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# Cortisol disrupts the neural correlates of extinction recall

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ABSTRACT

The renewal effect describes the recovery of extinguished responses that may occur after a change in context and indicates that extinction memory retrieval is sometimes prone to failure. Stress hormones have been implicated to modulate extinction processes, with mostly impairing effects on extinction retrieval. However, the neurobiological mechanisms mediating stress effects on extinction memory remain elusive. In this functional magnetic resonance imaging study, we investigated the effects of cortisol administration on the neural correlates of extinction memory retrieval in a predictive learning task. In this task, participants were required to predict whether certain food stimuli were associated with stomach trouble when presented in two different contexts. A two-day renewal paradigm was applied in which an association was acquired in context A and subsequently extinguished in context B. On the following day, participants received either cortisol or placebo 40 min before extinction memory retrieval was tested in both contexts. Behaviorally, cortisol impaired the retrieval of extinguished associations when presented in the extinction context. On the neural level, this effect was characterized by a reduced context differentiation for the extinguished stimulus in the ventromedial prefrontal cortex, but only in men. In the placebo group, ventromedial prefrontal cortex was functionally connected to the left cerebellum, the anterior cingulate and the right anterior parahippocampal gyrus to express extinction memory. This functional crosstalk was reduced under cortisol. These findings illustrate that the stress hormone cortisol disrupts ventromedial prefrontal cortex functioning and its communication with other brain regions implicated in extinction memory.



## Introduction

Extinction is defined as a process during which an organism learns that once acquired information is no longer valid and in consequence ceases to respond to it (Myers and Davis, 2007; Vervliet et al., 2013). However, the retrieval of extinction memory is sometimes prone to failure (Bouton, 2002). Extinguished responses do not disappear but may return for example after a change in context (Bouton and Bolles, 1979; Milad et al., 2005), a phenomenon known as the renewal effect. This recovery of once acquired responses indicates that extinction does not lead to forgetting or an erasure of the initial memory trace. It rather constitutes a new learning process in which a second, inhibitory association between a stimulus and another outcome is acquired (Bouton, 1993; Delamater, 2004; Myers and Davis, 2002). Which of the two now competing associations will be retrieved at a later point in time depends critically on the context (Bouton, 2002), which can either be identical to the one shown during acquisition (AAA or ABA), during extinction (ABB) or a novel one (ABC or AAB; see Rescorla, 2008).

Among these variants, the ABA renewal has been demonstrated in numerous different paradigms, such as appetitive conditioning (Bouton and Peck, 1989) and taste aversion learning (Rosas and Bouton, 1997) in rats, as well as in human fear conditioning (Alvarez et al., 2007; Effting and Kindt, 2007; Milad et al., 2005; Vansteenwegen et al., 2005) and predictive learning (Üngör and Lachnit, 2006, 2008). In particular, the predictive learning task provides a more systematic exploration of the basic mechanisms underlying associative learning and extinction processes using contextually gated changes in stimulus–outcome relations without an emotional component. The enhanced context sensitivity often observed after extinction is assumed to be caused by the unexpected change in stimulus–outcome relations occurring during this second learning phase (Rosas and Callejas-Aguilera, 2006, 2007), which in turn might draw attention to external stimuli that have been concurrently presented, such as the context (Bouton, 2002; Lucke et al., 2013; Nelson et al., 2013; Vervliet et al., 2013). In accordance, it has been proposed that contextual cues might serve to regulate the retrieval of ambiguous memories related to the same stimulus (Bouton, 1993).

*Abbreviations:* vmPFC, Ventromedial prefrontal cortex; GC, Glucocorticoid; PHG, Parahippocampal gyrus; ACC, Anterior cingulate cortex; OC, Oral contraceptives.

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With regard to the underlying brain structures, the hippocampal formation is known to be crucially relevant for contextual processing (Smith and Mizumori, 2006) and memory (Hirsh, 1974; Kennedy and Shapiro, 2004) and thus suggested to play a prominent role for extinction learning and the renewal effect alike (Kalisch et al., 2006; Milad et al., 2007). For instance, pharmacological inactivation of the hippocampus (Corcoran and Maren, 2001) and the ventromedial prefrontal cortex (vmPFC; Sierra-Mercado et al., 2006) disrupts the context-specific expression of extinction. Correspondingly, a recent study by Lissek et al. (2013) found increased hippocampal activity during extinction learning, whereas vmPFC was recruited during extinction recall in the predictive learning task.

Importantly, these brain structures are known to be specifically susceptible to the effects of stress hormones (Arnsten, 2009; Herry et al., 2010; Kim et al., 2006). Under stress, the consecutive activation of the sympathetic nervous system and the hypothalamus–pituitary–adrenocortical axis leads to the release of (nor)adrenaline and glucocorticoids (GCs; Joels and Baram, 2009). GCs bind to mineralocorticoid and glucocorticoid receptors (de Kloet et al., 2005) which are predominantly located in the PFC, hippocampus and amygdala (de Kloet, 2004) and activated by acute stress or cortisol administration alike. In particular, the main human GC cortisol has been shown to be a potent modulator of learning and memory (Joels et al., 2006; Schwabe et al., 2010; Wolf, 2009), with mostly impairing effects on memory retrieval (Roozendaal and McGaugh, 2011; Wolf, 2009).

First evidence from animal and human data indicates that acute stress also impairs the recall of extinction memory in fear conditioning (Deschaux et al., 2013; Raio et al., 2014) and in the non-aversive predictive learning task (Hamacher-Dang et al., 2013b). However, neuroimaging studies exploring the neural mechanisms underlying the impact of cortisol on extinction recall are lacking so far.

In the present study, we therefore aimed to investigate the potential modulatory role of cortisol on the neural correlates of extinction memory retrieval within an ABA renewal paradigm, applied in the predictive learning task (Hamacher-Dang et al., 2013a, 2013b; Üngör and Lachnit, 2006). On two consecutive days, the participants underwent acquisition and extinction in different contexts (context-dependent learning) and a renewal test (context-dependent recall of associations) prior to which participants either received an oral dose of cortisol or a placebo. In line with previous laboratory studies (Deschaux et al., 2013; Hamacher-Dang et al., 2013b; Raio et al., 2014), we expected cortisol to impair the retrieval of extinction memory. Similar to the well documented GC-induced reductions in hippocampal and prefrontal activation associated with impaired declarative memory retrieval (de Quervain et al., 2003; Oei et al., 2007; Weerda et al., 2010), this effect should be reflected in decreased activation of the hippocampus and the vmPFC during extinction recall as well.

Although there is a large body of neuroimaging literature concerning the regions involved in extinction processes, there are only few studies yet examining how these brain regions flexibly interact to express extinction (Milad et al., 2007; Schiller et al., 2013). Given the crucial role of vmPFC for extinction recall, aberrant functioning of this region or alterations in its connectivity to other structures of the extinction circuit might reflect the extinction retrieval deficits which have been observed after stress exposure. Consistent with this hypothesis, a recent study using resting-state functional connectivity analyses demonstrated a stress-induced disruption of interregional coupling between the vmPFC and the amygdala (Clewett et al., 2013). In order to enhance our understanding of the mechanisms mediating the expression of extinction and to further elucidate how they might be modulated by stress hormones, we investigated the functional connectivity of the emerging brain structures relevant for extinction recall. Since sex-dependent cortisol effects on brain activation in associative learning and extinction processes have been reported previously (for example Merz et al., 2010, 2012a) we additionally aimed to explore the potential interaction of sex and cortisol in the current study.

## Materials and methods

### *Participants and general procedure*

In total, 60 healthy, right-handed male and female students were recruited for participation in this study. Exclusion criteria were checked beforehand in a telephone interview and comprised chronic or acute illnesses, history of psychiatric or neurological treatment, a body mass index (BMI) outside the range of 18–27 kg/m$^2$, age outside the range of 18–40 years, drug use, smoking or regular intake of medicine, and standard fMRI exclusion criteria. All participants 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 3 months and were tested during pill intake to reduce potential influences of circulating sex hormones across the normal menstrual cycle (Merz et al., 2012b). In addition, the participants were instructed to refrain from physical exercise and consumption of food and drinks except water 2 hours prior to testing.

