Psychoneuroendocrinology 91 (2018) 79–85

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Cortisol increases the return of fear by strengthening amygdala signaling in men

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ARTICLE INFO

Keywords:
Amygdala
Fear extinction
Glucocorticoids
Relapse
Stress hormones
Return of fear

ABSTRACT

Relapses represent a major limitation to the long-term remission of pathological fear and anxiety. Stress modulates the acquisition and expression of fear memories and appears to promote fear recovery in patients with anxiety disorders. However, the neural correlates underlying stress hormone effects on the return of fear in humans remain unexplored. Likewise, little is known about the interactions between sex and stress hormones on return of fear phenomena. In this functional magnetic resonance imaging study, 32 men and 32 women were exposed to a fear renewal paradigm with fear acquisition in context A and extinction in context B. On the following day, participants received either cortisol or placebo 40 min before return of fear was tested in both contexts in a renewal and reinstatement test. Cortisol increased differential conditioned skin conductance responses in the extinction context B following reinstatement in men but not in women. On the neural level, this effect was characterized by enhanced fear-related activation in the right amygdala in men, while an activation decrement in this region was observed after cortisol treatment in women. Our results revealed that cortisol promotes the return of fear in men by strengthening a key node of the fear network – the amygdala. We thereby provide novel insights into a sex-specific mechanism mediating stress-induced fear recovery which may translate into different relapse risks and treatment strategies for men and women.

1. Introduction

Although exposure-based treatments for pathological fear and anxiety are very effective (Otte, 2011) fear relapses still pose a major challenge to long-term therapeutic efficacy (Yonkers et al., 2003). Unraveling factors promoting the recovery of extinguished fears is essential to achieve long-term remission. Fears can be experimentally modeled with conditioning paradigms in which a neutral stimulus (conditioned stimulus; CS) is followed by an aversive event (unconditioned stimulus; UCS). During extinction, the CS is repeatedly presented without the UCS leading to a decline of conditioned fear responses (Graham and Milad, 2011). However, extinguished responses do not disappear but may return after a change in context (renewal), by exposure to unsignaled UCS (reinstatement) or merely with the passage of time (spontaneous recovery; Bouton, 2002). These return of fear phenomena indicate that extinction does not erase the original fear memory but rather involves the formation of a new, context-dependent memory trace, inhibiting the expression of fear (Bouton, 2002).

Clinical observations suggest that stress exacerbates the reemergence of fears (Francis et al., 2012; Jacobs and Nadel, 1985). Stress activates the sympathetic nervous system and the hypothalamus-pituitary-adrenocortical axis leading to the release of (nor)adrenaline and glucocorticoids (GCs; cortisol in humans, Joëls and Baram, 2009). GCs exert their effects by binding to receptors expressed in the prefrontal cortex (PFC), hippocampus and amygdala (Wolf et al., 2016). These brain regions contribute to the formation and expression of fear and extinction memories (Sotres-Bayon and Quirk, 2010), making them highly susceptible to GCs (Maren and Holmes, 2016). Furthermore, cortisol is known to potently modulate learning and memory (Joëls et al., 2006; Wolf et al., 2016), with mostly impairing effects on memory retrieval (Buchanan et al., 2006; Roozendaal and McGaugh, 2011; Smeets, 2011; Wolf, 2017). Accumulating evidence indicates that acute stress also impairs extinction memory recall in fear conditioning (Deschaux et al., 2013; Raio et al., 2014) as well as in neutral associative learning paradigms (Hamacher-Dang et al., 2013). In a predictive learning task, pre-retrieval cortisol administration disrupted ventromedial PFC (vmPFC) functioning thereby diminishing the retention of extinguished associations (Kinner et al., 2016). In accordance, rodent studies suggest that elevated GC concentrations reduce inhibitory actions of the medial PFC on the amygdala, leading to exaggerated fear responses (Akirav and Maroun, 2007). Return of fear may therefore be mediated by a cortisol-induced activation boost in

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https://doi.org/10.1016/j.psyneuen.2018.02.020
Received 20 December 2017; Received in revised form 7 February 2018; Accepted 19 February 2018

fear-associated brain regions. However, the neural mechanisms underlying the impact of cortisol on the return of fear in humans remain unexplored. Moreover, sex differences are thought to play an important role in modulating fear and extinction processes (Maeng and Milad, 2015; Merz and Wolf, 2017; Stockhorst and Antov, 2016), but have been widely neglected in past research (Cahill and Aswad, 2015; Cover et al., 2014).

To address these issues, participants were subjected to a fear renewal design (Milad et al., 2007) in which they either received cortisol or placebo before the return of fear was tested. Based on initial evidence from laboratory stress studies (Deschaux et al., 2013; Hamacher-Dang et al., 2013; Raio et al., 2014), cortisol should impair extinction retrieval thereby enhancing the return of fear. On the neural level, this effect should be reflected by reduced activation in the vmPFC (Akirav and Maroun, 2007; Kinner et al., 2016) and increased activation of fear-associated brain regions such as amygdala, dorsal anterior cingulate cortex (dACC) and insula (Milad and Quirk, 2012). Due to sex hormone dependent cortisol effects on the neural correlates of fear acquisition and extinction (Merz and Wolf, 2017), we additionally sought to characterize the interaction of sex and cortisol.

