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What a difference timing makes: Cortisol effects on neural underpinnings of emotion regulation

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ABSTRACT

The ability of emotion regulation under stress is of crucial importance to psychosocial health. Yet, the dynamic function of stress hormones for the cognitive control of emotions over time via non-genomic and genomic cortisol effects remains to be elucidated. In this randomized, double-blind, placebo-controlled neuroimaging experiment, 105 participants (54 men, 51 women) received 20 mg hydrocortisone (cortisol) or a placebo either 30min (rapid, non-genomic cortisol effects) or 90min (slow, genomic cortisol effects) prior to a cognitive reappraisal task including different regulatory goals (i.e., downregulate vs. upregulate negative emotions). On the behavioral level, cortisol rapidly reduced and slowly enhanced emotional responsivity to negative pictures. However, only slow cortisol effects improved downregulation of negative emotions. On the neural level, cortisol rapidly enhanced, but slowly reduced amygdala and dorsolateral prefrontal activation as well as functional connectivity between both structures in the down- minus upregulate contrast. This interaction speaks for an effortful but ineffective regulation of negative emotions during rapid cortisol effects and improved emotion regulation capacities during slow cortisol effects. Taken together, these results indicate a functional shift of cortisol effects on emotion regulation processes over time which may foster successful adaptation to and recovery from stressful life events.

1. Introduction

Emotion regulation (ER) is crucial for quick and adequate coping with emotionally challenging events in daily life, thus representing an important prerequisite for mental health (Sheppes et al., 2015). ER competencies are particularly needed under acute stress protecting individuals from chronic stress states. Therefore, the influence of stress hormones on ER processes and the underlying neuroendocrine mechanisms must be understood more closely in order to develop related intervention and prevention approaches.

Cognitive ER is defined as all automatic or deliberate attempts to change the natural flow of an emotional response via cognitive processes (i.e., appraisal, attentional deployment; Ochsner and Gross, 2005). Cognitive reappraisal is one of the most effective ER strategies (Webb et al., 2012) and refers to a reinterpretation of a given stimulus to either up- or downregulate the emotional response according to the active regulatory goal. Executive functions like working memory, planning, and set shifting are essential for successful cognitive reappraisal (McRae et al., 2012). Several meta-analyses evidenced that cognitive reappraisal involves an extensive network of executive control regions (e.g., lateral and medial prefrontal cortex (PFC), orbitofrontal cortex) dampening activation in emotion-associated subcortical structures such as the amygdala (Kohn et al., 2014; Ochsner et al., 2012).

The stress hormone cortisol binds to mineralocorticoid (MR) and glucocorticoid receptors (GR) that are numerously located in the PFC and the amygdala thereby affecting cognitive and affective functioning timing-dependently (Hermans et al., 2014; Joëls et al., 2013). Generally, stress reduces prefrontal control functions while directing attentional resources towards salient, emotion-related stimuli (Arnsten, 2009). More specifically, stress hormones impair cognitive flexibility (Shields

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et al., 2016), weaken people's ability of goal-directed self-control (Maier et al., 2015; Quaedflieg et al., 2019) and amplify emotional attention biases (Rued et al., 2019) to ensure adaptive behavior towards threat related cues. Importantly, cortisol effects on cognitive and affective functions underly temporal dynamics comprising a rapid, non-genomic and a slow, genomic pathway. Non-genomic cortisol effects occur immediately and as long as cortisol levels are elevated, mediated by receptors located in the cell membrane. The genomic pathway acts via intracellular receptors and takes at least 60min to initiate its effects which then continue for several hours (Joëls et al., 2013). Imaging data points at non-genomic cortisol actions to promote salience network activation (including amygdala and thalamus) at the cost of executive control functioning while genomic cortisol effects might reverse these initial neural effects to restore homeostasis (Hermans et al., 2014). Consistently, on the behavioral level, cortisol reduced fear memory generalization (van Ast et al., 2013) and protected working memory performances (Henckens et al., 2011) when tested >210min but not 30min after cortisol administration. Similar timing-specific cortisol effects emerged for selective attention (Henckens et al., 2012b) or emotional face recognition (Henckens et al., 2010) with distinct brain activation patterns for rapid and slow cortisol effects (Henckens et al., 2012a). Taken together, timing-dependent cortisol effects have been observed for various cognitive and affective processes (de Kloet, 2004; Henckens et al., 2012b).

Existing evidence for stress hormone effects on cognitive ER is still scarce and inconsistent. For instance, stress has been either shown to impair (Raio et al., 2013), improve (Kinner et al., 2014) or having no effect at all on the ability to downregulate negative emotions (Ma et al., 2017; Sandner et al., 2021; Shermohammed et al., 2017). One potential moderator that might explain heterogenous findings is the timing between stress exposure and the ER task. In line with this notion, stress rapidly impaired the regulation of conditioned fear responses (Raio et al., 2013), while cortisol administration somewhat delayed reduced amygdala activation during reappraisal suggesting slow effects of cortisol to facilitate the downregulation of negative emotions (Jentsch et al., 2019). However, in contrast to these findings, there is also evidence from our lab that stress rapidly improves the effectiveness of reappraisal in men which was critically associated with cortisol increases (Langer et al., 2020). Following up on that, we directly compared rapid and slow cortisol effects on ER outcomes in a single study design and found a timing-independent cortisol-driven improvement of ER on the behavioral level (Langer et al., 2022). Despite some hints in favor of general (i.e., both, rapid and slow) beneficial cortisol effects on ER capacities, timing had never been varied systematically in a neuroimaging experiment before.

In addition, most research done so far has focused on pro-hedonic goals (e.g., downregulation of negative emotions, Jentsch et al., 2019; Langer et al., 2022; Raio et al., 2013; Sandner et al., 2021) without taking contra-hedonic goals (e.g., upregulation of negative emotions) into account. However, adequate adaptation to emotionally challenging situations may sometimes also require contra-hedonic regulation (Riediger, 2015; Tamir and Bigman, 2014). For instance, upregulation of anger helps an individual to assert his/her interest, fear protects from a bodily or psychological threat, guilt can help to learn from wrongdoings and may benefit social competencies and sadness is an important component of grief processes in response to a significant loss. In fact, there are effective psychotherapeutic interventions which act via upregulation of certain negative emotions (emotion-focused therapy; Greenberg, 2008) suggesting contra-hedonic regulation to be crucial for mental health. Beyond, downregulation of positive emotions (e.g., joy) is needed if a person has to concentrate on a certain working task or in situations where the expression of positive emotions would be socially inappropriate (e.g., downregulating laughter when witnessing another's misfortune; Webb et al., 2012). Together, in some situations contra-hedonic regulation can be at least equally important as pro-hedonic regulation.

In general, deliberate attempts to up- and downregulate negative emotions involve overlapping cognitive control regions affecting amygdala activity in the respective direction (Eippert et al., 2007; Frank et al., 2014). Yet, different regulatory goals have also been shown to act on distinct affect-generating structures. For instance, the downregulation of negative emotions more strongly targets interoceptive brain regions such as the postcentral gyrus, whereas upregulation more strongly modulates regions associated with emotional experience such as the amygdala, whereas the insula is related to both processes (Min et al., 2022). Given that cortisol rapidly strengthens emotional processing and attention towards threat-related information while slowly boosting cognitive control mechanisms (Hermans et al., 2014), it is reasonable to assume that cortisol may exert different effects on cognitive ER dependent on timing and the regulatory goal. Inclusion of opposite regulatory goals (i.e., up- vs. downregulation) may thus expand previous work exploring possible goal-specific cortisol effects on ER processes.

