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Endogenous cortisol is associated with functional connectivity between the amygdala and medial prefrontal cortex

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Summary Whether glucocorticoids mediate medial prefrontal cortex (mPFC) regulation of the amygdala in humans remains unclear. In the current study we investigated whether cortisol levels under relatively stress-free circumstances are related to amygdala resting-state functional connectivity with the mPFC. Resting-state fMRI data were acquired from 20 healthy male participants. Salivary cortisol was sampled at multiple times throughout the experiment. The cortisol area under the curve increase (AUCi) was calculated as a measure of cortisol dynamics. Next, seed based correlations were employed on the resting-state fMRI data to reveal regions of amygdala functional connectivity related to variations in cortisol AUCi. The resulting statistical maps were corrected for multiple comparisons using cluster based thresholding ($Z > 2.3$, $p < .05$). Two regions in the mPFC showed decreasing negative functional connectivity with the amygdala when a lesser decrease in cortisol AUCi was observed: the perigenual anterior cingulate cortex and medial frontal pole (BA10). Although we initially showed a relation with cortisol AUCi, it seemed that the baseline cortisol levels were actually driving this effect: higher baseline cortisol levels related to stronger negative functional connectivity with the mPFC. Endogenous cortisol levels may modulate amygdala functional connectivity with specific regions in the mPFC, even under relatively stress-free circumstances. Our results corroborate previous findings from both animal and human studies, suggesting cortisol-mediated regulation of the amygdala by the mPFC. We propose that through this feedback mechanism the stress response might be adjusted, pointing to the putative role of cortisol in modulating stress- and, more generally, emotional responses.

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1. Introduction

The release of glucocorticoids is one of the most prominent endocrine responses to a stressful situation. In humans, the glucocorticoid cortisol is secreted by the adrenal cortices after the hypothalamus–pituitary–adrenal (HPA) axis has been activated (Sapolsky et al., 2000; Ulrich-Lai and Herman, 2009). Whereas the autonomic nervous system supports a fast reaction to a stressful situation, cortisol typically reaches its peak plasma levels only after tens of minutes. Following its release, cortisol acts back on the HPA-axis in a negative feedback loop, thereby promoting inhibition of the stress response necessary to reach behavioral and physiological homeostasis (Herman et al., 2005; Ulrich-Lai and Herman, 2009).

Animal studies have provided ample evidence that the medial prefrontal cortex (mPFC) plays an important modulatory role within the stress circuitry (Diorio et al., 1993; Sullivan and Gratton, 2002; Cerqueira et al., 2008), either by stimulating or inhibiting HPA-axis activity, depending on which mPFC subdivision is involved (Radley et al., 2006; Ulrich-Lai and Herman, 2009). Whereas the ventral part of the mPFC has been attributed a more stimulatory role, the more dorsal part, in contrast, has rather been described as inhibiting HPA-axis activity. In addition, several studies suggest that this negative feedback circuit is mediated through the binding of cortisol to glucocorticoid receptors (GRs) in the mPFC (Diorio et al., 1993; Sanchez et al., 2000; Boyle et al., 2005; Furay et al., 2008; Ulrich-Lai and Herman, 2009).

The amygdala, a key region in facilitating stress responses, is an important target of such inhibitory feedback by the mPFC (Herman et al., 2005). In humans, the mPFC was found to be involved in modulating amygdala activity during emotional conflict and regulation of autonomic and affective responses, most notably the perigenual division of the anterior cingulate cortex (Pezawas et al., 2005; Etkin et al., 2006; Egner et al., 2008; Gianaros et al., 2008; Wager et al., 2009), but also the ventro- and dorsomedial (vm/dm) portions of the PFC (Urry et al., 2006; Banks et al., 2007). Based on the animal research reviewed above, cortisol might act as an important mediator in adjusting amygdala responses through the mPFC.

This notion is supported by the abnormal interactions between the mPFC and amygdala that have been reported frequently in stress-related psychiatric disorders, such as depression and posttraumatic stress disorder (PTSD) (Phillips et al., 2003b; Drevets et al., 2008; Liberzon and Sripada, 2008; Veer et al., 2010). Because of the concurrent HPA-axis dysregulation in these disorders (de Kloet et al., 2006; Pariante and Lightman, 2008), it is thought that prolonged exposure to abnormal cortisol levels is related to reduced top-down inhibition by the mPFC, thereby sustaining excessive amygdala activity (Liberzon et al., 2007).