Individual sessions were conducted in the afternoons of two consecutive days (between 1 and 6 pm) to guarantee relatively low and stable endogenous cortisol concentrations. After arrival on day 1, the participants received an explanation of the procedure, the pharmacological agents and the fMRI protocol. After signing the informed consent form they filled out questionnaires regarding their demographic data and were prepared for scanning. In a first fMRI session, the participants underwent acquisition and extinction in a computer-based predictive learning task. On the following day, the participants were tested for renewal 40 min after receiving either cortisol or placebo (described in detail below). At the end of the second testing session, the participants were reimbursed with 40€ for their participation and received additional information regarding the aim of the study. All procedures conformed to the Declaration of Helsinki and were approved by the ethics committee of the Medical Faculty of the Ruhr-University Bochum.

### *Predictive learning task*

A modified version of the predictive learning task (Hamacher-Dang et al., 2013a, 2013b) developed by Üngör and Lachnit (2006) was applied and adapted to the fMRI setting. In this task, the participants were asked to imagine being a doctor of a patient who sometimes suffers from stomach trouble after having meals in his two favorite restaurants. During scanning, the participants underwent three phases including acquisition and extinction on day 1 and a retrieval test on day 2.

In the acquisition phase, the participants learned to associate a food stimulus with a specific outcome. At the beginning of each trial, one of eight food stimuli (pictures of fruits and vegetables, for example strawberries or tomatoes) was presented for 3 s in one of the two contexts (indicated by a colored frame and the restaurant names “the bell” and “the dragon”). Afterwards, the participants had to predict whether the patient will experience stomach trouble or not after this meal (the question ‘Do you expect, that the patient will experience stomach trouble’ was superimposed, with the response options ‘Yes’ and ‘No’) by pressing the corresponding button on an fMRI-ready keyboard (Lumitouch, Photon Control Inc. Canada) within a response time window of 2.5 s. After expiration of the 2.5 s, feedback with the correct answer was presented for another 2.5 s. Feedback was displayed either in green color for correct predictions or in red color for wrong predictions and in case of a missing response. Inter-trial intervals depicting a white fixation cross on a black screen were randomly jittered between 5 and 7.5 s.

During extinction, two stimuli shown during acquisition were again presented but differed in regard to their context or changed both, its outcome and context (see Table 1). In particular, stimulus a+ had been associated with stomach trouble in context A during acquisition,

**Table 1**
Design of the predictive learning task. Stimuli presented during acquisition, extinction and renewal test. Letters a–l represent different food stimuli, symbols indicate the feedback given to the participant (+ causes stomach trouble, − does not cause stomach trouble, on day 2 feedback was omitted). The critical stimulus a and the corresponding control stimulus b are highlighted in bold. To test for renewal (ABA), stimuli a+ and b− were presented in context A and to test for extinction recall (ABB) stimuli a+ and b− were presented in context B. Results regarding stimuli c+ and d− are included in the supplementary material.

| | Day 1 | | Day 2 |
|---|---|---|---|
| | Acquisition | Extinction | Renewal test |
| Context A | **a+, b-,** c+, d- | c-, d-, i+, j+ | **a, b,** c, d, e, f |
| Context B | e+, f-,g+, h- | **a-, b-**, k+, l+ | **a, b,** c, d, e, f |
| Trials per stimulus | 8 | 8 | 4 |

whereas it was no longer associated with stomach trouble during extinction in context B. In contrast, stimulus b− was neither associated with stomach trouble in context A (acquisition) nor in context B (extinction).

During acquisition and extinction, eight stimuli were presented eight times each. Six additional distractor stimuli were introduced in each of the phases in order to make overall learning more difficult (Hamacher-Dang et al., 2013a, 2013b; Lissek et al., 2013). The trial order was randomized block-wise, so that each block contained two presentations of all stimuli of the respective learning phase. Within each block, the presentation order was randomized. Acquisition and extinction each comprised four blocks and were run within one fMRI session without a break in between the two phases (in sum 128 trials).

In the renewal phase on the following day, we tested memory for the two critical stimulus–outcome associations in both contexts. Therefore, stimuli a+ and b− and four additional distractor stimuli were presented in the former acquisition context (A) and the extinction context (B) without feedback. Each stimulus occurred four times in each context (in sum 48 trials). The resulting twelve stimulus–context combinations were completely randomized in two blocks containing two stimulus presentations in each context and matched between the cortisol and the placebo group. Analogous to classical fear conditioning studies and due to our particular interest in contextual renewal we focused on the four stimulus–context combinations resulting from the critical stimulus a+ and the corresponding control stimulus b− presented in context A and B (aA, bA, aB, bB).

*Cortisol administration, saliva sampling and analysis*

In a double-blind, randomized design 15 men and 15 women were administered three 10 mg tablets of cortisol (hydrocortisone; Hoechst) 40 min before the start of the functional scans for the renewal test on day 2. Visually identical placebos (tablettose and magnesium) were given to the remaining 15 men and 15 women.

To assess cortisol concentrations we collected saliva samples directly before tablet intake (baseline), as well as 30 min and 85 min after tablet intake (before and after the renewal test). Furthermore, saliva samples were taken before and after acquisition and extinction on day 1. Saliva samples were collected using Salivette sampling devices (Sarstedt, Nümbrecht, Germany) which were stored at −20 °C until assayed. Commercially available chemoluminescence immunoassays (CLIA; IBL International, Hamburg, Germany) subserved to measure free cortisol concentrations. Inter- and intra-assay variations were below 10%. Due to problems with saliva sampling and analyses, data from two participants had to be excluded from the cortisol analyses.

*Statistics*

All statistical analyses were performed using IBM SPSS 22 Statistics for Windows with the level of significance set to $\alpha = .05$. For repeated-measures analyses of variance (ANOVA), Greenhouse–Geisser corrected *p*-values were reported if the assumption of sphericity was violated. *p*-values of exploratory *t*-tests were corrected for unequal variances if appropriate. As between subject factors, treatment (cortisol vs. placebo) and sex (men vs. women) were always included. Since we were exclusively interested in cortisol effects and their modulation by sex, the main effects of sex were not analyzed.

For the cortisol concentrations on day 2, we conducted ANOVA with the repeated measurement factor time (baseline, +30 min, +85 min). To control for possible pre-existing group differences, we additionally analyzed cortisol concentrations before and after acquisition and extinction on day 1 using ANOVA with the factor time (baseline, +65 min). To assess behavioral performance in the predictive learning task, we calculated the mean percentage of stomach trouble predictions across the first two trials (beginning) and the last two trials (end) for acquisition and extinction (Hamacher-Dang et al., 2013a). An ANOVA with the within-subjects factor time (beginning vs. end) and stimulus (a+ vs. b−) was conducted. Performance on the renewal test was assessed by calculating the mean of all four stimulus presentations for the two critical stimuli a+ and b− in each context, respectively (results regarding stimuli c+ and d− are included in the supplementary material). An ANOVA with the within-subjects factors stimulus and context (acquisition context vs. extinction context) was conducted.

*fMRI data acquisition and analyses*

Functional and structural brain scans were acquired using a whole-body 3T scanner (Philips Achieva 3.0T X-Series, Philips, the Netherlands) with a 32-channel SENSE head coil. Structural images were obtained using an isotropic T1 TFE sequence (field of view = 240 × 240 $mm^2$; slice thickness = 1 mm; voxel size = 1 × 1 × 1 $mm^3$) with 220 transversally orientated slices covering the whole brain. Functional images were registered with a $T2^*$-weighted gradient echoplanar imaging sequence comprising 782 volumes for acquisition and extinction (first scan session) and 292 volumes for the renewal test phase (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 = 192 × 192$mm^2$; slice thickness = 3 mm; gap = 0.75 mm; ascending slice order; voxel size = 2 × 2 × 2$mm^3$). Three dummy scans preceded data acquisition, during which magnetization could reach steady state. To get information for unwarping $B_0$ distortions, a gradient echo field map sequence was measured before the functional run.