2. Methods and materials

2.1. Participants and general procedure

The required sample size was determined using G*Power 3.1 (Faul et al., 2009), assuming a medium-sized effect of cortisol on memory retrieval as reported in a meta-analysis by Het et al. (2005); average effect size of d = −0.49). Accordingly, the estimation of the sample size for a medium effect size of $f = 0.25$ (Cohen, 1969), an assumed correlation of $r = 0.30$ for repeated measurements and a given significance level of $\alpha = 0.05$, revealed a required sample size of 60 participants in order to achieve a power of $1\text{-}\beta \geq 0.90$ to detect a significant interaction comprising two between-subject factors and two within-subject factors.

Sixty-four healthy students (32 females) recruited at the Ruhr-University Bochum participated in this study. Compliance with inclusion criteria was checked beforehand in a standardized telephone interview. Students reporting MRI exclusion criteria, color blindness, chronic or acute illnesses, history of psychiatric or neurological treatment, drug use including smoking, regular intake of medicine, a body mass index (BMI) $< 18\,kg/m^2$ or $> 27\,kg/m^2$ and age < 18 or > 40 years were not eligible. All participants were right-handed and had normal or corrected-to-normal vision. Women were required to have been taking oral contraceptives (only monophasic preparations with an ethinylestradiol and a gestagenic component) for at least three months and were tested during the active pill phase to eliminate potential influences of circulating sex hormones across the menstrual cycle (Merz et al., 2012).

Individual sessions were conducted in the afternoons of two consecutive days (between 1 and 6 p.m.). We instructed participants to refrain from eating, physical exercise and drinking anything except water for at least two hours before the experimental sessions. Upon arrival, participants were informed about the general procedure, pharmacological agents and fMRI protocol. After providing written informed consent, participants were screened for color blindness using a selection of five Ishihara plates and completed questionnaires regarding demographic data. At the end of the experimental sessions, participants were reimbursed with 45€ and debriefed. All procedures were in accordance with the Declaration of Helsinki and approved by the ethics committee of the Medical Faculty of the Ruhr-University Bochum (see Fig. 1A for an overview of the general procedure).

2.2. Fear conditioning

A fear conditioning paradigm developed by Milad and colleagues (2007) was adopted as realized before (Merz et al., 2018). In this task, photos of two different rooms (office, conference room) were randomly assigned to serve as contexts A and B, both containing a desk lamp which indicated CS presence by different colors of the lamplight (blue, red, yellow; assignment of colors to the CSs was counterbalanced across participants). Each trial started with an initial presentation of a black screen with a white fixation cross (duration jittered between 0 and 1.875 s), after which the context was first presented alone for 3 s (during which the desk lamp was off) followed by 6 s of CS presentation (lamplight shining in one of the three colors within the context; see Fig. 1B). During reinforced CS+ trials, the UCS (100 ms) was delivered immediately after CS offset. An intertrial-interval depicting a white fixation cross on a black background was shown from CS offset until the start of the next context presentation for 9.125–11 s (total trial duration: 20 s).

During scanning, participants underwent fear acquisition and extinction on day 1 and a renewal and context-dependent reinstatement test on day 2. During fear acquisition in context A, two of the three stimuli (CS+E and CS+U) were followed by an aversive electrical stimulation (UCS; 100 ms) in five out of eight trials each (62.5% partial reinforcement rate), whereas the third stimulus (CS−) was never paired with the UCS. Each CS was presented eight times (see Fig. 1C). The first and last three trials of the acquisition phase always contained one presentation of each CS (CS+E, CS+U, CS−) and the corresponding CS+ were paired with the UCS. The stimulation electrodes remained attached during all subsequent phases of the experiment, but did not provide electrical stimulation during extinction, renewal and reinstatement test. Extinction in context B consisted of 16 unreinforced presentations of one CS+ (CS+E; extinguished CS+) intermixed with 16 presentations of the CS−. The other CS+ (CS+U; unextinguished CS+) was not shown (see Fig. 1C). CS+E and CS− were both shown in the first and last two trials of the extinction phase. During the renewal test, all three CS were presented four times each in both contexts A and B without any electrical stimulation. The resulting six CS-context combinations were shown in a pseudo-randomized order with no more than three consecutive presentations of the same context. The first six CS trials always consisted of all three CS in both contexts with a randomized allocation of the first CS. After the renewal test, reinstatement followed starting with the application of four unsignaled UCS separated by 5 s intervals. In order to avoid incidental conditioning to the background shown during inter-trial intervals, a gray screen was presented during the UCS application period (duration: 20 s). After that, all three CS were again presented four times each in both contexts A and B without electrical stimulation with the same stimulus presentation number and order as used during the renewal test (see Fig. 1C).