So far, three studies in healthy humans have found support not only for a mediating role of cortisol in connectivity between the amygdala and mPFC, either after ingestion of hydrocortisone (Henckens et al., 2010), or after social stress (Kern et al., 2008), but also pertaining to individual differences in normal diurnal cortisol patterns (Urry et al., 2006). Except for the study of Kern et al., who used task-free positron emission tomography to assess glucose metabolism in the brain after social stress, these results were obtained with task paradigms in which emotionally salient stimuli were used.

Resting-state functional connectivity (RSFC) analysis of the amygdala-mPFC circuit, on the other hand, might provide more insight on whether cortisol levels are related to interactions between these regions in humans in absence of task-induced activation, potentially providing a more intrinsic measure of cortisol mediated brain networks. In a recent study of our group we found that social stress increased amygdala RSFC with the mPFC compared to controls (Veer et al., 2011). However, this increased connectivity was not related to stress-induced cortisol levels, possibly due to a ceiling effect in the participants' cortisol responses or complex interactions with concurrent neuroendocrine responses to the stressor. Nonetheless, activation of the brain's stress circuitry was previously shown to be related even to subtle variations in stress-free cortisol fluctuations (Urry et al., 2006; Cunningham-Bussel et al., 2009). Therefore, we investigated whether such normal variations in endogenous cortisol also could be related to altered amygdala RSFC with the mPFC in a group of healthy young males under relatively stress-free circumstances.

2. Materials and methods

2.1. Participants

Twenty right-handed male volunteers (mean age 23.95 ± 2.52 years) from the general population were recruited by means of advertisements. All participants were screened before inclusion. Eligibility criteria were: no history of disease or chronic disease requiring medical attention, no dyslexia, no color blindness, no current use of prescribed medication and/or use of remedies containing corticosteroids, no use of psychotropic drugs, no current or past psychiatric problems, as was determined by the Amsterdam Biographical interview (ABV; Wilde, 1963), and the Dutch version of the Symptom checklist (SCL-90; Arrindell and Ettema, 1986). Furthermore, participants were required to have a body mass index (BMI; kg/m^2) between 19 and 26, and to be between 18 and 30 years old. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center and written informed consent was given by all participants.

2.2. Physiological assessments

Salivary cortisol was assessed using Salivettes (Sarstedt, Germany). Saliva sampling is a stress-free method to assess unbound cortisol (Kirschbaum and Hellhammer, 1994). Saliva samples were stored at $-20\ ^{\circ}C$ until assayed at Prof. Kirschbaum's laboratory (http://biopsychologie.tu-dresden.de). Cortisol concentrations in saliva (in nmol/L) were measured using a commercially available chemiluminescence-immunoassay kit with high sensitivity (IBL, Hamburg, Germany). Inter- and intra-assay coefficients of variation were below 10%. The cortisol area under the curve increase (AUCi) was determined for each participant, providing a measure of cortisol changes over the course of the experiment (Pruessner et al., 2003). Lastly, systolic blood pressure (SBP, mmHg),

diastolic blood pressure (DBP, mmHg), and heart rate (HR, bpm) were recorded using an automatic wrist blood pressure monitor (OMRON, R5-I) to assess activity of the autonomic nervous system. Repeated measures ANOVAs were carried out on the physiological data using SPSS (SPSS inc., IL).

2.3. FMRI data acquisition

Imaging data were acquired on a Philips 3.0-T Achieva MRI scanner using an eight-channel SENSE head coil for radiofrequency transmission and reception (Philips Medical Systems, Best, The Netherlands). Whole-brain RS-fMRI data were acquired using T_2*-weighted gradient-echo echo-planar imaging with the following scan parameters: 160 volumes; 38 axial slices scanned in ascending order; repetition time (TR) = 2200 ms; echo time (TE) = 30 ms; flip angle = 80°; FOV = 220 mm × 220 mm; 2.75 mm isotropic voxels with a 0.25 mm slice gap. A high-resolution anatomical image (T_1-weighted ultra-fast gradient-echo acquisition; TR = 9.75 ms; TE = 4.59 ms; flip angle = 8°; 140 axial slices; FOV = 224 mm × 224 mm; in-plane resolution 0.875 mm × 0.875 mm; slice thickness = 1.2 mm), and a high-resolution T_2*-weighted gradient-echo EPI scan (TR = 2.2 s; TE = 30 ms; flip angle = 80°; 84 axial slices; FOV = 220 mm × 220 mm; in-plane resolution 1.96 mm × 1.96 mm, slice thickness = 2 mm) were acquired for registration and normalization to standard space.