In the current study, we included two cortisol and their respective placebo groups with the first dataset testing rapid cortisol effects on ER (+30min) and a second dataset testing slow cortisol effects on ER (+90min). In addition to viewing neutral and negative pictures, we used two reappraisal conditions (up- and downregulation of negative pictures) as our primary focus, expecting most robust emotional intensity differences when contrasting these two opposing goals. Based on previous evidence for rapid detrimental effects of cortisol on executive control functions followed by delayed beneficial effects (e.g., Hermans et al., 2014; Shields et al., 2016), we predicted impaired ER (i.e., increased intensity ratings for downregulation/decreased intensity ratings for upregulation) in the rapid cortisol group and enhanced ER (i.e., decreased intensity ratings for downregulation/increased intensity ratings for upregulation) in the slow cortisol group. The PFC and the amygdala are consistently affected by reappraisal (Buhle et al., 2014; Frank et al., 2014) and sensitive to stress hormones (Henckens et al., 2010, 2012a, 2012b). Thus, on the neural level, we expected cortisol to rapidly decrease PFC and enhance amygdala activation and slowly increase PFC and decrease amygdala activity in the down- vs. upregulation contrasts. However, based on previous work from our lab suggesting timing-independent beneficial cortisol effects on the ability to downregulate negative emotions (Jentsch et al., 2019; Langer et al., 2020, 2022), cortisol could alternatively also enhance down- relative to upregulation in both time windows. Additionally, potential sex-dependent cortisol effects on ER were explored as before where acute stress affected ER in a sex-dependent manner (Kinner et al., 2014; Langer et al., 2020).

2. Material and methods

2.1. Participants

The current study is part of a larger project investigating cortisol effects on the neural basis of emotional processes, in which all current subjects participated (reported elsewhere: Hagedorn et al., 2022, 2021). They were all scanner experienced, since participants were already scanned the day before the ER paradigm was realized. This way, possible anticipatory stress due to the MRI environment (Gossett et al., 2018; Lueken et al., 2012; Muehlhan et al., 2011) was minimized, but might still play a role.

In total, 110 participants were recruited at the Ruhr University Bochum via flyers on the campus or online advertisements. In a standardized telephone interview, the following exclusion criteria were checked: chronic or acute illnesses, history of psychiatric or neurological treatment, standard MRI contraindications, drug use including smoking, regular medication, age <18 or >40 years, body mass index <18 or >27 kg/m^2, working in night shifts as well as vaccination, blood donation and traveling to a country with a time difference in the last month. All participants were right-handed, had normal or corrected-to-normal

vision and were not familiar with the used ER paradigm. In addition to men, naturally cycling women were included only and tested outside their menses to avoid potential confounds due to the intake of hormonal contraceptives (Jentsch et al., 2022).

Five participants (all from the second dataset) had to be excluded, due to a) not completion of the ER paradigm (one woman), b) zero values on cortisol data (one woman and one man), c) reported drug intake (one woman) and d) issues with fMRI data processing (one woman). Thus, a total of 105 participants remained with the following distribution: the first dataset tested rapid, immediate cortisol effects in 13 men and 11 women compared to placebo (12 men, 14 women), the second dataset tested slow, delayed cortisol effects in 15 men and 12 women compared to placebo (14 men, 14 women).

2.2. General procedure

The experiments were performed between 1 and 9p.m. After arrival, participants were informed about all experimental procedures including cortisol administration and fMRI. Afterwards, informed consent was given before demographic questionnaires were filled out. For the two immediate groups, the ER task was scheduled 25–30min after administration of cortisol or placebo. For the two delayed groups, the ER task took place 90–95min after tablet intake. Salivary cortisol samples were collected at different time points during the whole procedure (see below). Participants who completed the experiment received a compensation of 15€. The procedure was approved by the ethics committee of the Medical Faculty at the Ruhr University Bochum (registration no. 16–5789) and executed in accordance with the Declaration of Helsinki. In Fig. 1, the experimental procedure, changes in cortisol concentrations over time and an example trial of the ER task are illustrated.

2.3. Emotion regulation paradigm

A modified version of the ER paradigm used in previous studies (Jentsch et al., 2019; McRae et al., 2008; Ochsner et al., 2004) was applied. Participants were required to watch neutral and negative pictures or to regulate their emotions towards negative pictures using cognitive reappraisal. Four experimental conditions were defined: 1) view neutral (passively viewing neutral pictures), 2) view negative (passively viewing negative pictures), 3) downregulate (reappraise the presented situation on the picture by imagining a positive context or outcome to downregulate negative emotions), and 4) upregulate (reappraise the presented situation on the picture by putting oneself in the position of the observed person on the picture or imagining a disastrous outcome to upregulate negative emotions).

In each trial, the following instructional cues were first presented for 1.5s: “view”, “decrease” or “increase” indicating passively viewing