For preprocessing and statistical analyses we used the software package Statistical Parametric Mapping (SPM8; Wellcome Department of Cognitive Neurology, London, UK), implemented in MatLab R2012a (Mathworks Inc., Sherborn, MA). Preprocessing comprised the following steps: 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 (FWHM) kernel. For each participant the first (acquisition and extinction) and the second scan session (renewal test) were integrated in one first-level model including the following experimental conditions: the eight stimuli presented in the acquisition and in the extinction phase as well as the twelve stimulus-context combinations in the renewal test (see Table 1). We modeled regressors for the onset of each stimulus presentation, for the onset of the respective question about the participants' stomach trouble prediction following the stimulus, as well as the onset of the feedback that was given to the participant after a response separately for each of the three experimental phases. The button presses were introduced as additional regressors separated for the first and second scan session. 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). In order to account for movement

related variance, the six movement parameters from the realignment step were included as covariates in the analysis separately for each scan session. 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 effect group analyses. For acquisition and extinction the following contrasts were generated: [aA–bA] for acquisition in context A and [aB–bB] for extinction in context B. Analogous to the behavioral data analysis, we compared brain activation across the first two trials (beginning) with brain activation across the last two trials (end) for both phases. Regarding the renewal test phase, the overarching contrast [aA–bA − aB–bB] was set up to test for discrimination between the critical stimuli a+ and b− between the two contexts, as well as appropriate post hoc contrasts to further track significant activations deriving from this overarching contrast. ANOVA was conducted with the group factors treatment and sex in the full factorial model implemented in SPM8. In particular, we were interested in the interaction between cortisol and sex as well as the main effect of cortisol (main effects of sex were not analyzed separately). Moreover, we looked at the functional coupling of the extinction recall network by conducting psychophysiological interaction (PPI) analyses for each participant. Effective connectivity between a seed region and other brain areas in interaction with the experimental task were explored. Peak voxels of the brain regions subject to main and interaction effects in the ROI analyses were entered as seed regions (volume of interest; 5 mm sphere around the peak voxel; see Section 3).

For all statistical analyses, we used exploratory whole brain as well as region of interest (ROI) analyses including brain regions that were identified in previous experiments using the predictive learning task (Lissek et al., 2013, 2015) and studies examining fear conditioning in interaction with cortisol effects (Merz et al., 2010, 2012a, 2012b; Rodrigues et al., 2009): amygdala, vmPFC, orbitofrontal cortex, nucleus accumbens, hippocampus, and parahippocampal gyrus (PHG). The required masks were maximum probability masks with the probability threshold set to 0.25, taken from the Harvard–Oxford Cortical and Subcortical Structural Atlas provided by the 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). For the exploratory whole brain and the ROI analyses, the significance threshold was set to $p \leq .05$ on voxel-level corrected for multiple testing (family-wise error (FWE) correction); no minimal cluster size was applied. ROI analyses were conducted using the small volume correction option of SPM8 and with an initial intensity threshold of $p \leq .005$.

In order to test for positive associations between functional imaging and behavioral data, we extracted the individual contrast estimates of significant activations and correlated them with the corresponding behavioral data using Pearson product–moment correlations when both measures revealed significant effects.

## Results

### *Sample description*

The participants were aged between 19 and 32 years ($M$ = 24.0 years, $SD$ = 3.4) and had a mean body mass index of $M$ = 22.9 kg/m$^2$ ($SD$ = 1.9 kg/m$^2$). ANOVA with the between subject factors treatment and sex did not reveal any significant main or interaction effects concerning BMI (all $F_{(1, 56)} < .40$; $p > .53$) or age (all $F_{(1, 56)} < .73$; $p > .40$).

### *Salivary cortisol*

On day 2, the ANOVA of salivary cortisol concentrations revealed a significant main effect of time ($F_{(1.5, 80.5)} = 48.36$; $p < .001$), treatment ($F_{(1, 53)} = 62.03$; $p < .001$), and a time × treatment interaction ($F_{(1.5, 80.5)} = 47.69$; $p < .001$). Cortisol was elevated 30 and 85 min after hydrocortisone compared to placebo administration (both $p < .001$; Table 2). At baseline, cortisol concentrations did not differ between groups ($p = .40$). No significant interaction effects with sex were found.

On day 1, a general decrease in cortisol concentrations over time (from baseline to post scanning) was found (main effect of time: $F_{(1, 53)} = 6.51$; $p < .05$; Table 2) reflecting the normal circadian rhythm. The groups differed neither at baseline nor after scanning ($p = .38$ and $p = .81$, respectively). No interaction effects with sex occurred.

### *Predictive learning task*

#### *Acquisition*

The participants' ability to distinguish between stimulus a+ that was associated with stomach trouble and b− that was not associated with stomach trouble increased from the beginning (first two trials) to the end of acquisition (last two trials; time × stimulus interaction: $F_{(1,55)} = 70.28$; $p < .001$; main effect time: $F_{(1,55)} = 4.73$; $p < .05$; main effect stimulus: $F_{(1,55)} = 354.73$; $p < .001$; Fig. 1). On the neural level, this acquired stimulus discrimination was reflected by an enhanced activation in the right hippocampus at the end of acquisition when compared to the beginning (main effect time for the contrast [aA–bA]; $x = 28, y = -36, z = -6$; $T_{max} = 4.36$; $p_{corr} = .016$).

#### *Extinction*

Regarding extinction, a decreased percentage of stomach trouble predictions to stimulus a+ compared to b− was observed at the end of extinction (last two trials) as compared to the beginning (first two trials; time × stimulus interaction: $F_{(1,56)} = 19.01$; $p < .001$; main effect time: $F_{(1,56)} = 82.69$; $p < .001$; main effect stimulus: $F_{(1,56)} = 17.33$; $p < .001$; Fig. 1). Correspondingly, the right hippocampus tended to be activated at the beginning of extinction but was no longer activated at the end of extinction (main effect of time for the contrast [aB–bB]; $x = 14, y = -14, z = -18$; $T_{max} = 3.64$; $p_{corr} = .096$), indicating that the neural discrimination between stimulus a+ and b− decreased from the beginning to the end of this phase.

To specifically test the overall effect of contextual change on stomach trouble prediction we additionally compared the end of acquisition with the beginning of extinction in the behavioral data. For both stimuli the number of stomach trouble predictions differed between the phases after the context switch (main effect time: $F_{(1,56)} = 38.94$; $p < .001$; main effect stimulus: $F_{(1,56)} = 345.33$; $p < .001$; time × stimulus interaction: $F_{(1,56)} = 69.95$; $p < .001$). The predictions to a+ decreased from the end of acquisition to the beginning of extinction (main effect time: $F_{(1,56)} = 81.03$; $p < .001$), whereas the predictions to b− increased (main effect time: $F_{(1,56)} = 11.79$; $p < .05$).

For both, the behavioral data as well as the functional imaging data, analyses regarding acquisition and extinction did not reveal any main or interaction effects with the factor treatment, showing that the cortisol group did not differ from the control group with regard to their performance during acquisition or extinction on day 1 (prior to pharmacological treatment on day 2). For both phases, correlation analyses did not reveal significant associations between stimulus discrimination on the behavioral level (computed as the mean differential responding to stimuli a+ as compared to b−) and differential hippocampus activation in the contrast [aA–bA] for acquisition and in the contrast [aB–bB] for extinction (all $ps > .05$).

