated fear CRs in the nucleus accumbens (and differential SCRs in the SIR) in men, whereas the stressor facilitated it in women. This structure has been implicated in the development of contingency awareness (Klucken et al., 2009). Since all participants noticed the CS−UCS relationship in early acquisition (because of explicit instructions and the 100% reinforcement schedule, also reflected in the contingency ratings), it is very reasonable that the nucleus accumbens is involved in this initial part of the conditioning procedure. Stress reduced the recruitment of the nucleus accumbens in men (in the group as well as in the correlation analysis; also mirrored in the differential SCRs in the SIR), potentially pointing to a less rapid acquisition of the fear response on the neuronal level, but not on the subjective level (as indexed by the contingency ratings). More generally, the nucleus accumbens, as part of the striatum, is associated with habitual responding (Graybiel, 2008). A stress-induced shift to activation of the striatum has been proposed to reflect maladaptive recruitment of habitual actions as can be seen in drug abuse and addiction (Schwabe et al., 2011). These disorders are characterized by associative learning processes such as classical

as well as instrumental conditioning (Everitt and Robbins, 2005). The present study might extend this view in underlining not only the relevant vulnerability factors stress and conditioning processes, but also potential sex differences in the response of striatum-based learning mechanisms to stress.

The same sex-dependent stress effects emerged also in late acquisition of the experiment in the amygdala and the anterior cingulate. Accumulating evidence indicates that both structures are related to fear learning and expression (e.g. LeDoux, 2000; Mechias et al., 2010). In particular, the anterior cingulate is involved in anticipation of threat and aware fear conditioning (e.g. Büchel et al., 1998; Mechias et al., 2010), which is reflected in late acquisition of the current experiment. Besides, the anterior cingulate is also involved in pain modulation (e.g. Rainville, 2002), which integrates cognitive and emotional aspects of the fear conditioning design as well. Previous studies from our laboratory have already observed cortisol facilitating fear CRs in the anterior cingulate in OC women, but impairing differentiation in men (Stark et al., 2006; Tabbert et al., 2010). The present study confirms these previous pharmacological results and extends them to physiological stress-induced cortisol concentrations.

Further, the fear module centers around the amygdala (for a review: LeDoux, 2000), which orchestrates the stress response in activating the HPA axis (for a review: Rodrigues et al., 2009). High densities of stress, but also of sex hormone receptors exist in the amygdala, the anterior cingulate and the nucleus accumbens (for reviews: Östlund et al., 2003; Joels et al., 2011), thus enabling an interplay of sex and stress hormones in these regions. In the current paradigm, sex differences in fear processing following psychosocial stress emerged, particularly, differential amygdala responses were impaired in men, but facilitated in OC women. In men, acute cortisol administration led to reduced amygdala activation toward emotional input (Henckens et al., 2010), supporting a guardian function of glucocorticoids. Accordingly, hydrocortisone attenuated amygdala activation in resting participants (Lovallo et al., 2010). Besides, cortisol inhibited pathological fear (which implicates an exaggerated amygdala response) in spider and social phobia (Soravia et al., 2006) as well as phobia for heights (De Quervain et al., 2011) in men and women. However, information on OC intake or menstrual cycle status is not given in these publications. Our current results, together with our previous pharmacological studies (Stark et al., 2006; Merz et al., 2012b; Tabbert et al., 2010), indicate that stress or cortisol administration increase fear conditioning in OC women. This stress-induced mechanism might pave the way to exaggerated fear in OC women experiencing stress. A look into fear extinction reveals that OC women exhibit heightened responses in the neuronal extinction circuit, which might make them also more vulnerable to develop and maintain excessive fear (cf. Merz et al., 2012a; Graham and Milad, 2013). All in all, the critical impact of sex hormones on emotional learning must not be neglected, particularly regarding their implication in psychiatric disorders (Lebron-Milad and Milad, 2012; Lebron-Milad et al., 2012). Whether the effects of stress and sex hormones on fear learning translate into pathological fear in OC women should be elucidated in future clinical/epidemiological studies. More precisely, it has to be determined if OC women, free-cycling women and men differ from each other in terms of prevalence, maintenance and treatment of psychiatric disorders. For example, exposure therapy might be more successful in specific phases of the menstrual cycle. Besides, the differential influence of acute and chronic stress on persons with varying sex hormone status needs to be explored in more detail, both in clinical practice as well as in basic research. For example, the interplay between sex hormones and stress hormones affecting extinction learning, consolidation and recall has been largely neglected.