For all experimental phases, pseudo-randomized stimulus presentation orders were realized allowing no more than two consecutive presentations of the same CS. Additionally, stimulus presentation orders and CS and context allocation were matched between the cortisol and placebo group (Merz et al., 2018). Instructions were given as reported elsewhere (Hermann et al., 2016). Stimuli were presented via fMRI-ready LCD-goggles (Visuastim Digital, Resonance Technology Inc., Northridge, CA, USA) connected to a laptop using Presentation (Neurobehavioral Systems, Albany, CA).

2.3. Contingency awareness

Contingency awareness was assessed via a short interview containing one question for each CS regarding its relation to the UCS that immediately followed the acquisition phase and an additional questionnaire after the extinction phase (for a similar assessment, see; Tabbert et al., 2011). Participants were classified as contingency aware if they stated that the CS− was never followed by the UCS, whereas the CS+ always (or sometimes) preceded the UCS during fear acquisition.

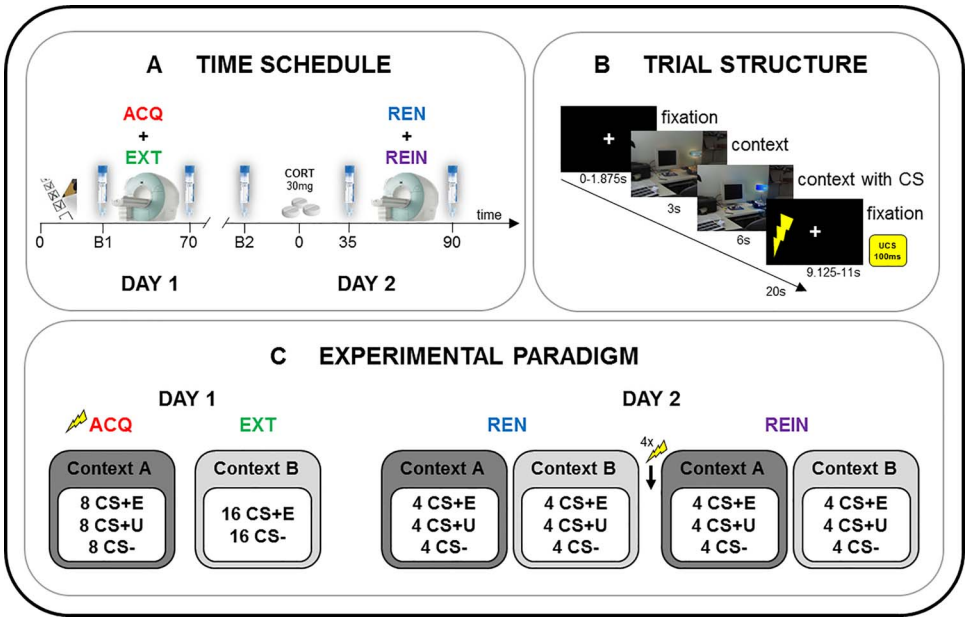

Fig. 1. (A) Time schedule. Day 1: Participants underwent fear acquisition (ACQ) and extinction (EXT) in a first fMRI session. Salivary probes were collected prior (B1 = baseline 1) and 70 min after scanning. Day 2: Participants received either 30 mg hydrocortisone or placebo 40 min before renewal (REN) and reinstatement (REIN) were tested in a second fMRI session. Salivary probes were collected upon arrival (B2 = baseline 2) as well as 35 (prior to scanning) and 90 min (after scanning) after the pharmacological treatment. **(B)** Description and timing of a typical trial structure including presentation of a fixation cross during the ITI, the context, the CS+, and the unconditioned stimulus (UCS; indicated by the yellow flash). **(C)** Experimental paradigm and amount of trials on day 1 and 2 (CS+E = CS+ extinguished; CS+U = CS+ unextinguished). For all experimental phases, stimulus presentation orders were pseudo-randomized allowing no more than two consecutive presentations of the same CS. Stimulus presentation orders and CS and context allocation were matched between the cortisol and placebo group. Reinstatement started with the application of four unsignaled UCS (yellow flash) presented on a gray background. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.4. Electrical stimulation, physiological recordings and SCR data analysis

A constant voltage stimulator (STM200; BIOPAC Systems, Inc., Goleta, CA, USA) was used to deliver transcutaneous electrical stimulation (UCS; 100 ms) via electrodes (surface size: 1 cm^2) attached to the fingertips of the second and third finger of the right hand. At the beginning of the first test session, stimulation intensity was set individually to be 'unpleasant but not painful' using a gradually increasing rating procedure. Electrical stimulation occurred immediately after CS+ offset. During all phases, stimulation electrodes remained attached but did not provide electrical stimulation during extinction, renewal and reinstatement test. For the unsignaled UCS during reinstatement, the same stimulus intensity was applied as calibrated on day 1.