2.4. FMRI data preprocessing

Prior to analysis, all resting-state fMRI data sets were submitted to a visual quality control check to ensure that no gross artifacts were present in the data. Next, data were analyzed using FSL Version 4.1.3 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004). The following preprocessing steps were applied to the EPI data sets: motion correction (Jenkinson et al., 2002), removal of non-brain tissue (Smith, 2002), spatial smoothing using a Gaussian kernel of 6 mm full width at half maximum (FWHM), grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor, a highpass temporal filter of 100 s (i.e., ≥0.01 Hz). The RS dataset was registered to the high resolution EPI image, the high resolution EPI image to the T_1-weighted image, and the T_1-weighted image to the 2 mm isotropic MNI-152 standard space image (T_1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada) (Jenkinson and Smith, 2001; Jenkinson et al., 2002). The resulting transformation matrices were then combined to obtain a native to MNI space transformation matrix.

2.5. FMRI time course extraction and statistical analysis

A seed based correlation approach (Fox and Raichle, 2007) was employed to reveal brain regions that are functionally connected to the amygdala during rest (e.g., Veer et al., 2011). To this end, binary masks of the bilateral amygdala were created using the Harvard-Oxford Subcortical Atlas, as provided in MNI standard space within FSL: the center voxel was determined for the left and right amygdala, and spherical regions of interest (ROIs) were subsequently created around these voxels using a radius of 4 mm. Next, using the inverse transformation matrix, the amygdala masks were registered to each participant's RS-fMRI preprocessed dataset. The mean time course was subsequently extracted from the voxels falling within each amygdala mask in native space. These time courses were entered as a regressor in a general linear model (GLM), together with nine nuisance regressors comprising the white matter signal, CSF signal, six motion parameters (rigid body: three translations and three rotations) and the global signal. The latter regressor was included to further reduce the influence of artifacts caused by physiological signal sources (i.e., cardiac and respiratory) on the results (Fox and Raichle, 2007). Each individual model was tested using FEAT version 5.98, part of FSL. The resulting individual parameter estimate (PE) maps, together with their corresponding within-subject variance maps, were then resliced into 2 mm isotropic MNI space and fed into a higher level mixed effects regression analysis (one-sample t-test), using the demeaned AUCi cortisol values as regressor of interest. Whole-brain Z (Gaussianized T/F) statistic images were thresholded using clusters determined by an initial cluster-forming threshold of $Z > 2.3$ ($p < .01$, one-tailed) and a corrected cluster significance threshold of $p < .05$ (Worsley, 2001).

2.6. Procedure

The current article reports on results obtained within a larger study addressing the effects of social stress on an emotional working memory task (Sternberg paradigm, using negative and neutral distracters during the delay period between target and probe; Oei et al., 2011) and resting-state functional connectivity (Veer et al., 2011). The results described here are based on the participants from the control group who were assigned to a non-stressful control condition (answering questions about a movie to their liking for 5 min, and counting backwards from 50 to zero) before entering the scanner. On the day of scanning participants arrived at either 8:30 or 10:30 AM, which was balanced within our participant group. Participants were asked to refrain from caffeine or sugar containing drinks, from smoking, and not to eat 2 h before arrival time to minimize unwanted effects on cortisol levels. The scanning protocol consisted of the task scan, several anatomical scans, and the RS scan which was acquired at the end of the scan protocol, 50 min after entering the scanner and 20 min after completing the task scan. For the RS scan, participants were instructed to lie still with their eyes closed during the entire scan in the darkened scanner room. Saliva was sampled at five time points throughout the procedure: before ("baseline", t = 0 min) and after preparation for the control condition ("post prep", t = 10 min), after completing the control condition just before entering the scanner ("pre scan", t = 20 min), immediately after finishing the emotional working memory task scan ("post task", t = 60 min), and immediately after the RS scan outside the scanner ("post RS", t = 90 min). At the exact same moments, a 10-point Likert scale was used to inquire about the subjectively perceived stress levels (see Fig. 1 for sampling time points and their relative timings). Blood pressure and heart rate were sampled at the same time points, except the fourth time point ("post task") when the participant was inside the