#### *Renewal test*

Regarding the renewal test on day 2, the ANOVA for a+ vs. b− revealed a main effect of stimulus ($F_{(1, 56)} = 136.19$; $p < .001$), showing a significantly higher percentage of stomach trouble predictions to stimulus a+ than to stimulus b−. Furthermore, a main effect of context ($F_{(1, 56)} = 35.54$; $p < .001$), a stimulus × context interaction ($F_{(1, 56)} =$

**Table 2**
Mean (SE) salivary cortisol concentrations at baseline and after scanning on day 1 as well as before, 30 min and 85 min after the administration of cortisol or placebo (30 mg) on day 2. Data is separately shown for men and women.

| | Cortisol | | Placebo | |
|---|---|---|---|---|
| | Men | Women | Men | Women |
| Salivary cortisol (nmol/l) | | | | |
| *Day1* | | | | |
| Baseline | 14.10 ± 1.90 | 11.97 ± 1.38 | 13.54 ± 1.52 | 9.70 ± .91 |
| After scanning | 11.20 ± 1.62 | 9.84 ± .91 | 12.25 ± 1.70 | 9.43 ± 1.15 |
| *Day2* | | | | |
| Before treatment | 11.64 ± 1.21 | 12.07 ± 1.56 | 10.23 ± .78 | 11.54 ± 1.16 |
| 30 min after treatment | 385.88 ± 67.85 | 258.74 ± 42.43 | 13.61 ± 1.71 | 11.36 ± 1.09 |
| 85 min after treatment | 225.47 ± 61.12 | 244.97 ± 17.86 | 9.80 ± 1.07 | 10.31 ± 1.60 |

42.72; $p < .001$) and a three-way interaction between stimulus, context and treatment ($F_{(1, 56)} = 9.08$; $p < .01$) occurred. To further characterize these interactions separate ANOVA for a+ and b− were conducted.

The participants made more stomach trouble predictions to stimulus a+ in the acquisition than in the extinction context, thus reflecting an ABA renewal effect (main effect of context: $F_{(1, 56)} = 43.89$; $p < .001$). Furthermore, the analysis revealed a context × treatment interaction ($F_{(1, 56)} = 4.82$; $p < .05$). The follow-up *t*-tests conducted separately for each context showed that in the extinction context, the participants in the cortisol group tended to make more stomach trouble predictions when compared with the placebo group, thus demonstrating impaired extinction memory retrieval (ABB; $t_{(58)} = 1.96$, $p = .055$; Fig. 2). In the acquisition context, no group differences occurred ($t_{(58)} = .90$, $p = .37$; Fig. 2).

Regarding stimulus b−, the percentage of stomach trouble predictions did not differ depending on the context in which the stimulus was presented during the renewal phase (main effect context: $p = .51$). Again a trend for a context × treatment interaction ($F_{(1, 56)} = 3.95$; $p = .052$) was found. In the acquisition context, the cortisol group tended to predict more stomach trouble for stimulus b− than the placebo group. However, the exploratory *t*-tests showed that the retrieval performance of the two groups differed neither significantly in the acquisition context ($p = .30$) nor in the extinction context ($p = .53$).

To test for differences in discrimination between a+ and b− in both contexts, separate ANOVA with both stimuli in either of each context were additionally conducted. In context A, a higher percentage of stomach trouble predictions occurred to a+ than to b− (main effect stimulus: $F_{(1, 56)} = 185.86$; $p < .001$). In context B, again the participants made generally more stomach trouble predictions to a+ than to b− (main effect stimulus: $F_{(1, 56)} = 24.90$; $p < .001$), which differed between groups (stimulus × treatment interaction: $F_{(1, 56)} = 5.02$; $p < .05$): The cortisol group tended to make more stomach trouble predictions to a+ than the placebo group ($t_{(58)} = 1.96$, $p = .055$; Fig. 2).

For the functional imaging data, the overarching contrast [aA–bA − aB–bB] was set up to test for discrimination between stimulus a+ which underwent a context and contingency shift during extinction and the control stimulus b− which only underwent a context change during extinction. Exploratory whole brain analyses did not reveal significant effects. However, ROI analyses for this contrast revealed a trend for enhanced activations in the right posterior PHG as well as in the right amygdala (Table 3) for stimulus discrimination in context A as compared to context B. Furthermore, a significant interaction between treatment and sex in the vmPFC was found, indicating that cortisol attenuated activation in this brain region in men, but tended to increase it in women (Table 3). For the reverse contrast [aB–bB – aA–bA], the left nucleus accumbens was significantly activated (Table 3).

To further track significant activations deriving from this overarching contrast, additional post hoc contrasts were analyzed. Therefore, we compared neural activation to stimulus a+ in the former acquisition context and the extinction context [aA–aB] to test for the renewal effect.

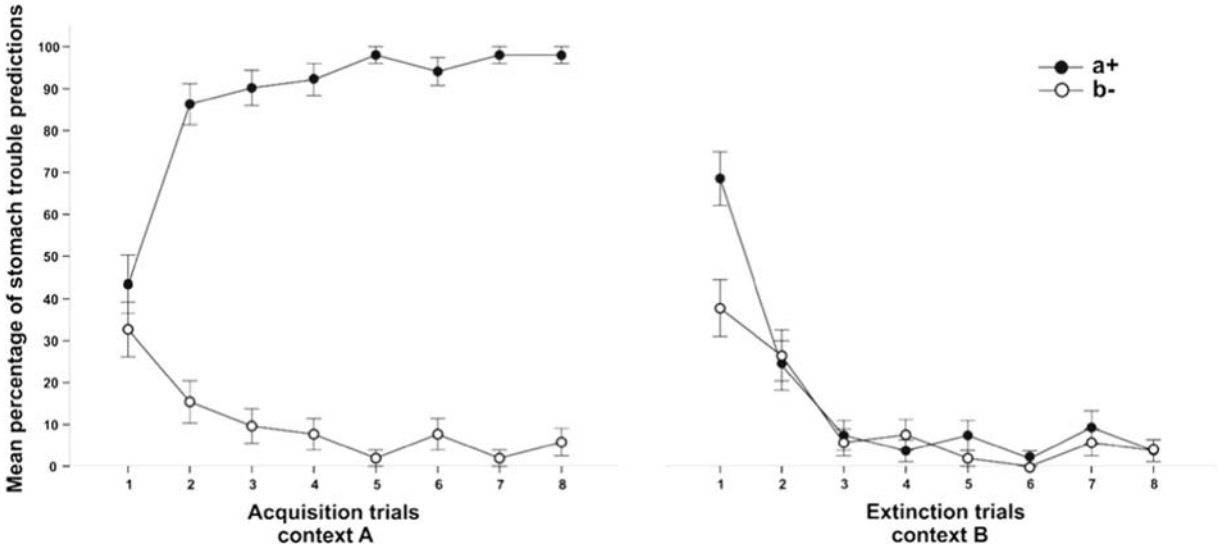


**Fig. 1.** Mean percentage of stomach trouble predictions to critical stimuli across all trials of acquisition (left side of the graph) and extinction (right side of the graph) on day 1. Signs indicate whether the stimulus had (+) or had not (−) been associated with stomach trouble during acquisition. Stimuli a+ (black) underwent a change in both, contingency and context, as it was associated with stomach trouble during acquisition in context A but was no longer associated with stomach trouble during extinction in context B. Stimulus b− (white) underwent a context shift during the extinction phase, but contingencies remained the same as during acquisition. The number of stomach trouble predictions were significantly higher for stimulus a+ and significantly lower for the stimulus b− at the end compared to the beginning of acquisition (left). For both stimuli mean percentage of stomach trouble predictions decreased significantly across extinction (right). Error bars denote standard errors of the mean.