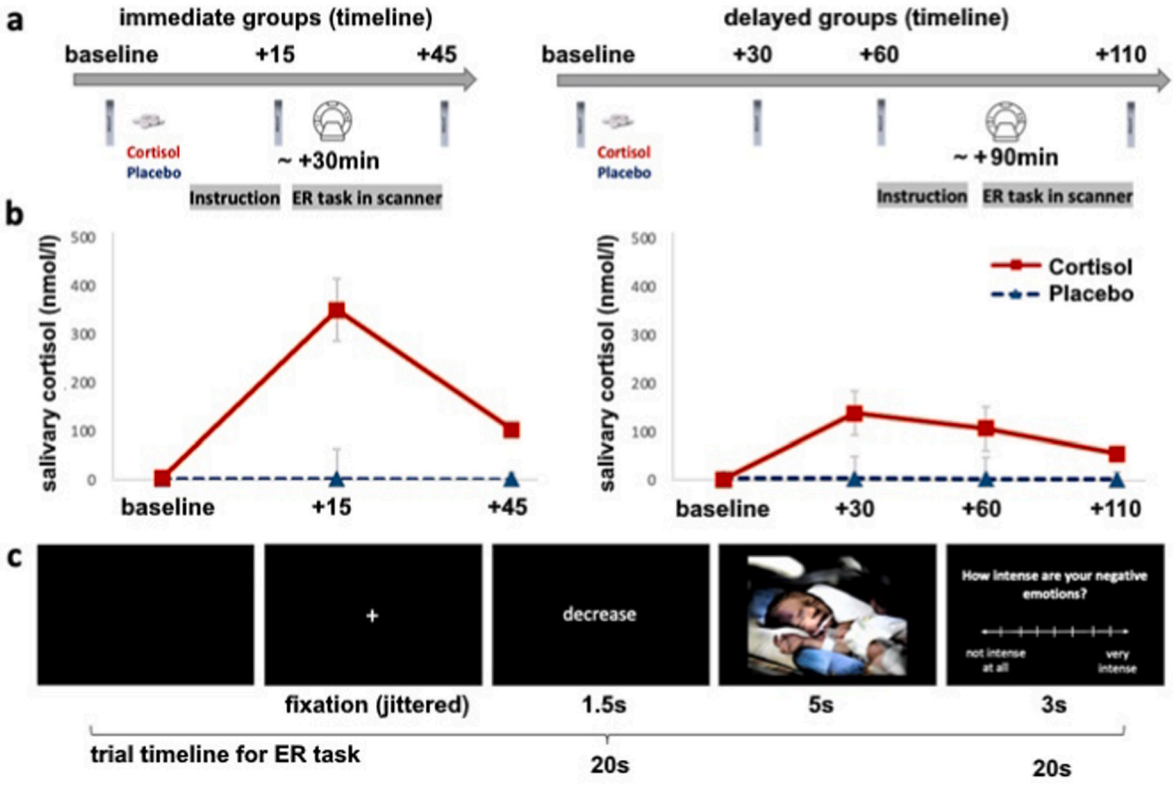

Fig. 1. Experimental design including the timing of cortisol/placebo administration with regard to the onset of the emotion regulation (ER) paradigm (a), cortisol concentrations over time (b) and an exemplary trial of the ER paradigm (c). a) The two immediate groups underwent the ER paradigm 25–30min after pharmacological manipulation. Participants of the two delayed groups were tested in the ER task 90–95min after tablet intake. Time points of saliva samples for cortisol concentrations slightly differed between the two datasets due to the different timing required for rapid vs. slow cortisol effects. b**)** In the line charts, mean cortisol concentrations (error bars represent standard errors of the mean) over time are illustrated separately for the cortisol and the placebo groups. Please note that peak cortisol concentrations of the two datasets were different, which reflect different sampling time points (15min (immediate group) and 30min (delayed group) after tablet intake) explaining the distinct result patterns. Of note, the observed cortisol concentrations after the intake of 20 mg hydrocortisone were elevated to supraphysiological levels. Thus, it is reasonable to assume that most mineralocorticoid and glucocorticoid receptors have been occupied in both datasets. Differences in these supraphysiological cortisol levels will most likely not be associated with any differential effect on ER processes. **c)** Trial timeline for the ER paradigm. The ER paradigm consisted of the following four conditions: view neutral, view negative, upregulate negative and downregulate negative. An instructional cue giving information about the condition was given before picture presentation (in this example, “decrease” for downregulate negative). Participants were required to rate the emotional intensity immediately after picture presentation.

(either neutral or negative pictures), down- or upregulation (of negative pictures) respectively. Afterwards, pictures were presented for 5s followed by a rating screen for additional 3s, asking participants to rate the intensity of the evoked negative emotions on a 7-point Likert scale (1 = "not at all", 7 = "very strong"). Inter-trial intervals depicting a white fixation cross on a black screen were randomly jittered leading to a total trial duration of 20s. Ten pictures were used for each of the four conditions and presented in eight blocks consisting of five pictures of the same condition in succession. One block of each condition was presented in each half of the experiment. Order of conditions was pseudorandomized and balanced between groups.

Before the start of the fMRI scan, participants were given detailed instructions about what to do when seeing the four instructional cues (one practice trial for each condition outside the fMRI scanner without time restrictions) with the possibility of asking questions anytime. Within the scanner, participants conducted six additional practice trials (1x view neutral, 1x view negative, 2x decrease, 2x increase) before the experimental run started. Pictures used for practice trials were not included in the actual ER task.

Pictures were selected from the Nencki Affective Picture System (Marchewka et al., 2014) based on available normative ratings. In total, 30 negative pictures (valence: $M = 2.99$, $SD = 0.51$; arousal: $M = 6.58$, $SD = 0.57$) and 10 neutral pictures (valence: $M = 5.97$, $SD = 0.37$; arousal: $M = 4.20$, $SD = 0.21$) were used. Arousal and valence ratings differed significantly between the sets of negative and neutral pictures (both $ps < .001$). All pictures were landscape (1024 pixels × 768 pixels) in orientation and matched for content and complexity.

Stimulus presentation and behavioral recordings were controlled by MATLAB R2012a (MathWorks Inc., Sherborn, MA) on an IBM compatible PC running Windows 7 and presented to the participants via fMRI-ready goggles (VisuaStim Digital; Resonance Technology Inc., Northridge, CA, USA). Emotional intensity ratings were given on an fMRI-ready keyboard (LUMItouch™ response pad; Photon Control Inc., BC, Canada).

2.4. Cortisol administration, saliva sampling and analyses

For both datasets, a double-blind, randomized, placebo-controlled design was realized. Participants in the cortisol groups received two 10 mg tablets of cortisol (hydrocortisone; Hoechst), whereas participants in the placebo groups received visually identical placebos. Saliva samples were taken using Salivettes (Sarstedt, Nümbrecht, Germany). Saliva sampling in the immediate group consisted of three times of measurement: prior to tablet intake as baseline, 15min (before ER) and 45min after tablet intake (after ER). Saliva sampling in the delayed group consisted of four times of measurement: prior to tablet intake (baseline), 30min, 60min (before ER) and 110min after tablet intake (after ER).

The dosage of 20 mg cortisol was chosen based on previous studies from our laboratory and other groups reporting a clear modulation of behavioral and brain responses with similar dosages, i.e., 10–30 mg (Hagedorn et al., 2021; Henckens et al., 2010, 2011, 2012a; Jentsch et al., 2019; Langer et al., 2022). Saliva samples were stored at −20 °C until assayed. Commercially available enzyme-linked immunosorbent assays (IBL, International, Hamburg, Germany) subserved to measure free cortisol concentrations. Inter- and intra-assay coefficients of variation were below 10%.

2.5. Statistical analyses

Statistical analyses were performed using IBM SPSS 22 Statistics for Windows (IBM Corp., Armonk, NY, USA) with the significance level set to $\alpha = 0.05$. Cortisol concentrations over time were analyzed separately due to the different collection times of saliva samples for the two datasets. A mixed analysis of variance (ANOVA) with the factors treatment (cortisol/placebo), sex (men/women), and time (baseline/+15min/+45min) was conducted for the immediate cortisol dataset. In the delayed cortisol dataset, the factor time comprised the factor levels baseline, +30min, +60min and +110min. For statistical analyses of cortisol concentrations, transformed values [natural logarithm transformation: LN (1 + nmol/l)] were used to attain a normal distribution.

To analyze intensity ratings during the ER task, mixed ANOVA with timing (immediate/delayed), treatment, sex, and condition (view neutral/view negative/downregulate negative/upregulate negative) was firstly conducted. To better examine potential interaction effects and considering that the fMRI data used contrasts between conditions, we also implemented comparable analyses with the following condition contrasts for intensity ratings: 1) emotion induction effect: rating of view negative – view neutral; 2) pure downregulation effect: rating of view negative – downregulate negative (note, unlike in our fMRI analysis where the contrast was anchored by downregulation, here the calculation was on viewing negative minus downregulation); 3) pure upregulation effect: upregulate negative – view negative; 4) regulation differentiation effect: upregulate negative – downregulate negative (again, unlike in our fMRI analysis where the contrast was anchored by downregulation). Mixed ANOVA with the factors timing, treatment and sex was conducted for each contrast. More detailed analyses encompassed the placebo groups only, the cortisol groups only and the comparison including three groups (placebo/immediate cortisol/delayed cortisol) for all results, in which a treatment effect occurred. Greenhouse-Geisser corrected p-values were used if the assumption of sphericity was violated and partial η^2 were reported as estimations of effect sizes.

2.6. fMRI data acquisition and analyses

Functional and structural brain scans were acquired using a whole-body 3T scanner (Philips Achieva 3.0 T X-Series, Philips, the Netherlands) with a 32-channel SENSE head coil. Structural images were obtained with an isotropic T1 TFE sequence (FOV = 240mm × 240mm; voxel size = 1mm × 1mm × 1mm) and comprised 220 transversally orientated slices covering the whole brain. Functional images encompassed 40 ascending slices measured parallel to the orbitofrontal bone transition (FOV: 192mm × 192mm, voxel size: 2mm × 2mm × 3mm) obtained with a T2 weighted gradient echoplanar imaging sequence (TR: 2.5s, TE: 30ms, flip angle: 67°, slice gap: 0.75mm). During the ER scanning session 335 volumes were registered. Three dummy scans preceded data acquisition during which magnetization could reach steady state (in addition, the first three volumes of the functional data were discarded).

For preprocessing and statistical analyses, we used the software Statistical Parametric Mapping (SPM12; Wellcome Department of Cognitive Neurology, London, UK), implemented in Matlab 2017a, 2020a (Mathworks Inc., Sherborn, MA). Preprocessing contained realignment, slice time correction, co-registration to the participant's structural image, normalization to MNI standard space, and smoothing using an 8 mm FWHM Gaussian kernel.