It has to be mentioned that stress induction led to different conditioning findings in rodents (for a review: Dalla and Shors, 2009) as well as humans (at the electrodermal level: Jackson et al., 2006; Zorawski et al., 2006). These studies related stress to increased conditioning in males and to impaired CRs in females. Along with important methodological differences (e.g. single cue vs. differential conditioning; eye-blink vs. context vs. fear conditioning; neutral vs. biological salient CS, cf. Merz et al., 2010, 2012a), OC usage and menstrual cycle were not controlled for in the mentioned human studies.

Nonetheless, our current results in men correspond to preceding experiments exploring stress effects on eyeblink conditioning and conditional discrimination learning in humans (Wolf et al., 2009, 2012). Besides, pharmacological studies using 30 mg hydrocortisone also consistently revealed attenuated conditioned neuronal activation in men and enhanced CRs in OC women (Stark et al., 2006; Merz et al., 2010, 2012b; Tabbert et al., 2010). But administration of 30 mg hydrocortisone evokes supraphysiological cortisol concentrations and only mimics activation of the HPA axis, but not of the SNS. The use of a psychosocial stressor is more appropriate to investigate real-life circumstances and the concerted action of both stress axes. The highly similar results obtained with pharmacological cortisol administration and stress-induced cortisol elevations support the notion that the effects observed in the current study indeed reflect the impact of cortisol on the central nervous system and are not secondary to stress-induced changes in affect or in other central neurotransmitters (e.g. CRH).

At first sight, it seems problematic that OC women did not display the same salivary cortisol response to stress compared to men, because the stressor might not be equally effective in men and OC women. However, sAA concentrations as well as negative affect were equally affected. Besides, the altered pattern in salivary cortisol was frequently observed in OC women (e.g. Kirschbaum et al., 1999) and should therefore closely match real-life situations. Apparently, our current data (also reflected in the results after inclusion of the AUC_G as a measure of total cortisol concentrations) together with those using 30 mg hydrocortisone (Stark et al., 2006; Merz et al., 2010, 2012b; Tabbert et al., 2010) suggest that the exact amount of cortisol increase (different rises in men and OC women after stress, but comparable after cortisol administration) is not the driver of the interaction effects, but rather OC intake per se. This assumption should be tested in future dose—response experiments.

Sex-independently, stress facilitated the CS+/CS− differentiation in the hippocampus in early acquisition, but

attenuated CRs in the MFC in late acquisition. These two structures host a high density of stress hormone receptors (e.g. Joels et al., 2011) and have been crucially implicated in the regulation of conditioned fear (e.g. Shin and Liberzon, 2010). Notably, stress hormones reduced activation of the hippocampus and medial prefrontal areas in rodents and healthy humans (e.g. Oei et al., 2007; for reviews: Kim and Diamond, 2002; Wolf, 2008). Our findings of enhanced hippocampal differentiation after acute stress contradict these reports; however, these previous studies tested effects of stress hormones on hippocampus activation during memory retrieval. In contrast, fear conditioning refers to the memory stage of encoding. Previously, psychosocial stress led to enhanced hippocampal activation during encoding of a working memory task, but to reduced hippocampal responses during retrieval (Weerda et al., 2010). These results support the temporal dynamics model of emotional memory processing (Diamond et al., 2007) proposing an initial enhancement of hippocampal functioning after stress, which later switches to an impairing mode. In contrast, medial prefrontal areas are inhibited during high cortisol concentrations (e.g. Oei et al., 2007), which is in line with our current results. A hypoactive medial prefrontal cortex is assumed to play a role in the pathogenesis of PTSD, potentially resulting from traumatic stress experience or reflecting a predisposing factor increasing the risk for certain individuals to develop a psychiatric disorder (Milad et al., 2009; for a review: Shin and Liberzon, 2010). In the present study, acute stress attenuated regulatory MFC activation; together with heightened BOLD responses in the anterior cingulate and in the amygdala a critical pathway might be open to exaggerated fear in OC women, but not in men.