SCRs were sampled at 5000 Hz with a GSR sensor and a 16-channel BrainAmp amplifier (Brain Products GmbH, Munich, Germany) using Ag/AgCl electrodes filled with isotonic (0.05 M NaCl) electrolyte medium attached to the hypothenar surface of the left hand. Raw SCR data were acquired and saved via BrainVision Recorder software (Brain Products GmbH) and afterwards low-pass filtered with a cutoff frequency of 10 Hz. Conditioned SCRs were defined as maximum amplitudes (in μS) starting within a window of 1–6.5 s after CS onset using the trough-to-peak analysis implemented in Ledalab 3.4.4 (Benedek and Kaernbach, 2010). Data were log-transformed to obtain a normal distribution.

2.5. Cortisol administration, saliva sampling and analysis

In a double-blind, randomized design 16 men and 16 women received three 10 mg tablets of cortisol (hydrocortisone; Hoechst) 40 min before the start of the functional scans for the renewal and reinstatement test on day 2. Visually identical placebos (tablettose and magnesium) were given to the other 16 men and 16 women. Saliva samples for the assessment of cortisol concentrations were collected via Salivette sampling devices (Sarstedt, Nümbrecht, Germany) directly before tablet intake (baseline), as well as 35 min (before the renewal test) and 90 min after tablet intake (after the reinstatement test) on day 2 (see Fig. 1A). Furthermore, samples were taken before acquisition and after extinction on day 1 and stored at −20 °C until assayed. Commercially available enzyme-linked immunosorbent assays (ELISA; Demeditec, Kiel, Germany) subserved to measure free cortisol concentrations. Inter- and intra-assay variations were below 10%.

2.6. Statistics

Statistical analyses were performed using IBM SPSS Statistics for Windows 22.0 with the significance level set to $\alpha = 0.05$. For repeated-measures analyses of variance, Greenhouse-Geisser corrected *p*-values were used if assumptions of sphericity were violated and partial eta-square (η_p^2) were reported as estimations of effect sizes. The between-subjects factors treatment (cortisol vs. placebo) and sex (men vs. women) were included in all analyses to control for the variance related to these factors. Only for day 2, we report cortisol effects and their modulation by sex to directly test our hypotheses. For cortisol concentrations, ANOVA with the repeated measurement factor time (day 1: baseline, +70 min; day 2: baseline, +35 min, +90 min) were conducted.

Statistical comparisons of SCRs were performed separately for fear acquisition, extinction, renewal and reinstatement test. Since we were particularly interested in the context-dependent retrieval of extinguished associations, we focused on the comparisons between CS+E and CS−. For fear acquisition and extinction, ANOVA with the within-subjects factors CS (CS+E vs. CS−) and trial (acquisition: 8 trials, extinction: 16 trials) were conducted. For the renewal and reinstatement test, the within-subjects factors CS (CS+E vs. CS−) as well as trial (4 trials) and context (A vs. B) were entered to test for differences in conditioned responding between the acquisition and extinction context.

2.7. fMRI data analyses

Functional and structural brain scans were acquired using a whole-body 3 T scanner (Philips Achieva 3.0 T X-Series, Philips, Netherlands) with a 32-channel SENSE head coil. Structural images were obtained with an isotropic T1 TFE sequence (field of view = 240mm × 240 mm; voxel size = 1mm × 1mm × 1 mm) and encompassed 220 transversally orientated slices covering the whole brain. Functional images were registered with a T2*-weighted gradient echoplanar imaging sequence comprising 207 vols for fear acquisition, 271 vols for extinction (first scan session) and 425 vols for the renewal and reinstatement test (second scan session) with 40 transaxial slices parallel to the orbitofrontal cortex-bone transition (TR = 2.5 s; TE = 30 ms; flip angle 67°;

field of view = 192mm × 192 mm; voxel size = 2mm × 2mm × 3 mm; gap = 0.75 mm; ascending slice order). Three dummy scans preceded functional data acquisition during which magnetization could reach steady state (in addition the first three volumes of the functional data were discarded). To get information for unwarping B0 distortions, we measured a gradient echo field map sequence prior to all functional runs.

For preprocessing and statistical analyses the software package Statistical Parametric Mapping (SPM8; Wellcome Department of Cognitive Neurology, London, UK), implemented in MatLab R2012a (Mathworks Inc., Sherborn, MA) was used. Preprocessing encompassed unwarping and realignment, slice time correction, 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 with a 6 mm full-width half-maximum kernel.

For each participant, fear acquisition, extinction, renewal and reinstatement test were integrated as separate sessions in one first-level model including the following experimental conditions: context alone, blocks of four trials for CS+E, CS+U and CS− (separately for each context during the renewal and reinstatement test), UCS, UCS omission (after CS+ presentation), and non-UCS (after CS− presentation). All regressors were modeled by a stick function convolved with the canonical hemodynamic response function in the general linear model, without specifically modeling the duration of the different events (i.e. event-related design). The six movement parameters from the realignment step served as covariates in the analysis separately for each scan session. A high pass filter with a time constant of 128 s was implemented.

Random effect group analyses were conducted and focused on the contrast [CS+E minus CS−]. To capture time-dependent changes during extinction learning differential brain activation across the first eight trials (early extinction) was compared with brain activation across the last eight trials (late extinction). For the renewal and reinstatement test, the overarching contrasts [RenA $_{(CS+E\ minus\ CS-)}$] minus [RenB $_{(CS+E\ minus\ CS-)}$] and [ReinA $_{(CS+E\ minus\ CS-)}$] minus [ReinB $_{(CS+E\ minus\ CS-)}$] were set up to test for context-dependent differential conditioned fear responses. ANOVA was conducted with the group factors treatment and sex in the full factorial model implemented in SPM8.