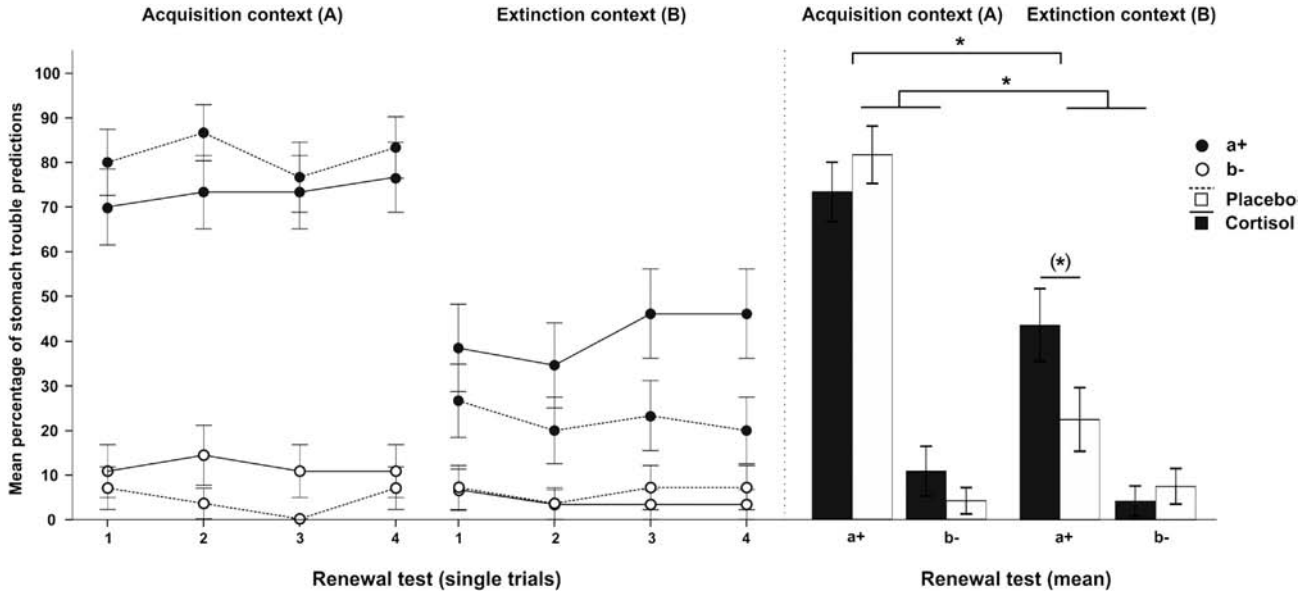


**Fig. 2.** Results of the renewal test on day 2, representing the mean percentage of stomach trouble predictions to the critical stimuli in the acquisition context and the extinction context. The single trial data is displayed on the left side. On the right side data is averaged over all four trials of the renewal test. Signs indicate whether the stimulus had (+) or had not (−) been associated with stomach trouble during acquisition. Stimuli a + (left side: black circles) and b − (left side: white circles) were presented in both, the former acquisition context (A) and the extinction context (B). Overall, the percentage of stomach trouble predictions was significantly higher to stimulus a+ than to stimulus b−. For the critical stimulus a+, which was associated with stomach trouble in context A and extinguished in context B, there was a significant difference in stomach trouble predictions between the two contexts indicative of an ABA renewal effect. Moreover, in the extinction context, the participants in the cortisol group (left side: solid lines; right side: black bars) showed impaired memory retrieval for the extinguished stimulus (a+) when compared to the placebo group (left side: dashed lines; right side: white bars; ABB; right side, left bars). Error bars denote standard errors of the mean. $^{*}p < .05$; $^{(*)}p = .055$.

Again, a significant main effect of context was found in the right posterior PHG, indicating greater activation to stimulus a+ when it was presented in the acquisition context (aA) than in the extinction context (aB; see Fig. 3B; Table 3). Furthermore, the significant treatment × sex interaction in the vmPFC, which already appeared in the overarching contrast, could be detected in this contrast as well. As illustrated in Fig. 4C, cortisol attenuated neural differentiation between the two contexts in men, but tended to increase it in women. The right amygdala deriving from the overarching contrast did not appear in the contrast aA–aB again, nor did the remaining post hoc contrasts (bA–bB, aA–bA and aB–bB) yield any further significant effects. Likewise, activation in the left accumbens which was initially revealed in the reverse contrast [aB–bB − aA–bA] could not be further tracked by the post hoc contrasts.

To have a closer look at the treatment × sex interaction, correlations between differential neural activation in the vmPFC and behavioral responses during the renewal test were conducted for men and women separately. In men, differential activation in the vmPFC deriving from the overarching contrast [aA–bA − aB–bB] tended to be positively associated with differential stomach trouble predictions on the behavioral level ($r = .33$, $p = .071$). In women, however, no significant correlation was found. For the post hoc contrast [aA–aB], again a trend for a positive correlation between differential vmPFC activation and responding to

**Table 3**
Localization and statistics of the peak voxel in the ROI analyses for the main effects as well as for the treatment × sex interactions in the overarching contrast [aA-bA minus aB-bB], and the respective post hoc contrasts [aA-aB], [bA-bB], [aA-bA], and [aB-bB]. In addition, localization and statistics of the peak voxel of the effective connectivity analyses of the main and interaction effect emerging from the post hoc contrast [aA-aB] are given.

| Contrast | Brain structure | x | y | z | $T_{max}$ | $P_{corr}$ |
|---|---|---|---|---|---|---|
| [aA–bA − aB–bB] | R posterior parahippocampal gyrus | *22* | *−38* | *−14* | 3.58 | *.065* |
| | R amygdala | *32* | *0* | *−18* | *3.57* | *.066* |
| Treatment × sex | R ventromedial prefrontal cortex | 4 | 40 | −2 | 3.12 | .048 |
| aB–bB − aA–bA | L nucleus accumbens | −12 | 8 | −10 | 3.39 | .037 |
| [aA–aB] | R posterior parahippocampal gyrus | 22 | −26 | −22 | 4.59 | .004 |
| Treatment × sex | R ventromedial prefrontal cortex | 4 | 40 | −2 | 3.10 | .049 |
| [bA–bB] | No significant activations | | | | | |
| [aA–bA] | No significant activations | | | | | |
| [aB–bB] | No significant activations | | | | | |
| Effective connectivity of the R posterior parahippocampal gyrus in the main contrast [aA–aB] | | | | | | |
| aB–aA | L amygdala | −16 | −6 | −20 | 3.96 | .021 |
| | L anterior parahippocampal gyrus | −16 | −4 | −24 | 3.90 | .045 |
| Effective connectivity of the R ventromedial prefrontal cortex in the treatment × sex interaction contrast [aA-aB] | | | | | | |
| Placebo - Cortisol | L cerebellum (WB) | −20 | −58 | −48 | 6.14 | .010 |
| | anterior cingulate gyrus | −2 | 4 | 34 | 4.39 | .046 |
| | R anterior parahippocampal gyrus | 24 | −22 | −28 | 4.06 | .033 |

The significance threshold was $p_{corr} \leq .05$ (FWE-corrected; small volume correction in SPM8). All coordinates (x, y, z) are given in the MNI space. L = left, R = right, WB = whole brain. Trends up to a threshold of $p_{corr} < .07$ are written in italics for the activation contrasts. The peak voxel from the WB analysis was labeled based on the Harvard–Oxford Cortical Structural Atlas.