The statistical model for each participant (first level) included the following regressors: view neutral (NEU), view negative (NEG), downregulate, upregulate), with each condition consisting of two separated halves, button presses, instructional cues and intensity ratings. 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. A high pass filter with a time constant of 128s was used to remove slow signal drifts. Similar to previous ER studies (Buhle et al., 2014; Frank et al., 2014; Jentsch et al., 2019), random effect group analyses were conducted and focused on the following contrasts: [emotion induction effect: view NEG vs. view NEU], [downregulation effect: downregulate vs. view NEG], [upregulation effect: upregulate vs. view NEG] and [regulation differentiation effect:

downregulate vs. upregulation]. An ANOVA was conducted with the group factors timing, treatment and sex in the full factorial model implemented in SPM12. More detailed analyses encompassed the placebo groups only, the cortisol groups only and the comparison including three groups (placebo/immediate cortisol/delayed cortisol) for all results, in which a treatment effect occurred.

For all statistical analyses, we used region of interest (ROI) analyses targeting brain regions identified in previous meta-analyses examining ER processes indicating amygdala regulation by the PFC (Berboth and Morawetz, 2021; Buhle et al., 2014; Webb et al., 2012). Masks for the dorsolateral PFC (dlPFC), ventrolateral PFC (vlPFC) and dorsomedial PFC (dmPFC) were based on the anatomical parcellation of the MNI brain as described by Tzourio-Mazoyer et al. (2002) and created with MARINA (Walter, 2002). Considering the amygdala as the brain region showing the strongest evidence to be modulated by both up- and downregulation (Buhle et al., 2014; Frank et al., 2014) and affected by cortisol administration (Jentsch et al., 2019), we employed the following integrated and extensive approach to better define cortisol-related effects on ER. We firstly obtained the amygdala mask from the online meta-analysis platform Neurosynth (https://neurosynth.org) using the term "emotion regulation" (leading to an automated meta-analysis of 247 studies with $p_{FDR} < 0.01$). Then, we included published meta-analyses of fMRI studies on ER indicating that the amygdala is modulated by reappraisal (consisting of a 6 mm sphere around the reported peak voxel of these meta-analyses; right amygdala MNI: x = 27, y = 6, z = −12; left amygdala MNI: x = −18, y = −3, z = −15, Buhle et al., 2014; right amygdala MNI: x = 20, y = 0, z = −14; left amygdala MNI: x = −20, y = −6, z = −14, Frank et al., 2014. Finally, we incorporated amygdala coordinates previously shown to be modulated by cortisol during ER (defined as 6 mm sphere around the reported peak voxel of empirical research; MNI: x = 26, y = 2, z = −26; Jentsch et al., 2019). All these regions were binarized and merged to serve as (bilateral) amygdala ROI in the current study. In addition, brain regions typically involved in emotional arousal and more goal-specific regulation such as the insula and anterior cingulate cortex (ACC) were also included and defined as ROIs using maximum probability masks (1 mm; threshold: 0.25) from the Harvard-Oxford Cortical- and Subcortical-Atlases.

Moreover, functional connectivity was investigated using psychophysiological interaction (PPI) analyses as implemented in SPM12 (Friston et al., 1997; Gitelman et al., 2003; O'Reilly et al., 2012). For this purpose, significantly activated ROIs derived from the main contrasts were entered as seed regions (volume of interest with a 5 mm sphere around the peak voxel) in PPI analyses along with the interaction timeseries. Correction for multiple comparisons at a significance level of $p \leq .05$ was restricted to the predefined ROIs using small volume correction with family-wise error (FWE) correction. In addition, results of complementary exploratory whole brain analyses with a minimal cluster size of 10 voxels are provided. For the full factorial design, we strictly refer to the F statistics (including main and interaction effects). Subsequent T-tests and the extracted contrast estimates provided information regarding the direction of the obtained effects.

3. Results

3.1. Cortisol dynamics after pharmacological manipulation

For the immediate cortisol dataset, a significant main effect of time ($F_{(1.4,66.5)} = 117.45$, $p < .001$, $\eta_p^2 = .72$), treatment ($F_{(1,46)} = 379.97$, $p < .001$, $\eta_p^2 = .89$), as well as a time by treatment interaction occurred ($F_{(1.4,66.5)} = 135.50$, $p < .001$, $\eta_p^2 = .75$). Post hoc t-tests showed that the increase in cortisol concentrations was significantly higher in the cortisol than in the placebo group from baseline to 15min ($t_{(48)} = 11.58$, $p < .001$) as well as from baseline to 45min after tablet administration ($t_{(48)} = 13.48$, $p < .001$), indicating a successful cortisol manipulation (Fig. 1b).

Similarly for the delayed cortisol dataset, a significant main effect of time ($F_{(1.9,96.3)} = 66.93$, $p < .001$, $\eta_p^2 = .57$), treatment ($F_{(1,50)} = 56.13$, $p < .001$, $\eta_p^2 = .52$), as well as a time by treatment interaction emerged ($F_{(1.9,96.3)} = 94.47$, $p < .001$, $\eta_p^2 = .65$). Post hoc t-tests showed that cortisol increases were significantly higher in the cortisol compared to the placebo group from baseline to 30min ($t_{(52)} = 11.35$, $p < .001$), from baseline to 60min ($t_{(52)} = 10.33$, $p < .001$) as well as from baseline to 110min after tablet intake ($t_{(52)} = 11.12$, $p < .001$), again indicating a successful cortisol manipulation (Fig. 1b). No significant main or interaction effects with sex occurred (all $ps > .05$).

3.2. Intensity ratings

For the primary analysis of the intensity ratings, there was a main effect of condition ($F_{(2.7,264.4)} = 623.50$, $p < .001$, $\eta_p^2 = .87$) showing that the intensity of negative emotions differed significantly between the conditions (negative emotion intensity: upregulate > view negative > downregulate > view neutral, all $ps < .001$). A significant main effect of sex ($F_{(1,97)} = 4.51$, $p = .036$, $\eta_p^2 = .04$) furthermore indicated an overall higher intensity of negative emotions rated by women as compared to men. In addition, a condition by sex interaction ($F_{(2.7,264.4)} = 3.05$, $p = .033$, $\eta_p^2 = .03$) revealed higher intensity ratings for the upregulate condition in women compared to men ($t_{(103)} = 2.99$, $p = .003$).

Moreover, a condition by treatment by timing interaction was found ($F_{(2.7,264.4)} = 2.93$, $p = .039$, $\eta_p^2 = .03$), which was driven by the cortisol groups (condition by timing interaction: $F_{(2.7,124.5)} = 3.52$, $p = .021$, $\eta_p^2 = .07$) and not by the placebo groups ($p_s > .63$). To solve this interaction according to our planned analyses, additional ANOVAs with difference scores were conducted (i.e., emotion induction effect, pure downregulation effect, pure upregulation effect, regulation differentiation effect). We found a timing by treatment interaction for the emotion induction effect (rating of view negative – view neutral; $F_{(1,97)} = 8.64$, $p = .004$, $\eta_p^2 = .08$), which was driven by the cortisol groups (main effect timing: $F_{(1,47)} = 10.53$, $p = .002$, $\eta_p^2 = .18$) and not by the placebo groups ($p_s > .26$). Post hoc t-tests revealed that in the immediate dataset, cortisol reduced the emotion induction effect ($t_{(48)} = 2.25$, $p = .029$), while in the delayed set, cortisol increased the emotion induction effect compared to placebo ($t_{(53)} = 2.03$, $p = .047$; Fig. 2). Moreover, analyses revealed a significant timing by treatment interaction for the downregulation contrast (rating of view negative – downregulate; $F_{(1,97)} = 4.06$, $p = .047$, $\eta_p^2 = .04$) which was driven by the cortisol groups (main effect timing: $F_{(1,47)} = 5.09$, $p = .029$, $\eta_p^2 = .10$) and not by the placebo groups ($p_s > .63$). Cortisol enhanced downregulation of negative emotions in the delayed dataset ($t_{(53)} = 2.03$, $p = .047$), while this effect was not observed in the immediate dataset ($t_{(53)} = 0.72$, $p = .475$; Fig. 2). For the pure upregulation effect and regulation differentiation effect, no significant differences were found ($p_s > .142$).