For all statistical analyses, we used region of interest (ROI) analyses targeting brain regions identified in previous studies examining fear acquisition and extinction in general (Sotres-Bayon and Quirk, 2010) and in interaction with cortisol (Merz et al., 2012; Rodrigues et al., 2009): amygdala, vmPFC, orbitofrontal cortex (OFC), dACC, insula, nucleus accumbens, and hippocampus (maximum probability masks; probability threshold set to 0.25, Harvard–Oxford Cortical and Subcortical Structural Atlases, Harvard Center for Morphometric Analysis; http://www.cma.mgh.harvard.edu/fsl_atlas.html).

The vmPFC mask consisted of a 5 mm sphere surrounding the peak voxel for extinction-related neural responses in the vmPFC (MNI coordinates $x = 0, y = 40, z = -3$), as indicated in a review of extinction and regulation of fear studies (Schiller and Delgado, 2010). The dACC mask consisted of a 5 mm sphere surrounding the peak voxel for fear-related neural responses in the dACC (MNI coordinates $x = 0, y = 16, z = 36$) derived from a meta-analysis on fear conditioning (Mechias et al., 2010). Correction for multiple comparisons at a significance level of $p \leq .05$ was restricted to pre-defined ROIs and used the small volume correction (SVC) based on the Gaussian random field theory (family-wise error (FWE) rate method; Friston, 2007).

2.8. Exclusion of participants

Data from one male participant from the placebo group had to be excluded from all analyses due to a failure to show contingency awareness after fear acquisition. For three participants (two female-cortisol; one female-placebo), SCR data of all phases were excluded from analyses due to exceptionally low responding to the UCS (less than four detectable responses) during acquisition or poor data quality during the renewal test (e.g. due to random noise). One additional participant (female-placebo) had to be excluded from SCR analyses of the acquisition and extinction phase due to technical failure during data recording and another one (male-cortisol) was excluded from SCR analysis of the reinstatement phase because of fallen off electrodes. Functional imaging data for the reinstatement test from two participants (male-cortisol; female-cortisol) were excluded due to excessive movements during this phase.

3. Results

3.1. Sample description

Participants were aged between 18 and 36 years ($M = 23.67$, $SD = 3.26$) and had a mean BMI of $M = 22.44\ kg/m^2$ ($SD = 2.36$). ANOVA with the between-subjects factors treatment and sex revealed that overall men ($M = 24.55$, $SD = 3.91$) were slightly older than women ($M = 22.81$, $SD = 2.22$; $F_{(1,59)} = 4.58$, $p < .05$, $\eta_p^2 = .07$) and had a higher BMI ($M = 23.67$, $SD = 2.48$) than women ($M = 21.25$, $SD = 1.52$; $F_{(1,59)} = 21.47$, $p < .001$, $\eta_p^2 = .27$). No interaction effects with treatment occurred (all $ps > .10$).

3.2. Salivary cortisol

For day 2, ANOVA revealed a significant main effect of time ($F_{(2,118)} = 36.734$; $p < .001$; $\eta_p^2 = .38$), treatment ($F_{(1,59)} = 59.54$; $p < .001$; $\eta_p^2 = .50$) and a time × treatment interaction ($F_{(2,118)} = 36.86$; $p < .001$; $\eta_p^2 = .39$). Whereas groups did not differ at baseline ($p > .10$), cortisol was elevated 30 and 90 min after hydrocortisone compared to placebo administration (both $ps < .001$; Table 1). In addition, a significant treatment × sex interaction occurred ($F_{(1,59)} = 9.50$; $p < .01$; $\eta_p^2 = .14$), revealing higher cortisol concentrations in cortisol treated women relative to cortisol treated men ($F_{(1,30)} = 9.70$; $p < .01$; $\eta_p^2 = .24$), whereas no sex difference occurred in the placebo group ($p > .05$).[1] On day 1, groups differed neither at baseline nor after scanning (both $ps > .05$).

3.3. Fear conditioning

Fear acquisition and extinction were both successful (see the supplemental information for a detailed results section and Fig. S1 for an illustration of SCRs during all phases of the fear conditioning paradigm).

3.3.1. SCRs

3.3.1.1. Renewal test. Regarding the renewal test on day 2, ANOVA for SCRs revealed significant main effects of CS ($F_{(1,56)} = 56.58$; $p < .001$; $\eta_p^2 = .50$) and context ($F_{(1,56)} = 10.46$; $p < .05$; $\eta_p^2 = .16$) and a CS × context interaction ($F_{(1,56)} = 4.57$; $p < .05$; $\eta_p^2 = .08$), indicative of a renewal effect, as participants showed stronger differential responding to the CS+E relative to the CS− in context A as compared to context B ($t_{(59)} = 2.16$, $p < .05$; $\eta_p^2 = .07$; Fig. S1). In addition, a significant main effect of trial ($F_{(3,168)} = 51.13$; $p < .001$; $\eta_p^2 = .48$) and a CS × trial interaction ($F_{(3,168)} = 23.67$; $p < .001$; $\eta_p^2 = .30$) reflected habituation of responding from the first to the last trial. No main or interaction effects with treatment occurred (all $ps > .10$).