In addition to cortisol, the central effects of CRH should be mentioned as well. CRH is produced in the paraventricular nucleus (PVN) of the hypothalamus and the amygdala and both regions also express CRH receptors. Previously, it has been shown that estrogens activate, whereas androgens inhibit CRH transcription (Bao et al., 2005, 2006; Bao and Swaab, 2011). Consequently, the results of the present study as well as our pharmacological results (Stark et al., 2006; Merz et al., 2010, 2012b; Tabbert et al., 2010) could partly reflect the involvement of CRH neurons on sex differences after heightened cortisol concentrations. It should be noted that at present, fMRI is not capable of measuring reliably activation of the PVN, therefore we refrained from including this further ROI. A further limiting factor is that it remains to be shown if the current results in OC women after psychosocial stress can be replicated in free-cycling women. According to our previous report regarding cortisol effects on fear conditioning (Merz et al., 2012a,b), we speculate that free-cycling women most likely exhibit reduced CRs after stress, i.e. a pattern similar to men.

In conclusion, our data demonstrate that exposure to the same stressor can have opposing effects on the neuronal and electrodermal correlates of fear learning in men and women using OCs. This might contribute to the higher prevalence of several anxiety disorders in women compared to men. The respective symptomatologies are connected to the formation of conditioned fear during acute stress. Future studies are necessary to investigate the role of sex hormones, in particular of OCs, on the effect of psychosocial stress on fear conditioning. Such studies may foster our understanding of the factors underlying sex differences in the prevalence of anxiety disorders.

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Conflict of interest

All authors declare no conflict of interest.

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References

Bao, A.M., Swaab, D.F., 2011. Sexual differentiation of the human brain: relation to gender identity, sexual orientation and neuropsychiatric disorders. Front. Neuroendocrinol. 32, 214–226.

Bao, A.M., Hestiantoro, A., van Someren, E.J., Swaab, D.F., Zhou, J.N., 2005. Colocalization of corticotropin-releasing hormone and oestrogen receptor-alpha in the paraventricular nucleus of the hypothalamus in mood disorders. Brain 128, 1301–1313.

Bao, A.M., Fischer, D.F., Wu, Y.H., Hol, E.M., Balesar, R., Unmehopa, U.A., Zhou, J.N., Swaab, D.F., 2006. A direct androgenic involvement in the expression of human corticotropin-releasing hormone. Mol. Psychiatry 11, 567–576.

Bonne, O., Grillon, C., Vythilingam, M., Neumeister, A., Charney, D.S., 2004. Adaptive and maladaptive psychobiological responses to severe psychological stress: implications for the discovery of novel pharmacotherapy. Neurosci. Biobehav. Rev. 28, 65–94.

Büchel, C., Morris, J.S., Dolan, R.J., Friston, K.J., 1998. Brain systems mediating aversive conditioning: an event-related fMRI study. Neuron 20, 947–957.

Dalla, C., Shors, T.J., 2009. Sex differences in learning processes of classical and operant conditioning. Physiol. Behav. 97, 229–238.

De Quervain, D.J.-F., Bentz, D., Michael, T., Bolt, O.C., Wiederhold, B.K., Margraf, J., Wilhelm, F.H., 2011. Glucocorticoids enhance extinction-based psychotherapy. Proc. Natl. Acad. Sci. U.S.A. 108, 6621–6625.

Diamond, D.M., Campbell, A.M., Park, C.R., Halonen, J., Zoladz, P.R., 2007. The temporal dynamics model of emotional memory processing: a synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes–Dodson law. Neural Plast., http://dx.doi.org/10.1155/2007/60803.

Eickhoff, S.B., Stephan, K.E., Mohlberg, H., Grefkes, C., Fink, G.R., Amunts, K., Zilles, K., 2005. A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. NeuroImage 25, 1325—1335.

Everitt, B.J., Robbins, T.W., 2005. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat. Neurosci. 8, 1481—1489.

Graham, B.M., Milad, M.R., 2013. Blockade of estrogen by hormonal contraceptives impairs fear extinction in female rats and women. Biol. Psychiatry 73, 371—378.

Graybiel, A.M., 2008. Habits, rituals, and the evaluative brain. Annu. Rev. Neurosci. 31, 359—387.

Henckens, M.J.A.G., van Wingen, G.A., Joëls, M., Fernandez, G., 2010. Time-dependent effects of corticosteroids on human amygdala processing. J. Neurosci. 30, 12725—12732.