Analyses putting together the two placebo groups and thus comparing three groups (placebo/immediate cortisol/delayed cortisol) revealed similar results. In particular, a condition by group interaction occurred at trend-level ($F_{(5.4,269.4)} = 1.90$, $p = .089$, $\eta_p^2 = .04$) with significant differences between the immediate and the delayed cortisol group only ($p = .010$), but not for the comparisons including the placebo group ($p > .118$). As shown in the main results, follow-up analyses revealed group effects for the emotion induction effect ($F_{(2,99)} = 4.28$, $p = .017$, $\eta_p^2 = .08$) with higher intensity ratings in the delayed compared to the immediate cortisol group ($p = .008$), but no differences regarding comparisons with the placebo group ($p > .202$). Additionally, group effects occurred in the downregulation contrast ($F_{(2,99)} = 3.63$, $p = .030$, $\eta_p^2 = .07$) with slightly higher intensity ratings in the delayed compared to the immediate cortisol group ($p = .059$) and in the delayed cortisol compared to the placebo group ($p = .068$), but not between the immediate cortisol and the placebo group ($p > .99$).

No further main or interaction effects were observed. Importantly, analyses restricted to the two placebo groups did not reveal any significant differences between datasets ($p_s > .309$). Thus, placebo groups

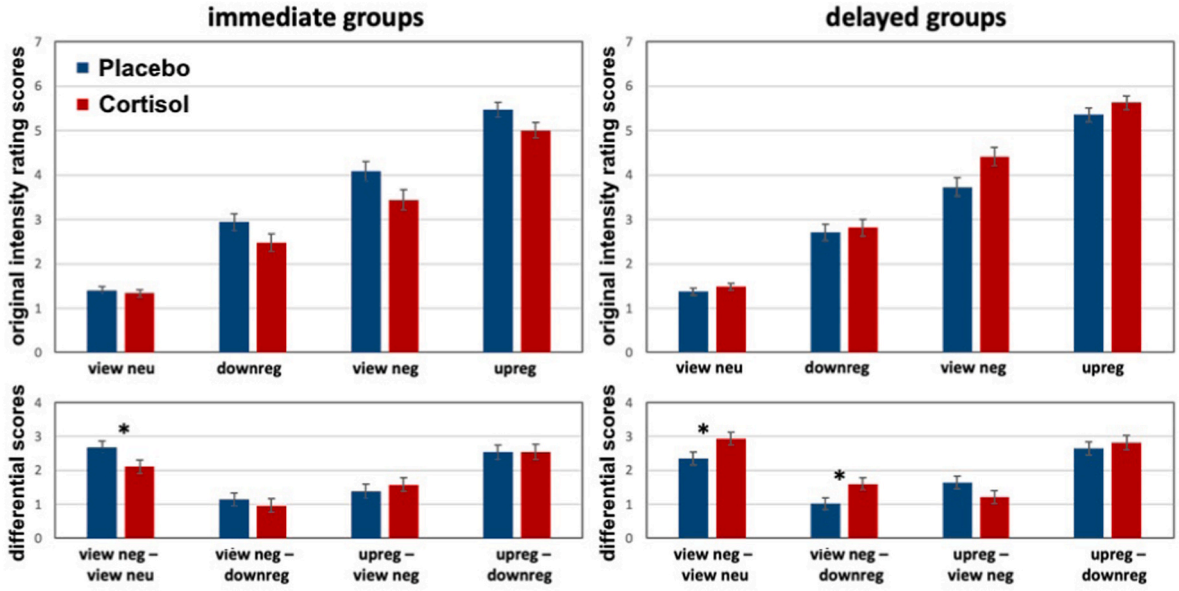

Fig. 2. Immediate and delayed cortisol effects on intensity ratings of the ER task. The left panel is from the immediate dataset including rapid cortisol effects, the right panel is from the delayed dataset including slow cortisol effects on intensity ratings. The top panel shows the original intensity ratings, the lower panel depicts difference scores (comparable to the fMRI contrasts). Opposing rapid and slow cortisol effects occurred for emotion induction (view neg – view neu). In addition, slow cortisol effects promote downregulation of negative emotions (view neg – downreg). Error bars represent standard errors of the mean, $^*p < .05$. An alternative post hoc approach was realized with separate ANOVAs for each condition resulting in significant timing × treatment interactions in the view negative ($F_{(1,97)} = 9.28$, $p = .003$, $\eta_p^2 = .09$) and upregulate condition ($F_{(1,97)} = 4.94$, $p = .029$, $\eta_p^2 = .05$). When viewing negative pictures, intensity ratings were significantly lower in the cortisol relative to the placebo immediate group ($F_{(1,46)} = 4.58$, $p = .038$, $\eta_p^2 = .09$), but higher in the cortisol relative to the placebo delayed group ($F_{(1,51)} = 4.87$, $p = .032$, $\eta_p^2 = .09$). This pattern of results is consistent with our statistical approach reported above. For upregulate, post hoc tests conducted separately for the immediate and delayed group did not reveal any significant differences (all $ps > .05$). Separate analyses regarding the factor treatment revealed no differences between the immediate and delayed placebo group ($p > .66$), but higher intensity ratings for the delayed relative to the immediate cortisol group ($F_{(1,47)} = 7.75$, $p = .008$, $\eta_p^2 = .14$). For view neutral and downregulate, no significant main or interaction effects were observed.

were comparable in their intensity ratings.

3.3. Neural responses

3.3.1. Whole brain analyses

Viewing negative as compared to viewing neutral pictures led to more activation in the temporal and occipital cortex, which extended to the precuneus. When downregulating negative emotions, a wide range of brain regions were involved including the prefrontal, posterior parietal (angular gyrus), and temporal lobes as well as the cerebellum. When upregulating negative emotions, activated brain areas included posterior parietal, prefrontal, temporal, and occipital lobes, as well as the cerebellum and cingulate cortex. Directly contrasting downregulation vs. upregulation further revealed specific activation patterns for both regulatory goals, respectively. While downregulation more strongly involved regions related to inhibition and cognitive control like the dlPFC (superior/inferior frontal gyrus) and parietal lobe (angular gyrus), upregulation recruited more visual brain regions (middle occipital gyrus; cf. Table 1). No significant effects of treatment, timing or sex occurred for whole brain analyses. Additionally, analyses restricted to the two placebo groups did not reveal any significant differences between datasets.

3.3.2. ROI analyses

3.3.2.1. Emotion induction effect [view negative vs. view neutral]. The right amygdala was significantly activated when viewing negative as compared to neutral pictures (x = 22, y = 8, z = −10, $F = 15.51$, $p_{(FWE)} = 0.017$), indicating a successful induction of negative emotions. In addition, increased left dmPFC activation (x = −6, y = 16, z = 42, $F = 24.92$, $p_{(FWE)} = 0.002$) was observed for this contrast. A main effect of sex furthermore revealed stronger activation in the left amygdala (x = −18, y = −10, z = −12, $F = 18.34$, $p_{(FWE)} = 0.007$) and left dmPFC (x = −6, y = 16, z = 42, $F = 17.99$, $p_{(FWE)} = 0.029$) when viewing negative relative to neutral pictures in women as compared to men. No timing- or treatment-related main effects or their interactions were observed.