[1] Due to this sex difference in the cortisol group, we ran additional analyses regarding the renewal and reinstatement test for SCRs and BOLD-fMRI, including cortisol concentrations on day two as a covariate. Results for the reported main effect of treatment and treatment × sex interactions in both measures were highly similar to the original analyses.

Table 1
Mean (± SEM) salivary cortisol concentrations at baseline and after scanning on day 1 as well as before, 35 min and 90 min after the administration of cortisol (30 mg) or placebo on day 2. Data is separately shown for men and women.

	men		women	
	cortisol	placebo	cortisol	placebo
salivary cortisol (nmol/l)				
day 1				
baseline	8.23 ± 1.04	11.00 ± 1.56	6.82 ± 0.59	7.92 ± 0.64
after scanning	9.05 ± 2.01	9.27 ± 1.24	6.20 ± 0.63	8.68 ± 0.77
day 2				
before treatment	8.29 ± 1.46*	7.81 ± 0.84	7.13 ± 0.57	7.28 ± 0.67
35 min after treatment	265.15 ± 85.46*	7.98 ± 1.12	450.21 ± 72.35*	6.99 ± 0.61
90 min after treatment	166.62 ± 38.44*	7.14 ± 0.93	364.54 ± 30.65*	6.26 ± 0.56

* $p < .001$ (*t*-tests), significant difference between cortisol and placebo group.

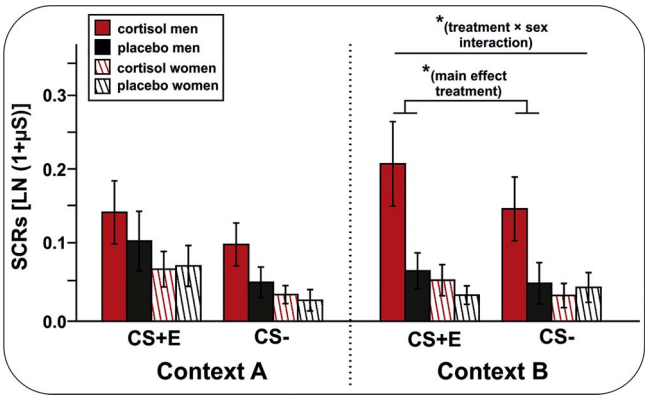

Fig. 2. Mean (± SEM) conditioned SCRs for the CS + E and CS − during the reinstatement test in context A and B on day 2. Main and interaction effects with the factor treatment are indicated with *$p < .05$. Data is depicted for the cortisol and placebo group separately in men and women. In context B, cortisol generally enhanced SCRs in men.

3.3.1.2. Reinstatement test. During the context-dependent reinstatement test, participants showed significantly higher conditioned SCRs to the CS + E relative to the CS − (main effect CS: $F_{(1,55)} = 5.83$; $p < .05$; $\eta_p^2 = .10$) again declining from the first to the last reinstatement trial (CS × trial interaction: $F_{(3,165)} = 5.10$; $p < .01$; $\eta_p^2 = .09$; main effect trial: $F_{(3,165)} = 9.79$; $p < .001$; $\eta_p^2 = .15$; Fig. S1). Moreover, context-dependent SCRs were modulated by treatment and sex (main effect treatment: $F_{(1,55)} = 3.89$; $p = .054$; $\eta_p^2 = .07$; context × treatment interaction: $F_{(1,55)} = 5.10$; $p < .05$; $\eta_p^2 = .09$; context × treatment × sex: $F_{(1,55)} = 4.39$; $p < .05$; $\eta_p^2 = .07$). As illustrated in Fig. 2, cortisol generally enhanced SCRs in context B in men (treatment × sex interaction: $F_{(1,55)} = 5.49$; $p < .05$; $\eta_p^2 = .09$; men: main effect treatment: $F_{(1,28)} = 6.95$; $p < .05$; $\eta_p^2 = .20$), whereas no such an effect occurred in women or in context A (*ps* > .10).

3.3.2. Neural responses

3.3.2.1. Renewal test. For the renewal contrast [(RenA $_{(CS+E\ minus\ CS-)}$) minus (RenB $_{(CS+E\ minus\ CS-)}$)], we found stronger differential activation of the left OFC in the acquisition context A as compared to the extinction context B, most likely representing the neural signature of fear renewal (Table 2). No modulations by treatment or sex were found.