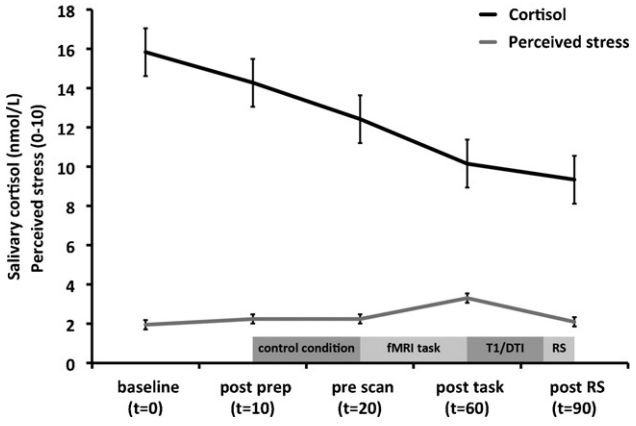

Figure 1 Mean salivary cortisol levels (nmol/L) and Likert scores (0–10) together with their standard error of the mean at each of the sampling time points (t = time in minutes from baseline). RS = resting-state scan, T_1/DTI = anatomical scans.

scanner room, due to MR-incompatibility of the equipment. An exit-interview followed at the end of the procedure. Subsequently, participants were thanked and paid for their participation in the study.

3. Results

3.1. Physiological and behavioral results

3.1.1. Cortisol

See Fig. 1 for average cortisol values at each sampling time point. A gradual decrease of endogenous cortisol levels over the course of the experiment was observed in our participants. This was confirmed by a main effect of Time, $F(1.38; 26.3) = 8.91$, $p = .003$, and a linear contrast post hoc, $F(1; 19) = 10.57$, $p = .004$. Nonetheless, a number of participants demonstrated only a minor decrease ($N = 9$) or even an increase ($N = 5$) in cortisol levels, as was reflected by the cortisol AUCi. Although the distribution of cortisol AUCi is skewed, no outliers were identified. No difference was found between the "pre scan" and "post RS" time points ($p > .1$).

3.1.2. Heart rate

Over the course of the experiment heart rate decreased, as expressed in a main effect of Time, $F(3; 57) = 3.25$, $p = .028$. No difference was found between the "pre scan" and "post RS" time points ($p > .1$).

3.1.3. Blood pressure

Blood pressure showed a different pattern in anticipation of scanning, participants had a decrease in both systolic (SBP) and diastolic (DBP) blood pressure, yet both were increased after scanning to values even above baseline (main effect of time: $F(3; 57) = 4.19$, $p = .009$, and $F(3; 57) = 15.78$, $p < .001$, SBP and DBP, respectively; "post RS" larger than "pre scan": $t(19) = 3.05$, $p = .007$ and $t(19) = 4.07$, $p < .001$, SBP and DBP, respectively). It must be noted, however, that the "post RS" measurement took place directly after the scans, when participants were seated in another room. This could have increased blood pressure markedly because the participant suddenly had to stand upwards after a long period of laying still inside the scanner. Therefore, it is conceivable that this in fact is the cause of the increase in blood pressure.

3.1.4. Behavior

See Fig. 1 for the perceived stress scores. Subjective stress ratings demonstrated a main effect of time, $F(4; 76) = 10.26$, $p < .001$, with higher ratings "post task" than "pre scan", $t(19) = -3.8$, $p = .001$, but not at the "post RS" measurement compared to "post task" ($p > .1$).

3.2. Functional connectivity results

The pattern of amygdala functional connectivity within our participant group largely overlaps with previously described functional and anatomical connections of the amygdala (Stein et al., 2007; Roy et al., 2009; Robinson et al., 2010). The areas involved include: brainstem, hippocampus, hypothalamus, subgenual cingulate cortex, dorsal cingulate cortex, posterior lateral orbitofrontal cortex, insula, temporal poles, primary visual cortex (see Table 1). The majority of these regions together form the "emotional brain" circuitry, dedicated to the processing and regulation of emotion (Pessoa, 2008).

Fig. 2 shows the two clusters of resting-state functional connectivity with the joint amygdala seeds that are positively correlated with cortisol AUCi ($p < .05$, cluster corrected): the perigenual anterior cingulate cortex (pgACC) and medial frontal pole (BA10). That is, less decrease of cortisol levels over the course of the experiment is associated with less negative RSFC with the two mPFC regions. Moreover, mild cortisol AUCi increases appear to relate to an increase in positive amygdala RSFC with the pgACC and BA10. We did not observe an effect of cortisol AUCi looking at either left or right amygdala RSFC alone.