3.3.2.2. Pure downregulation effect [downregulate vs. view negative]. Significant activations in all prefrontal regions including bilateral dlPFC, dmPFC and vlPFC ($ps_{(FWE)} < 0.001$) for downregulating negative emotions as compared to simply viewing negative pictures indicate that downregulation recruits additional prefrontal resources. In addition, stronger right amygdala (x = 28, y = 2, z = −16, $F = 31.70$, $p_{(FWE)} < 0.001$) activation was found in men as compared to women. No timing- or treatment-related main effects or interactions occurred.

3.3.2.3. Pure upregulation effect [upregulate vs. view negative]. Analyses revealed increased activation of the bilateral amygdala (right amygdala: x = 16, y = −6, z = −16, $F = 19.02$, $p_{(FWE)} = 0.005$; left amygdala: x = −18, y = 0, z = −14, $F = 29.26$, $p_{(FWE)} < 0.001$) for the contrast upregulate minus view negative. Increased activations were also observed in prefrontal regions including the left dlPFC (x = −38, y = 10, z = 52, $F = 71.12$, $p_{(FWE)} < 0.001$), left dmPFC (x = −14, y = 62, z = 28, $F = 51.73$, $p_{(FWE)} < 0.001$) and bilateral vlPFC (left vlPFC: x = −52, y = 16, z = −2, $F = 46.32$, $p_{(FWE)} < 0.001$; right vlPFC: x = 44, y = 36, z = 16, $F = 21.21$, $p_{(FWE)} = 0.010$). Again, a higher activation, but this time in the left amygdala (x = −18, y = −8, z = −12, $F = 15.37$, $p_{(FWE)} = 0.022$), occurred in men compared to women. There were no significant voxels related to timing or treatment effects.

3.3.2.4. Regulation differentiation effect [downregulate vs. upregulate]. Downregulation recruited more right hemispheric regions (right dlPFC, right dmPFC, right vlPFC, $ps_{(FWE)} < 0.003$), while upregulation involved more left hemispheric regions (left dlPFC, left dmPFC, left insula, left

Table 1
MNI coordinates of peak voxels and corresponding T and $p_{(FWE)}$ values of activation clusters showing significant activation when contrasting the experimental conditions in the whole sample.

Brain structure		MNI coordinates			Statistical values		
		x	*y*	*z*	*K*	*T*	$p_{(FWE)}$
(A) Emotion induction contrast							
[view negative vs. view neutral]							
Middle Occipital Gyrus	L	−44	−78	6	8360	11.57	<.001
Precuneus	R	2	−60	24	402	6.63	<.001
Brainstem	R	4	−26	−6	22	5.71	.005
Precuneus	L	−4	−76	46	18	5.42	.014
Superior Frontal Gyrus	L	−20	2	58	11	5.40	.015
[view neutral vs. view negative] No suprathreshold clusters							
(B) Pure downregulation contrast							
[downregulate vs. view negative]							
Superior Frontal Gyrus	L	−2	4	66	2384	9.66	<.001
Angular Gyrus	L	−48	−58	26	1874	8.38	<.001
Inferior Frontal Gyrus	L	−50	22	−6	978	7.46	<.001
Middle Temporal Gyrus	R	66	−16	−16	221	7.39	<.001
Superior Temporal Gyrus	R	52	12	−26	138	7.17	<.001
Inferior Frontal Gyrus	R	54	34	−6	424	6.69	<.001
Cerebellum Posterior Lobe Declive	R	20	−74	−24	244	6.44	<.001
Cerebellum Posterior Lobe Pyramis	R	20	−82	−42	173	6.42	<.001
Inferior Semi-Lunar Lobule	R	30	−64	−54	39	6.30	<.001
Angular Gyrus	R	56	−60	26	260	6.23	.001
Superior Frontal Gyrus	L	−18	52	30	37	5.84	.003
Superior Frontal Gyrus	R	12	52	40	26	5.77	.004
Middle Temporal Gyrus	R	46	−32	−2	63	5.59	.007
Superior Frontal Gyrus	R	22	44	42	15	5.56	.008
Precuneus	L	−2	−58	38	26	5.39	.015
[view negative vs. downregulate] No suprathreshold clusters							
(C) Pure upregulation contrast							
[upregulate vs. view negative]							
Angular Gyrus	L	−50	−60	24	1812	9.74	<.001
Cerebellum Posterior Lobe Declive	R	24	−80	−24	3706	9.20	<.001
Middle Frontal Gyrus	L	−40	8	52	3064	9.20	<.001
Superior Frontal Gyrus	L	−4	4	70	773	8.65	<.001
Inferior Semi-Lunar Lobule	R	30	−66	−54	217	8.36	<.001
Precuneus	L	−4	−52	36	1133	8.25	<.001
Superior Temporal Gyrus	L	−52	18	−8	607	7.34	<.001
Superior Occipital Gyrus	R	12	−96	24	386	6.85	<.001
Middle Temporal Gyrus	L	−60	0	−18	241	6.74	<.001
Hippocampus	L	−20	−20	−14	58	6.73	<.001
Cerebellum	L	−46	−70	−26	143	6.19	.001
Cingulate Gyrus	L	−2	20	34	111	6.06	.001
Precuneus	L	−2	−56	72	108	6.05	.001
Middle Occipital Gyrus	L	−12	−104	8	54	5.94	.002
Middle Occipital Gyrus	R	50	−76	4	23	5.77	.004
Parahippocampal Gyrus	R	16	−12	−18	15	5.77	.004
Temporal Lobe	R	32	−40	8	15	5.74	.004
Middle Temporal Gyrus	L	−56	−36	0	63	5.73	.004
Precuneus	L	−6	−84	46	23	5.49	.011
Calcarine	R	18	−84	10	13	5.44	.013
[view negative vs. upregulate] No suprathreshold clusters							
(D) Regulation differentiation contrast							
[downregulate vs. upregulate]							
Superior Frontal Gyrus	R	16	30	56	216	7.16	<.001
Angular Gyrus	R	50	−60	38	311	6.99	<.001
Inferior Frontal Gyrus	R	52	30	−8	99	5.99	<.001
[upregulate vs. downregulate]							
Middle Occipital Gyrus	R	20	−100	12	108	5.96	.002
Calcarine	R	32	−58	10	16	5.92	.002

vlPFC, all $ps_{(FWE)} < 0.028$), suggesting a hemispheric asymmetry in ER processes targeting different regulatory goals (such as approach-avoidance for example; Kelley et al., 2017). Analyses restricted to both placebo groups revealed a similar hemispheric pattern: Downregulation recruited more right hemispheric regions (right dlPFC, right dmPFC, $ps_{(FWE)} < 0.027$), while upregulation involved more left hemispheric regions (left dmPFC, left amygdala, all $ps_{(FWE)} < 0.031$) and the anterior cingulate cortex ($p_{(FWE)} = 0.044$).