3.3.2.2. Reinstatement test. During the context-dependent reinstatement test, the contrast [(ReinB $_{(CS+E\ minus\ CS-)}$) minus (ReinA $_{(CS+E\ minus\ CS-)}$)] revealed generally stronger differential activation in the left OFC in context B as compared to context A (Table 2). Additionally, a main effect of treatment and a treatment × sex interaction emerged for the right amygdala

Table 2
Localization and statistics of the peak voxel for the contrast [CS + E minus CS −] during **(A)** the renewal test and **(B)** the reinstatement test. Both test phases comprised four trials of each CS in both contexts A and B. Differential neural responses were tested in the acquisition context A as compared to the extinction context B. Main and interaction effects with cortisol are shown.

Contrast	Brain structure	*x*	*y*	*z*	T_{max}	p_{corr}
(A) renewal test						
[RenA minus RenB]						
CS + E minus CS −	L orbitofrontal cortex	−12	10	−24	4.06	.035
[RenB minus RenA]						
CS + E minus CS −	no significant activations					
(B) reinstatement test						
[ReinA minus ReinB]						
CS + E minus CS −	no significant activations					
[ReinB minus ReinA]						
CS + E minus CS −	L orbitofrontal cortex	−36	28	−18	4.07	.037
placebo minus cortisol	R amygdala	14	−4	−14	3.51	.041
treatment × sex	R amygdala	26	−10	−16	4.10	.009

The significance threshold was $p_{corr.} \leq .05$ (FWE-corrected for small volume correction). All coordinates (*x, y, z*) are given in MNI space. L = left, R = right.

(Table 2). This interaction was driven by higher differential neural responses in context B relative to context A in cortisol treated men compared to men receiving placebo, while the opposite picture emerged in women (Fig. 3).

4. Discussion

The present study provides converging evidence from autonomic and neural measures for a sex-dependent cortisol-induced return of fear. Cortisol specifically amplified the return of fear after re-exposure to unsignaled UCS (reinstatement) in men, which was characterized by enhanced differential amygdala signaling in the extinction compared to the acquisition context. Elevated stress hormone levels strengthen amygdala functioning but dampen activity in fear-inhibitory regions such as the PFC (Akirav and Maroun, 2007). In line with this notion, it has been shown that cortisol diminished vmPFC activation thereby impairing the retrieval of extinguished associations in a neutral predictive learning task (Kinner et al., 2016). Work in animals and humans further suggests that acute stress impairs fear extinction memory recall as well, resulting in a reemergence of conditioned fear responses (Deschaux et al., 2013; Raio et al., 2014). Together with these findings, our results illustrate the detrimental effects of GCs on extinction memory ultimately leading to a stronger return of fear in men.

As expected, a renewal effect generally occurred, reflecting an increase of conditioned fear SCRs and stronger differential neural signaling in the left OFC in context A compared to context B. Importantly,

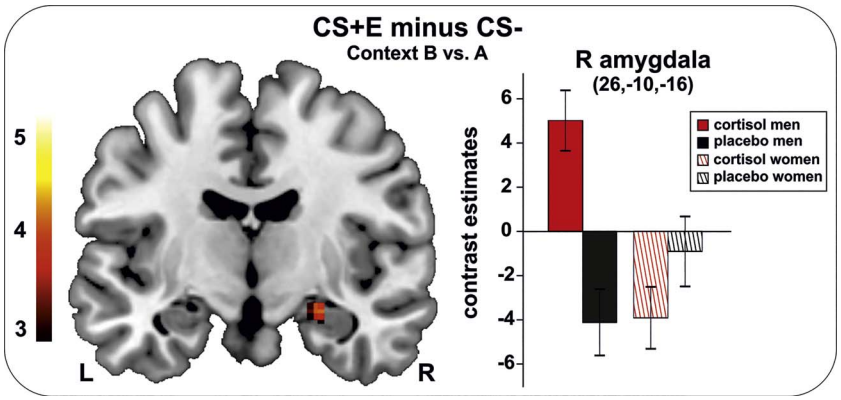

Fig. 3. Neural activation for differential responding (CS + E minus CS −) during the reinstatement test in the extinction context B compared to the acquisition context A. The depicted coronal slice was selected according to the reported treatment × sex interaction in the right amygdala. For demonstration purposes, data were thresholded with $T \geq 3$ (see color bar for exact T-values). In the bar graphs, mean (± SEM) differential contrast estimates are additionally given in the respective peak voxel for the cortisol and placebo group, separately for men and women. All coordinates (x, y, z) are given in MNI space. L = left, R = right.
Cortisol enhanced differential neural responses in the right amygdala in context B compared to context A in men, while attenuating it in women.

we also found stronger differential activation of the left OFC after reinstatement, but this time in context B. Consistently, the OFC is known to be critically involved in the acquisition and expression of fear memories (Milad and Quirk, 2012) and more generally implicated in the evaluation of contingencies between different stimuli that may modulate behaviors in response to threat or punishment (Milad and Rauch, 2007; Rolls and Grabenhorst, 2008). In accordance, phobic patients appear to show greater OFC activation when exposed to phobia-related pictures, underscoring the role of the OFC in fear processing (Dilger et al., 2003).