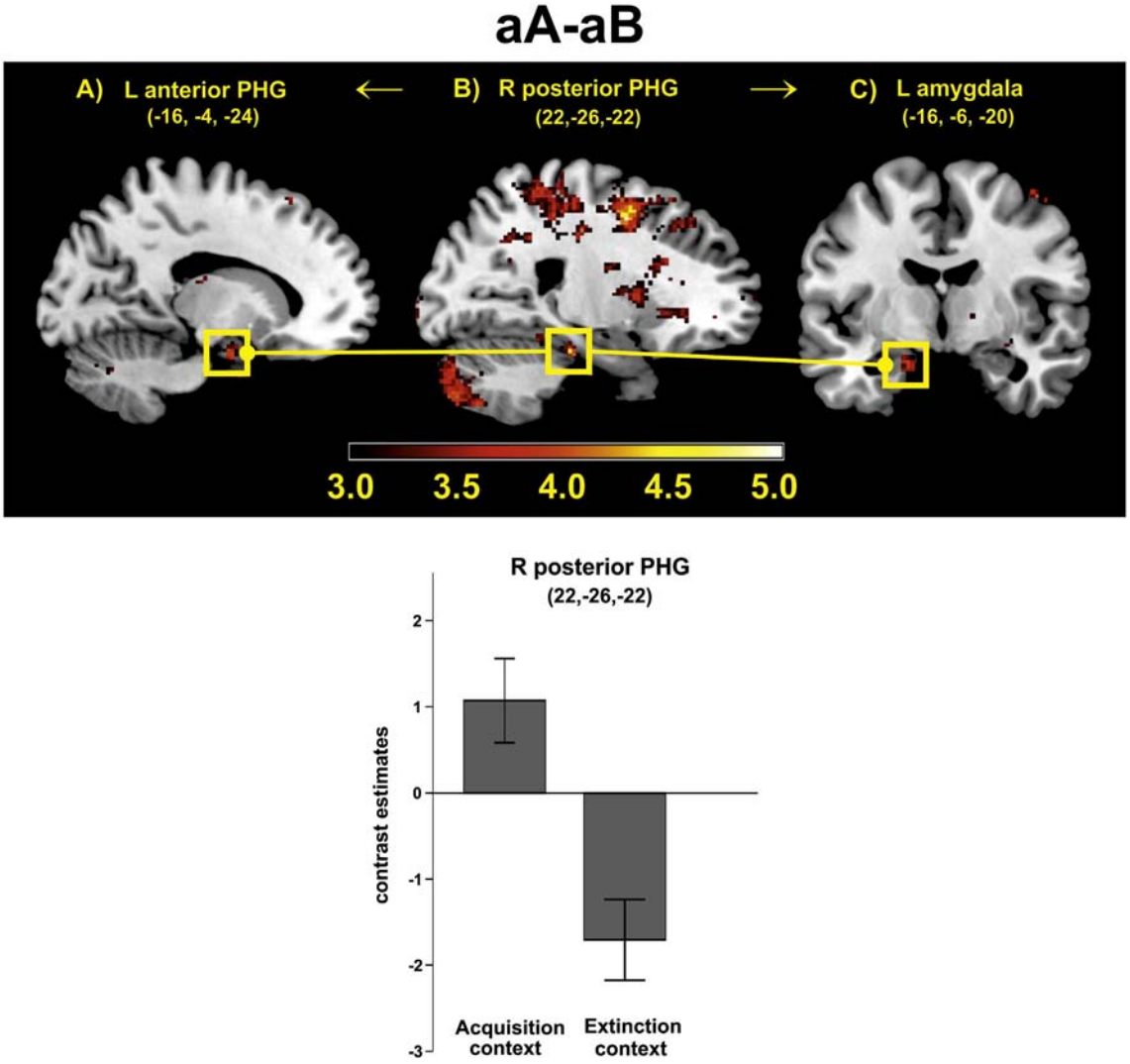


**Fig. 3.** Neural activations for the main effect of context B) and the related effective connectivities A), C) are shown for the post hoc contrast [aA–aB] (derived from the overarching contrast [aA–bA − aB–bB]) during the renewal test phase on day 2. The depicted coronal and sagittal slices were selected according to the reported activation in the right posterior parahippocampal gyrus (PHG; entered as seed region for psychophysiological interaction analyses) B) and the respective peak voxels of brain regions which were commonly activated: A) the left anterior PHG and C) the left amygdala. For demonstration purposes, data were thresholded with $T \geq 3.0$ (see color bar for exact $T$ values) and displayed on the standard MNI brain template. L = left, R = right. In the bar graphs, mean differential contrast estimates for [aA–aB] are additionally given in the respective peak voxel. B) During the renewal test, the right posterior PHG showed greater activation to the extinguished stimulus a + when it was retrieved in the acquisition context (aA) when compared to the extinction context (aB). Furthermore, the right posterior PHG was functionally connected to the left anterior PHG A) and the left amygdala C) during the renewal test. Error bars are standard errors of the mean.

stimulus a + in context A as compared to context B emerged only in men, but not in women ($r = .36$, $p = .053$).

Functional connectivity analyses were additionally realized using the peak voxels of the main and interaction effect from the contrast [aA–aB] as seeds (see Table 3). To reduce subsequent testing, we only analyzed functional connectivity for brain regions found significant in this contrast and restricted results to a threshold of $p_{corr.} < .05$. Whereas the right posterior PHG projected directly to the left amygdala and the left anterior PHG (Fig. 3A, C), functional connectivity between vmPFC and other brain regions was modulated by cortisol. In particular, the vmPFC was functionally connected to the left cerebellum (whole brain analysis), anterior cingulate and the right anterior PHG in the participants treated with placebo. However, this functional crosstalk was blocked under cortisol (Fig. 4A, B, D).

## Discussion

In the current study, we investigated the effect of the stress hormone cortisol on the neural correlates of extinction memory retrieval in the predictive learning task. Our results indicate that cortisol disrupts vmPFC functioning and its communication with PHG, ACC and cerebellum, thereby impairing the retrieval of an extinguished association.

As previously shown (Hamacher-Dang et al., 2013a, 2013b), the participants exhibited successful acquisition and extinction which did not differ between groups. Functional imaging data further supported the behavioral findings. Enhanced stimulus discrimination was found in the right hippocampus at the end of acquisition when compared to the beginning of this phase. Correspondingly, the right hippocampus was still activated at the beginning of extinction but not at the end of this phase, indicating that neural discrimination between stimuli a + and b − significantly decreased during extinction. Given the role of the hippocampus for context processing (Smith and Mizumori, 2006) and in particular for encoding a specific cue-outcome relation in a certain context (Maren, 2011), our findings add to the existing evidence that the hippocampus is involved in both the acquisition and extinction of aversive as well as neutral stimulus–outcome associations (Herry et al., 2010; Ji and Maren, 2007; Knight et al., 2004; Lissek et al., 2013; Maren, 2011; Maren and Quirk, 2004; Milad and Quirk, 2012).