Importantly, we found a significant treatment by timing interaction in the right amygdala (x = 26, y = 2, z = −30, $F = 13.72$, $p_{(FWE)} = 0.034$) and right dlPFC (x = 22, y = 40, z = 38, $F = 19.79$, $p_{(FWE)} = 0.026$) for the contrast downregulate minus upregulate. Restricting this analysis to both cortisol groups revealed a trend only for higher right amygdala activation in the immediate compared to the delayed group (x = 24, y = 2, z = −28, $T = 3.26$, $p_{(FWE)} = 0.078$), but not for the right DLPFC. In the immediate dataset, cortisol increased dlPFC activity when downregulating relative to upregulating negative emotions (Fig. 3a). Whereas the amygdala was more strongly recruited during up- relative to downregulation under placebo (down < up), this goal-specific difference in amygdala activation almost vanished in the cortisol group (down = up; Fig. 3b). This cortisol effect could be either driven by an enhanced amygdala activation during downregulation or reduced amygdala recruitment during upregulation (or both), both rather indicating a reduced impact of reappraisal on amygdala activation. By contrast, in the delayed dataset, cortisol dampened amygdala activation for down- vs. upregulation, while a stronger recruitment of the dlPFC was found under placebo conditions (down > up). This difference in dlPFC activity between regulatory goals was again cancelled out under cortisol treatment (down = up; Fig. 3b). Analyses putting together the two placebo groups and thus comparing three groups (placebo/immediate cortisol/delayed cortisol) could not confirm these results in the right amygdala and right DLPFC overall or in the comparison of the placebo and the immediate cortisol group. Comparing the placebo group with the delayed cortisol group at least revealed significant group differences in the right amygdala (x = 34, y = −4, z = −18, $T = 3.36$, $p_{(FWE)} = 0.049$).

PPI analyses with the right amygdala as seed (VOI with a 5 mm sphere around the peak voxel x = 26, y = 2, z = −30), revealed a significant treatment by timing interaction for its functional connectivity with the dlPFC (x = −20, y = 52, z = 8, $t = 4.23$, $p_{(FWE)} = 0.027$). Immediate cortisol effects increased the amygdala-dlPFC functional connectivity in the downregulate vs. upregulate contrast, while delayed cortisol effects showed the opposite pattern (i.e., decreased amygdala-dlPFC functional connectivity for down- vs. upregulation; Fig. 3). Further PPI analyses showed that no other main or interaction effects occurred for any of the other seed regions.

Likewise, there were no other significant main or interaction effects with treatment, timing or sex for the remaining (reverse) contrasts, neither in the activation nor in the PPI analyses. Furthermore, analyses restricted to the two placebo groups did not reveal any significant differences between datasets.

4. Discussion

In the current study, we investigated the role of timing for cortisol effects on ER by administering cortisol at two different time points prior to an emotional reappraisal task asking participants to up- and downregulate negative emotions. As expected, cortisol acted on brain regions involved in cognitive ER in dependence of timing, especially when contrasting the opposite regulation conditions. More specifically, cortisol rapidly reduced the effectiveness of cognitive reappraisal to change amygdala activation in the intended direction (i.e., cortisol enhanced amygdala activation during downregulation and/or reduced amygdala activation during upregulation). Moreover, cortisol rapidly increased dlPFC activation and the amygdala-dlPFC functional connectivity when downregulating relative to upregulating negative emotions.

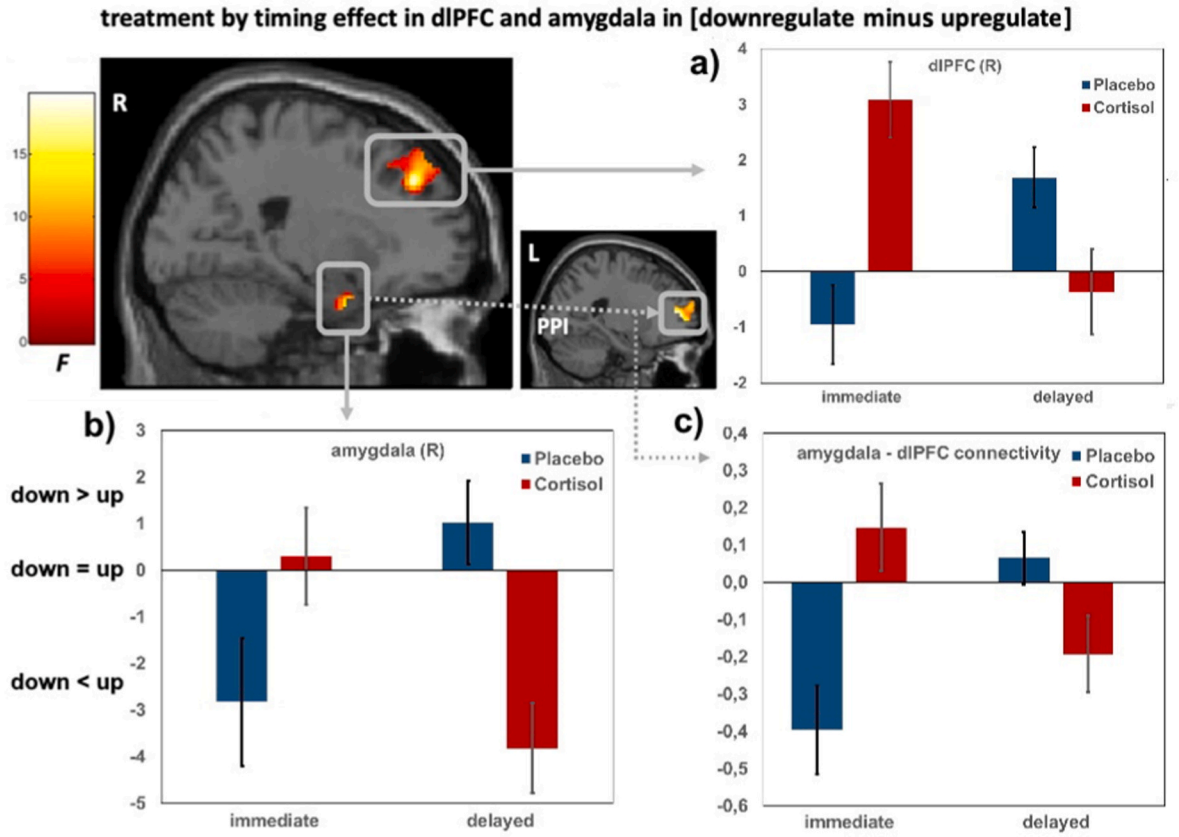

Fig. 3. Treatment by timing interaction in brain activation and functional connectivity in the downregulate minus upregulate contrast. **a)** cortisol administration induced a higher dlPFC activation in the immediate (i.e., immediate cortisol minus immediate placebo) compared to the delayed group (i.e., delayed cortisol – delayed placebo) in the contrast downregulate minus upregulate; **b)** cortisol (relative to placebo) increased amygdala activation in the immediate compared to the delayed cortisol group in the same contrast; **c)** cortisol (relative to placebo) increased amygdala-dlPFC functional connectivity in the immediate compared to the delayed group with downregulation as anchor of interpretation. Alternatively, cortisol (relative to placebo) induced more amygdala-dlPFC functional connectivity in the delayed group for upregulation. The average contrast estimates for significant peak voxels were extracted as bar graphs, with error bars representing standard errors of the mean. Please note that due to the arbitrary baseline, activations or deactivations cannot be inferred. But positive values reflect higher activations for downregulate relative to upregulate (down > up), negative values represent higher activations for upregulate compared to downregulate (down < up), values near zero reflect no activation differences between down- and upregulate (down = up).

Slow cortisol effects exhibited the opposite pattern: they increased the effectiveness of reappraisal to modulate amygdala activation in a goal-specific manner (i.e., reduced activation during downregulation and/or increased activation during upregulation), but diminished differences in dlPFC recruitment between the regulatory goals and amygdala-dlPFC connectivity. Intensity ratings partly confirmed these results in showing cortisol to slowly facilitate the downregulation of negative emotions, whereas no rapid cortisol effects on ER occurred on the behavioral level.