Interestingly, during the context-dependent reinstatement test cortisol augmented fear responding particularly in the originally safe extinction context B, indicating an inability to use contextual information to express extinction memories. Consistently, cortisol also impaired the contextualization of fear memories resulting in fear generalization to other CSs and contexts (van Ast et al., 2012). Conversely, it has been shown that the effects of stress on declarative memory can also be modulated by contextual cues (Schwabe and Wolf, 2009). For the current results, it is therefore reasonable that the presentation of a neutral background (gray screen) during reinstatement constituted a novel situation for the participants, which lacked appropriate contextual cues to successfully retrieve the extinction memory during the succeeding test phase. Moreover, the reinstatement procedure itself (unpredictable UCS) may have evoked a general uncertainty about when and where UCS will recur. This uncertainty might in turn have compromised the predictive value of previously learned associations (Haaker et al., 2014) and at the same time have also challenged the occasion-setting properties of the extinction context to gate the retrieval of discrete CS-UCS associations (Bouton, 2002). This explanation could also account for the generally enhanced SCRs after reinstatement, particularly found in men after cortisol treatment. Apparently, cortisol generally enhanced anticipatory anxiety by either increasing reinstatement-induced uncertainty or compromising contextual extinction retrieval or both. Interestingly, a recent study revealed that exposure to life adversity promotes fear generalization in the amygdala after reinstatement (Scharfenort et al., 2016). This lines up with results showing acute stress to shift the amygdala towards heightened sensitivity but lower specificity, leading to augmented amygdala responsiveness in general (van Marle et al., 2009). Together with these findings, our data suggest that the ability to discriminate safety from danger cues may be compromised under conditions of high GC levels.

However, even though cortisol enhanced conditioned fear responses following reinstatement, the renewal test remained unaffected by cortisol treatment. Contrary to that, evidence from previous laboratory stress and pharmacological studies indicated that extinction recall was also impaired during this initial test phase (Kinner et al., 2016; Raio et al., 2014), particularly after changing the context (renewal; Hamacher-Dang et al., 2013). Yet, on the other hand, exposure to acute stress has also been shown to abolish fear renewal (Merz et al., 2014). In line with that, GCs administered before exposure based therapy appear to enhance treatment efficacy by reducing fear memory retrieval (de Quervain et al., 2017; Soravia et al., 2006). Moreover, it is also reasonable that the intensity of the procedure itself may influence how stress hormones interact with extinction recall. According to that, reinstatement could be regarded as a 'strong' manipulation probably also producing stronger cortisol effects on extinction memory expression than a contextual change during the renewal test. Nevertheless, as both renewal and reinstatement are clinically relevant phenomena that could serve to explain the reemergence of phobic fears in contexts that induce feelings of anxiety or insecurity, more experimental work is needed to delineate potential factors that may modulate how stress hormones interact with the retrieval of fear and extinction memories, respectively.

Of note, cortisol largely exerted opposing effects in women showing activation decrements in the right amygdala during the context-dependent reinstatement test. Sex-dependent stress hormone effects on fear conditioning have been reported in rodents and humans (Maeng and Milad, 2015; Merz and Wolf, 2017; Stockhorst and Antov, 2016) and moreover shown to manifest as a function of menstrual cycle stage and hormonal contraceptive usage (Merz and Wolf, 2017). In addition, sexual dimorphisms are well documented for brain structures implicated in stress and fear learning alike (amygdala, dACC, hippocampus, vmPFC; Maeng and Milad, 2015). Our data extend these findings by providing first evidence of a sex-dependent cortisol effect on experimentally induced context-dependent fear reinstatement. However, whether these findings obtained in women taking oral contraceptives can be extended to free-cycling women remains open and hence emphasize the need to further explore the interplay of sex and stress hormones in the modulation of fear and extinction memories (Merz and Wolf, 2017; Stockhorst and Antov, 2016).

5. Conclusion

To conclude, cortisol promoted the return of fear following reinstatement, which was associated with enhanced SCRs and stronger differential neural responses in the right amygdala. A cortisol-induced return of autonomic and neural fear responses was found in men but not in women. We therefore provide first evidence for a sex-specific cortisol effect on the return of fear that may translate into different vulnerabilities to fear relapse in men and women. As cortisol was specifically related to the context-dependent reinstatement of fear responses, our results thus characterize a neuroendocrine mechanism explaining how stress may promote relapses in the face of adverse events.

Financial disclosure

All authors reported no biomedical financial interests or potential conflicts of interest.

Acknowledgments

We gratefully acknowledge the help of Eve-Mariek Hessas, Andreas Haltermann, Alina Renner, Farina Helmke, Annegret Last, Nuria Mackes, Judith Hollenhorst and Tobias Zwadlo during data collection and recruitment of participants. Moreover, we thank Tobias Otto and PHILIPS Germany for technical support.

Funding for this study was provided by the German Research Foundation (DFG; Research Unit 1581 Extinction Learning; Project P5 (WO 733/13-2) and SFB 1280 Extinction Learning; Project A09 (OTW, CJM)). The DFG had no role in study design, collection, analysis and interpretation of data, writing of the manuscript or in the decision to submit the paper for publication.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.psyneuen.2018.02.020.

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