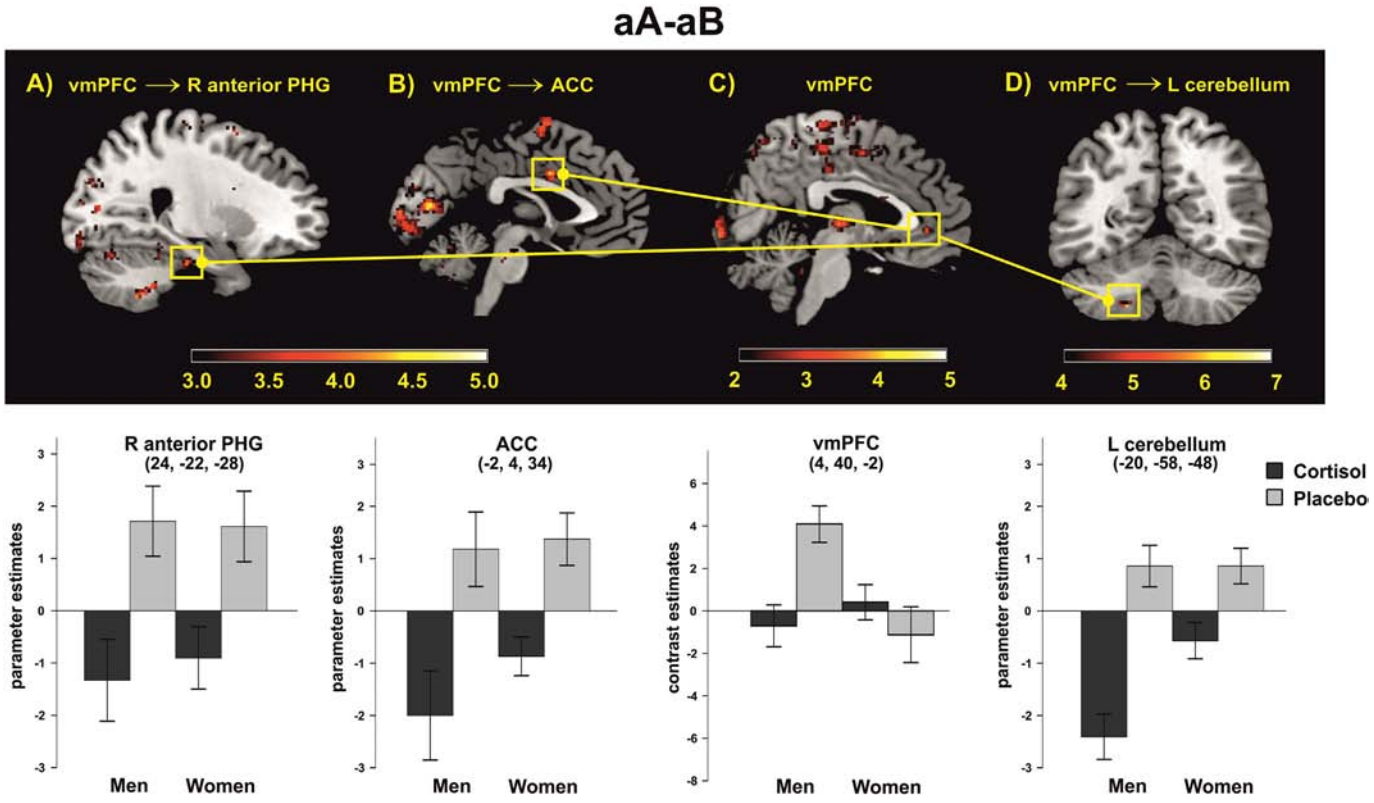


**Fig. 4.** Neural activation for the treatment × sex interaction C) and the related effective connectivities A), B), D) are shown for the post hoc contrast [aA–aB] (derived from the overarching contrast [aA–bA – aB–bB]) during the renewal test on day 2. The depicted coronal and sagittal slices were selected according to the reported activation in the ventromedial prefrontal cortex (vmPFC; entered as seed region for psychophysiological interaction analyses) C) and the respective peak voxels of brain regions which were commonly activated: A) right anterior parahippocampal gyrus (PHG), B) anterior cingulate (ACC) and D) left cerebellum. For demonstration purposes, data were thresholded with $T \geq 3.0$ A), B), $T \geq 2.0$ C) and $T \geq 4.0$ D) (see color bar for exact $T$ values) and displayed on the standard MNI brain template. L = left, R = right. In the bar graphs, mean differential contrast or parameter estimates are additionally given for the cortisol and placebo group, separately for men and women in the respective peak voxel. C) In men, cortisol significantly attenuated vmPFC activation to the extinguished stimulus a+ when presented in the acquisition context (aA) as compared to the extinction context (aB), whereas in women the opposite effect was observed. Moreover, in the placebo group the vmPFC was functionally connected to the right anterior PHG A), the ACC B) and the left cerebellum D). However, under cortisol administration functional crosstalk between vmPFC and these brain regions was diminished in both, men and women. Error bars are standard errors of the mean.

Moreover, an overall effect of contextual change occurred at the beginning of extinction. Even though contingency for the control stimulus b− did not change from acquisition to extinction, stomach trouble predictions for this stimulus increased at the beginning of extinction. Probably, some participants inferred that a context shift might be associated with a shift in contingencies (Sjouwerman et al., 2015). Correspondingly, a marked decrease of stomach trouble predictions to stimulus a+ was apparent right at the beginning of extinction after the contextual change.

During the retrieval test phase, a renewal effect was observed as indicated by more stomach trouble predictions to the extinguished stimulus in the acquisition compared to the extinction context. Consistent with that, previous studies using predictive learning tasks (Hamacher-Dang et al., 2013a, 2013b; Üngör and Lachnit, 2006) as well as fear conditioning paradigms (Bouton and Bolles, 1979; Milad et al., 2005) already demonstrated a return of conditioned responses after a context change.

Correspondingly, our data provide evidence for PHG involvement in mediating the context-specificity of extinguished memories and its renewal after a context change. Greater right posterior PHG activation was found to the extinguished stimulus when it was presented in the former acquisition context as compared to the extinction context. This altered BOLD-response suggests that the PHG may be recruited specifically during the recall phase to differentiate between the two contexts that are indicative of the acquisition or extinction memory and therefore might trigger the retrieval of the respective stimulus–outcome association. Importantly, the enhanced activation to the extinguished stimulus in the acquisition context seems to reflect the neural signature of the renewal effect that we have observed on the behavioral level. Functional connectivity analyses further revealed that right posterior PHG projected to the left anterior PHG and the left amygdala, indicating that these structures receive direct input from the PHG about the informational value of the two contexts, and thus might contribute in producing the renewal effect. Consistent with our findings, the hippocampal formation is considered to be crucially involved in context-dependent extinction memory, particularly in associating a specific context with an altered cue–outcome relation (Herry et al., 2010; Ji and Maren, 2007; Milad and Quirk, 2012; Quirk and Müller, 2008). For instance, rodent studies have demonstrated that temporary inactivation of the hippocampus prior to testing prevents contextual shifts to modulate extinction recall (Corcoran and Maren, 2001; Hobin et al., 2006). Consequently, the renewal effect which usually occurs when testing takes place outside the extinction context was completely eliminated after lesions to the hippocampus (Ji and Maren, 2005). Our results, furthermore, expand human imaging data showing that extinction memory retrieval is mediated by a prefrontal–hippocampal network (Kalisch et al., 2006; Milad et al., 2007). Interestingly, hippocampal and parahippocampal activation during extinction learning predicted the occurrence of a renewal effect during extinction recall in the predictive learning task (Lissek et al., 2013). Consistently, Merz et al. (2014a, 2014b) reported enhanced posterior PHG activation during the first trials of extinction learning, probably reflecting the establishment of a new inhibitory memory trace. Therefore, we suggest that extinction processes are not necessarily restricted to the hippocampus itself, but might expand to surrounding structures of the hippocampal formation.

Obviously, our results cannot be directly transferred to classical fear conditioning in which the participants experience an aversive event

themselves. Nevertheless, besides differences, there are also fundamental similarities between both learning experiences. In either case, the occurrence of an outcome has to be predicted on the basis of the presence or absence of specific stimuli. Moreover, phenomena occurring after successful extinction such as spontaneous recovery, reinstatement or renewal have been observed in both learning types (Bouton, 2002; Üngör and Lachnit, 2006; Vila and Rosas, 2001a, 2001b). Thus, predictive learning and fear conditioning seems to be governed by similar basic associative learning mechanisms that are apparently in part independent from task valence.

In line with our hypothesis, cortisol impaired the retrieval of extinguished associations, as reflected by a stronger return of the initially acquired behavioral response after cortisol administration. Thus, our results, confirm previous findings from laboratory studies reporting a reemergence of extinguished fear after acute stress in rats (Deschaux et al., 2013) and humans (Raio et al., 2014; but see Merz et al., 2014a) and extend them to pharmacologically elevated cortisol concentrations. Similarly, a recent work from our lab has shown a detrimental effect of stress on the retrieval of extinction memory in the predictive learning paradigm as well (Hamacher-Dang et al., 2013b). Moreover, the cortisol-induced memory impairments observed in the present study parallel evidence regarding GC effects on the retrieval of declarative memory (Buchanan et al., 2006; Kuhlmann et al., 2005; Smeets, 2011).

Of note, a pharmacological administration of cortisol cannot be directly translated to the effects of acute stress which always entails both, noradrenergic activity and glucocorticoid release. Beyond, psychological factors that characterize a stressful situation but fail to appear in pharmacological studies might play an important role. Due to the mechanistic approach used in the present study, we provide evidence that the stress hormone cortisol is directly related to impaired extinction memory retrieval which is in line with our previous behavioral stress study (Hamacher-Dang et al., 2013b). Nevertheless, the effects of a stress-induced physiological cortisol response on the neural correlates of extinction memory should be tested to complement the picture.