Previous work mostly from animals indicated that stress hormones exert timing-dependent effects (Karst et al., 2005, 2010) with both rapid, non-genomic and slow, genomic cortisol effects supporting adequate stress adaptation (Joëls et al., 2013; Vogel et al., 2016). Congruently, there is evidence in humans for timing-dependent cortisol effects on cognitive and emotional functions, such as working memory (Henckens et al., 2011), fear generalization (van Ast et al., 2013) and emotional distraction tasks (Henckens et al., 2012a). Here, we sought to experimentally dissociate rapid from slow cortisol effects on ER by administering cortisol either 30 or 90min prior to the task. Cortisol rapidly amplified dlPFC as well as the amygdala-dlPFC functional connectivity during down- relative to upregulation. Deliberate attempts to downregulate emotions require cognitive effort (Gyurak et al., 2011) associated with increased involvement of the executive control network (Etkin et al., 2015; Ochsner and Gross, 2005). In line with previous behavioral results (Langer et al., 2020), our data thus imply that cortisol rapidly boosts the cognitive regulatory engagement during deliberate attempts to downregulate negative emotions. However, despite this stronger recruitment of cognitive control resources during ER, cortisol rapidly reduced the effectiveness of reappraisal to down- and upregulate amygdala activation. More specifically, while we found reduced amygdala activation during downregulation and/or enhanced amygdala activation during upregulation under placebo, this goal-specific difference in amygdala activation almost vanished in the immediate cortisol group. In line, Henckens et al. (2012b) showed cortisol to enhance prefrontal-amygdala connectivity specifically in a time window of rapid, non-genomic actions. Reduced regulatory changes in amygdala activation together with tightened coupling with the executive network as observed in the current study might thus suggest that rapid cortisol actions impair a flexible regulation of negative emotions despite a higher expenditure of cognitive resources. Of note, cortisol effects on neural ER correlates were specifically found for the down -vs. upregulation contrast with no equivalent emerging in the intensity ratings.

In contrast to the rapid effects, cortisol slowly improved cognitive attempts to regulate negative emotions as reflected by dampened activation of the amygdala and its connectivity to the dlPFC in the down- vs. upregulate contrast. This finding is consistent with previous work providing evidence for slow, genomic cortisol actions to initiate a shift from a vigilant, arousing state to a cognitive controlled mode helping the organism to recover from the stressor (de Kloet et al., 2008; Hermans et al., 2014; Joëls et al., 2013). In addition, earlier work on ER supports this notion by showing slow cortisol effects to reduce emotion-related

amygdala activation while decreasing negative emotions via cognitive reappraisal (Jentsch et al., 2019). In line with these findings and in accord with the decreased amygdala activation found in the current study, a cortisol-induced delayed improvement of reappraisal to specifically downregulate negative emotions was also evident in our intensity ratings. Taken together, this neural and behavioral pattern could speak for a specific improvement of downregulatory reappraisal processes.

Another important aspect consists of cortisol slowly diminishing the goal-specific regulatory activation of the dlPFC which was present under placebo (down > up), leading to a more balanced recruitment of the dlPFC during the down- and upregulation of negative emotions. The dlPFC is a key structure for executive processes, especially for continuous updating and manipulation of stimuli in working memory (Wager and Smith, 2003). Here, participants had to manipulate the meaning of the perceived situation by imagining a positive outcome to downregulate negative emotions. Other than putting oneself into the position of an observed person and to imagine an even worse outcome, which was required during upregulation trials, the complete reversal of an emotional state might be more effortful and could thus explain the stronger recruitment of the dlPFC during downregulation trials in the placebo group. Under cortisol, this goal-specific dlPFC activation vanished possibly due to a particular facilitation of downregulatory processes. Altogether, our data suggest that slow cortisol effects either facilitate downregulation or upregulation of negative emotions (or both), thus enabling individuals to adequately and flexibly cope with negative experiences: downregulation is improved with reduced emotional involvement and/or upregulation is improved with enhanced emotional involvement in times of slow cortisol effects.

Analyses of the intensity ratings revealed that cortisol-treated participants rated negative relative to neutral pictures as more negative in the delayed group and as less negative in the immediate group. The rapid cortisol effects are in line with previous evidence for immediate buffering effects of cortisol on negative emotional reactivity (Het and Wolf, 2007; Reuter, 2002). However, cortisol has also repeatedly been shown to slowly diminish emotional processing by reducing activation in emotion-related brain regions such as the amygdala (Hermans et al., 2014). The enhanced emotional responsivity in the delayed group in the current study was thus somewhat unexpected. Of note, imaging data of Henckens et al. (2010) suggested cortisol to rapidly reduce and slowly normalize amygdala reactivity to negative stimuli. The current opposing timing-specific effects of cortisol on emotional responsivity might therefore be attributed to a rapid suppression of negative reactivity, which is partially compensated by a delayed, enhanced processing of negative emotions.

Some limitations should be noted. Firstly, timing-specific cortisol-driven changes in reported emotional intensity and neural activity did not correspond perfectly. Even though self-reports provide a reliable measure of subjective emotional experiences, these data might be influenced by awareness of and willingness to report the current emotional state as well as demand characteristics (Mauss and Robinson, 2009). Moreover, in line with previous imaging studies (Jentsch et al., 2019) but in contrast to behavioral studies (Langer et al., 2020, 2022), we used intensity ratings as a subjective marker of ER performance. This scale might not be fully sensitive to assess all emotional changes when applying reappraisal, a strategy particularly potent to change the valence of the emotional response. Future studies might benefit from additional ER related rating scales (e.g., arousal, valence, difficulty/effort, success) and/or inclusion of psychophysiological measures (e.g., heart rate, pupil or electromyographic responses; Zaehringer et al., 2020) as objective ER markers. Secondly, given that cortisol levels did not fully return to baseline after 110min, we cannot rule out the possibility that non-genomic and genomic cortisol effects both had been active in the delayed group. Future studies may thus benefit from even larger time intervals between cortisol intake and delayed ER assessment (e.g., Henckens et al., 2012b; Henckens et al., 2012a; Henckens et al., 2011, 2010). Thirdly, cortisol administration allows for a mechanistic investigation of the contribution of cortisol on ER, but this approach cannot be directly compared with exposure to acute stress (Langer et al., 2020). In addition to cortisol release (and prior to the secretion of corticotropin-releasing hormone from the hypothalamus and adrenocorticotropic hormone from the pituitary gland), acute stress includes activation of the sympathetic nervous system quickly initiating the release of (nor)epinephrine as well as psychological factors characterizing a stressful situation such as negative affect.

5. Conclusions

In sum, the present study provides initial evidence for opposing rapid and slow cortisol effects on the neural correlates of ER when contrasting different regulatory goals. Rapid cortisol effects impaired the effectiveness of reappraisal to regulate amygdala activation despite increased dlPFC involvement suggesting reduced capacities to flexibly down- and/or upregulate negative emotions. By contrast, slow cortisol effects enhanced the effectiveness of reappraisal to regulate amygdala activity (up, down or both), which was accompanied by reduced subjective emotional intensity when downregulating negative emotions. These results are in line with evidence for cortisol to promote adaptation to and coping with stressful situations via rapid impairments and slow improvements of a flexible regulation of negative emotions to restore homeostasis.

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CRediT authorship contribution statement

Dong-ni Pan: Formal analysis, Data curation, Writing – original draft, Visualization. **Valerie L. Jentsch:** Conceptualization, Funding acquisition, Writing – review & editing. **Katja Langer:** Writing – review & editing. **Bianca Hagedorn:** Conceptualization, Data curation, Investigation, Writing – review & editing. **Oliver Höffken:** Writing – review & editing. **Oliver T. Wolf:** Conceptualization, Writing – review & editing, Funding acquisition. **Christian J. Merz:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition, Validation, Visualization.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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