Het, S., Rohleder, N., Schoofs, D., Kirschbaum, C., Wolf, O.T., 2009. Neuroendocrine and psychometric evaluation of a placebo version of the 'Trier Social Stress Test'. Psychoneuroendocrinology 34, 1075—1086.

Jackson, E.D., Payne, J.D., Nadel, L., Jacobs, W.J., 2006. Stress differentially modulates fear conditioning in healthy men and women. Biol. Psychiatry 59, 516—522.

Joels, M., Fernandez, G., Roozendaal, B., 2011. Stress and emotional memory: a matter of timing. Trends Cogn. Sci. 15, 280—288.

Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R., Walters, E.E., 2005. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. Arch. Gen. Psychiatry 62, 593—602.

Kim, J.J., Diamond, D.M., 2002. The stressed hippocampus, synaptic plasticity and lost memories. Nat. Rev. Neurosci. 3, 453—462.

Kirschbaum, C., Pirke, K.-M., Hellhammer, D.H., 1993. The 'Trier Social Stress Test' — a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28, 76—81.

Kirschbaum, C., Prüssner, J.C., Stone, A.A., Federenko, I., Gaab, J., Lintz, D., Schommer, N., Hellhammer, D.H., 1995. Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men. Psychosom. Med. 57, 468—474.

Kirschbaum, C., Kudielka, B.M., Gaab, J., Schommer, N.C., Hellhammer, D.H., 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus—pituitary—adrenal axis. Psychosom. Med. 61, 154—162.

Klucken, T., Tabbert, K., Schweckendiek, J., Merz, C.J., Kagerer, S., Vaitl, D., Stark, R., 2009. Contingency learning in human fear conditioning involves the ventral striatum. Hum. Brain Mapp. 30, 3636—3644.

Lebron-Milad, K., Milad, M.R., 2012. Sex differences, gonadal hormones and the fear extinction network: implications for anxiety disorders. Biol. Mood Anx. Dis. 2, 3.

Lebron-Milad, K., Graham, B.M., Milad, M.R., 2012. Low estradiol levels: a vulnerability factor for the development of posttraumatic stress disorder. Biol. Psychiatry 72, 6—7.

LeDoux, J.E., 2000. Emotion circuits in the brain. Annu. Rev. Neurosci. 23, 155—184.

Lovallo, W.R., Robinson, J.L., Glahn, D.C., Fox, P.T., 2010. Acute effects of hydrocortisone on the human brain: an fMRI study. Psychoneuroendocrinology 35, 15—20.

Mechias, M.-L., Etkin, A., Kalisch, R., 2010. A meta-analysis of instructed fear studies: implications for conscious appraisal of threat. NeuroImage 49, 1760—1768.

Merz, C.J., Tabbert, K., Schweckendiek, J., Klucken, T., Vaitl, D., Stark, R., Wolf, O.T., 2010. Investigating the impact of sex and cortisol on implicit fear conditioning with fMRI. Psychoneuroendocrinology 35, 33—46.

Merz, C.J., Tabbert, K., Schweckendiek, J., Klucken, T., Vaitl, D., Stark, R., Wolf, O.T., 2012a. Neuronal correlates of extinction learning are modulated by sex hormones. Soc. Cogn. Affect. Neurosci. 7, 819—830.

Merz, C.J., Tabbert, K., Schweckendiek, J., Klucken, T., Vaitl, D., Stark, R., Wolf, O.T., 2012b. Oral contraceptive usage alters the effects of cortisol on implicit fear learning. Horm. Behav. 62, 531—538.

Milad, M.R., Pitman, R.K., Ellis, C.B., Gold, A.L., Shin, L.M., Lasko, N.B., Zeidan, M.A., Handwerger, K., Orr, S.P., Rauch, S.L., 2009. Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. Biol. Psychiatry 66, 1075—1082.

Nater, U.M., Rohleder, N., 2009. Salivary alpha-amylase as a noninvasive biomarker for the sympathetic nervous system: current state of research. Psychoneuroendocrinology 34, 486—496.

Oei, N.Y.L., Elzinga, B.M., Wolf, O.T., de Ruiter, M.B., Damoiseaux, J.S., Kuijer, J.P.A., Veltman, D.J., Scheltens, P., Rombouts, S.A.R.B., 2007. Glucocorticoids decrease hippocampal and prefrontal activation during declarative memory retrieval in young men. Brain Imaging Behav. 1, 31—41.

Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97—113.

Östlund, H., Keller, E., Hurd, Y.L., 2003. Estrogen receptor gene expression in relation to neuropsychiatric disorders. Ann. N. Y. Acad. Sci. 1007, 54—63.

Prokasy, W.F., Ebel, H.C., 1967. Three components of the classically conditioned GSR in human subjects. J. Exp. Psychol. 73, 247—256.

Prüssner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28, 916—931.

Rainville, P., 2002. Brain mechanisms of pain affect and pain modulation. Curr. Opin. Neurobiol. 12, 195—204.

Rodrigues, S.M., LeDoux, J.E., Sapolsky, R.M., 2009. The influence of stress hormones on fear circuitry. Annu. Rev. Neurosci. 32, 289—313.

Rohleder, N., Nater, U.M., 2009. Determinants of salivary alpha-amylase in humans and methodological considerations. Psychoneuroendocrinology 34, 469—485.

Schiller, D., Levy, I., Niv, Y., LeDoux, J.E., Phelps, E.A., 2008. From fear to safety and back: reversal of fear in the human brain. J. Neurosci. 28, 11517—11525.

Schwabe, L., Dickinson, A., Wolf, O.T., 2011. Stress, habits, and drug addiction: a psychoneuroendocrinological perspective. Exp. Clin. Psychopharmacol. 19, 53—63.

Shin, L.M., Liberzon, I., 2010. The neurocircuitry of fear, stress, and anxiety disorders. Neuropsychopharmacology 35, 169—191.

Soravia, L.M., Heinrichs, M., Aerni, A., Maroni, C., Schelling, G., Ehlert, U., Roozendaal, B., de Quervain, D.J.-F., 2006. Glucocorticoids reduce phobic fear in humans. Proc. Natl. Acad. Sci. U.S.A. 103, 5585—5590.

Sotres-Bayon, F., Quirk, G.J., 2010. Prefrontal control of fear: more than just extinction. Curr. Opin. Neurobiol. 20, 1—5.

Stark, R., Wolf, O.T., Tabbert, K., Kagerer, S., Zimmermann, M., Kirsch, P., Schienle, A., Vaitl, D., 2006. Influence of the stress hormone cortisol on fear conditioning in humans: evidence for sex differences in the response of the prefrontal cortex. NeuroImage 32, 1290—1298.

Tabbert, K., Merz, C.J., Klucken, T., Schweckendiek, J., Vaitl, D., Wolf, O.T., Stark, R., 2010. Cortisol enhances neural differentiation during fear acquisition and extinction in contingency aware young women. Neurobiol. Learn. Mem. 94, 392—401.

Tabbert, K., Merz, C.J., Klucken, T., Schweckendiek, J., Vaitl, D., Wolf, O.T., Stark, R., 2011. Influence of contingency awareness on neural, electrodermal and evaluative responses during fear conditioning. Soc. Cogn. Affect. Neurosci. 6, 495—506.

Watson, D., Clark, L.A., Tellegen, A., 1988. Development and validation of brief measures of positive and negative affect: the PANAS Scales. J. Pers. Soc. Psychol. 54, 1063—1070.

Weerda, R., Muehlhan, M., Wolf, O.T., Thiel, C.M., 2010. Effects of acute psychosocial stress on working memory related brain activity in men. Hum. Brain Mapp. 31, 1418—1429.

Wolf, O.T., 2008. The influence of stress hormones on emotional memory: relevance for psychopathology. Acta Psychol. 127, 513—531.

Wolf, O.T., 2009. Stress and memory in humans: twelve years of progress. Brain Res. 1293, 142—154.

Wolf, O.T., Minnebusch, D., Daum, I., 2009. Stress impairs acquisition of delay eyeblink conditioning in men and women. Neurobiol. Learn. Mem. 91, 431—436.

Wolf, O.T., Bauser, D.S., Daum, I., 2012. Eyeblink conditional discrimination learning in healthy young men is impaired after stress exposure. Psychophysiology 49, 164—171.

Zorawski, M., Blanding, N.Q., Kuhn, C.M., LaBar, K.S., 2006. Effects of stress and sex on acquisition and consolidation of human fear conditioning. Learn. Mem. 13, 441—450.