Importantly, cortisol attenuated context differentiation in the vmPFC particularly in men, as indicated by decreased BOLD-responses to the extinguished stimulus in the acquisition compared to the extinction context. Moreover, correlation analyses revealed that neural differentiation in the vmPFC was positively associated with differential stomach trouble predictions to stimulus a+ in context A as compared to context B, but again only in men. Accordingly, Lissek et al. (2015, 2013) found vmPFC activation to be associated with recall performance in the predictive learning task. A recent fMRI-study further revealed that cortisol administration disrupted medial frontal cortex activation during fear extinction in men (Merz et al., 2014b). In accordance with these findings, GCs have been implicated in reducing prefrontal activation in general (Arnsten, 2009) and in particular during memory retrieval (Oei et al., 2007).

Interestingly, greater vmPFC signaling to the extinguished stimulus was detected when retrieval took place in the former acquisition context as compared to the extinction context in placebo men (cf. Fig. 4C). Thus, the vmPFC seems to mirror the same activation pattern we had already observed in the PHG. Given the proposed interplay between prefrontal and hippocampal structures in mediating extinction recall, the current results therefore indicate that contextual information might be communicated between the PHG and the vmPFC. Supporting this line of argumentation, effective connectivity analyses in the present study revealed that the vmPFC was functionally connected to the PHG, the cerebellum and the ACC in the placebo group. However, this functional crosstalk was blocked under cortisol treatment. In consequence, extinction memory retrieval was disrupted, as confirmed by a stronger return of the initially acquired response. These results parallel previous findings, showing that vmPFC interacts with hippocampal structures to regulate contextual gating of extinction recall (Milad and Quirk, 2012; Milad et al., 2007).

Correspondingly, the anterior cingulate and the cerebellum are also associated with extinction processes (Kattoor et al., 2014; Lissek et al., 2013; Merz et al., 2012a; Utz et al., 2015). For instance, ACC activation has been recently detected during extinction recall but predominantly during ABA test trials, suggesting a role of this structure in mediating the renewal effect (Lissek et al., 2013). In a broader sense, the ACC has been related to error monitoring (Iannaccone et al., 2015) and response selection, especially when two conflicting response tendencies are available (Kennerley et al., 2006; Walton et al., 2007). Given its role in these evaluative processes, the increased functional coupling between vmPFC and ACC we observed during ABA trials might therefore reflect an integration of a decision process that has to be made whether to respond according to the combination of context and cue or to the cue alone.

Concerning the prefrontal–cerebellar projections, human lesion studies provide initial evidence that the cerebellum considerably contributes to emotional and cognitive associative learning as well (Timmann et al., 2010). Consistent with that view, the cerebellum was recently shown to be involved in both, the acquisition and extinction of conditioned eyeblink (Gerwig et al., 2006; Thürling et al., 2015) and fear responses (Utz et al., 2015) as well as in abdominal pain-related associative learning processes (Kattoor et al., 2013, 2014). Cerebellar involvement in associative learning and extinction processes might be based on the ability of this structure to provide correct predictions about the relationship between sensory stimuli (Timmann et al., 2010).

Taken together, our functional connectivity data suggest the vmPFC to be one of the key players initiating the recruitment of secondary brain regions such as the PHG, the ACC and the cerebellum to successfully retrieve the association between a context and the respective cue–outcome relation during memory retrieval. Importantly, cortisol interferes with this interregional communication and thereby impairs extinction retrieval. In accordance with these results, evidence from resting-state connectivity analyses indicates that stress hormones mainly disrupt functional crosstalk between structures implicated in extinction (Clewett et al., 2013).

For the current study, it is however important to note that the cortisol effects on regional vmPFC activation predominated in men, whereas in women, this effect was hardly detectable or rather tended to oppose the activation pattern observed in men (slight increase in vmPFC signaling). Consistent with that, sex hormones and specifically OC usage has been shown to alter the neural correlates of extinction learning (Graham and Milad, 2013; Merz et al., 2012a; Milad et al., 2006; Zeidan et al., 2011). Moreover, sex hormones are known to modulate how stress influences vmPFC functioning (Maeng et al., 2010; Shansky et al., 2010). Interestingly, previous work revealed the same sex-specific cortisol/stress effect on the neural correlates of fear conditioning, with BOLD-signal reductions in men and increases specifically in OC women but not free-cycling women (Merz et al., 2012b, 2013). Thus, OC usage and not sex per se appears to substantially modulate cortisol effects on the neural correlates of conditioning and extinction processes. In accordance, cortisol administration has been found to impair declarative memory retrieval in naturally cycling women but not in OC women, suggesting that OC usage might be associated with a generally reduced sensitivity of the brain to acute cortisol elevations (Kuhlmann and Wolf, 2005).

Sparse evidence for such a global neurobiological mechanism of OC effects can be derived from the animal literature. OCs contain exogenous sex hormones such as ethinylestradiol which act peripherally and centrally to suppress the production of endogenous sex steroids (Lobo and Stanczyk, 1994; Nielsen et al., 2014). The gestagenic compound in OCs binds to progestin receptors, whereas the main estrogenic component binds to estrogen receptors (ERs). In the vmPFC, GRs co-localize with ER expression which might render this structure particularly susceptible to sex-stress hormone interactions (Cover et al., 2014; de Kloet, 2004; Montague et al., 2008). Furthermore, the altered hypothalamus–pituitary–gonadal axis activity in OC women, which has bidirectional interactions with the HPA axis (Turner et al., 2012),

could account for the distinct effects of cortisol on brain activation in men and women. Due to the high percentage of women using OCs, future studies are warranted dissecting how these endogenous hormones affect extinction processes and their modulation by stress.

Another important issue that needs consideration is the time between extinction and recall phase. In fact, recent works in rodents (Woods and Bouton, 2008) as well as in humans suggest that the timing of extinction training considerably influences extinction retention (Maren, 2014). However, different designs and variation in the timing of stress or cortisol administration relative to acquisition, extinction or extinction recall complicates the picture of cortisol effects on extinction and extinction recall. We applied a two-day paradigm with successive phases of acquisition and extinction learning on day 1 and a retrieval test phase on the following day. Thus, the mechanisms that affect the consolidation of the two acquired memories could also account for differences in the renewal test phase. For instance, it is known that sleep strongly influences memory consolidation (Diekelmann and Born, 2010; Diekelmann et al., 2009; Marshall and Born, 2007; Stickgold, 2005). Accordingly, animal and human studies demonstrated that sleep can serve to consolidate memory for fear and fear extinction (Menz et al., 2013; Pace-Schott et al., 2015). Thus, information on sleep duration and sleep quality would have provided important additional information for the interpretation of the current results. Moreover, cortisol plays an important role both in modulating sleep and memory function (Born and Wagner, 2009; Payne, 2011). Correspondingly, HPA-activity and in particular cortisol release has been shown to affect memory consolidation during sleep (Born and Wagner, 2004; Wagner and Born, 2008). Although we sampled salivary cortisol on day 1, we cannot rule out that alterations in diurnal/nocturnal cortisol secretion could have modulated the consolidation of acquisition or/and extinction memory and thus affected memory performance on day 2. To address this issue, the assessment of a cortisol day profile could provide interesting information to be included in future studies. Altogether, the exact timing between cortisol administration, acquisition, extinction and extinction recall (also in relation to sleep) should be considered in future studies.

## Conclusions

In conclusion, our findings demonstrate that administration of cortisol substantially disrupts vmPFC functioning and its communication with PHG, ACC and cerebellum, leading to an impairment of extinction memory retrieval in the predictive learning task. The PHG appears to have a crucial role in providing contextual information about the learned associations which is then used by the vmPFC to gate a correct response to the specific cue–outcome relation. Cortisol, however interferes with these processes and thereby impairs extinction memory retrieval. Whether our results can be extended to more emotional learning tasks such as fear conditioning remains an open question. Furthermore, the sex-specific cortisol-effects observed in the current study eminently emphasize the need to further explore the interplay of stress and sex hormones in the modulation of extinction memory. Such studies may foster our understanding of the basic learning and memory processes involved in extinction and may further elucidate how stress is becoming a potential risk factor for relapses in patients with psychiatric disorders.

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