We furthermore tested whether cortisol levels at baseline were in fact driving the steepness of the AUCi slopes, and thereby possibly the effects on amygdala RSFC. That is, did higher cortisol levels at baseline relate to a larger cortisol decrease over the course of the experiment? This was indeed the case, as was illustrated by the negative correlation between baseline cortisol and AUCi ($r(20) = -0.87$, $p < .05$). In addition, when using the baseline values as predictor instead of cortisol AUCi, we found the exact same results, although being inverted. That is, higher baseline cortisol was associated with stronger negative amygdala RSFC with the two mPFC regions.

Lastly, to distinguish between delayed and more direct effects of cortisol, we averaged the absolute cortisol levels on time points 4 (post task) and 5 (post RS) and used these as a predictor of amygdala RSFC. However, no effect was observed.

4. Discussion

Here we show that basal variations in endogenous cortisol in healthy young male participants are related to the strength of amygdala resting-state functional connectivity with two regions in the mPFC, specifically the pgACC and medial frontal pole (BA10). This result is in line with our hypothesis and the notion that cortisol impacts crosstalk between the mPFC and amygdala. Therefore, our findings potentially

Table 1 Resting-state functional connectivity results.

Region	Hemisphere	Cluster size 2 mm voxels	Peak voxel coordinates (MNI)			Z-value	p-Value (one-tailed)
			x	y	z		
Amygdala							
Positive							
Lateral orbitofrontal cortex	R	35,890	30	34	−18	5.09	<.0001
	L		−30	34	−16	5.29	<.0001
Hippocampus	R		28	−22	−16	6.03	<.0001
	L		−26	−20	−16	6.19	<.0001
Putamen	R		30	−14	−4	6.39	<.0001
	L		−30	−16	0	6.14	<.0001
Globus pallidus	R		24	−4	0	6.2	<.0001
	L		−20	0	2	5.62	<.0001
Insula	R		42	−2	−8	5.55	<.0001
	L		−40	−6	−8	4.81	<.0001
hypothalamus	R		6	−4	−12	4.04	<.0001
	L		−6	−2	−26	4.95	<.0001
Subcallosal cortex	R		8	10	−14	4.99	<.0001
	L		−6	16	−14	4.54	<.0001
Temporal pole	R		46	10	−16	5.35	<.0001
	L		−52	10	−16	5.36	<.0001
Superior temporal gyrus	R		54	−34	4	3.76	.0001
	R		48	−24	−4	3.57	.0002
	L		−54	−14	−8	4.54	<.0001
	L		−52	−34	2	4.04	<.0001
Middle temporal gyrus	R		56	−12	−14	5	<.0001
	L		−56	−14	−10	4.34	<.0001
Occipital cortex	R		14	−86	4	3.6	.00015
	L		−6	−92	4	4.52	<.0001
Brainstem	R		−2	−34	−16	6.13	<.0001
Dorsal anterior cingulate cortex	R	7318	8	−8	40	4.27	<.0001
	L		−8	−8	44	4.23	<.0001
Postcentral gyrus	R		62	−16	38	4.82	<.0001
	L		−46	−16	36	4.76	<.0001
Precentral gyrus	R		60	4	32	4.67	<.0001
	L		−52	6	28	4.21	<.0001
Negative							
Posterior cingulate cortex	R	12,325	4	−36	26	4.41	<.0001
	L		−4	−36	26	4.3	<.0001
Precuneus	R		6	−66	30	3.13	.0009
	L		−8	−70	32	3.67	.0001
Lateral frontal pole	R	4027	26	58	10	4.08	<.0001
	L		−34	58	6	3.75	.0001
Perigenual anterior cingulate cortex	R	1300	4	36	10	2.94	.0017
Medial superior frontal gyrus			−2	26	50	3	.0014
Cortisol							
Perigenual anterior cingulate cortex		584	−2	36	2	3.61	.00015
medial frontal pole (BA10)			−2	64	−4	3.2	.0007

Note: All Z-values are cluster corrected for multiple comparisons ($p < .05$), using an initial cluster forming threshold of $Z > 2.3$.

reflect a modulatory pathway within the human brain's stress and emotion circuitry that is mediated by cortisol.

Cortisol exerts its influence through both mineralocorticoid (MRs) and glucocorticoid receptors (GRs), which are differentially distributed throughout the brain (de Kloet et al., 2005; Joëls and Baram, 2009): Whereas MRs are predominantly found in the hippocampal formation, GRs are more ubiquitously located in the brain, though high concentrations of this receptor type have been located particularly in the medial prefrontal cortex (mPFC) (Diorio et al., 1993; Sanchez et al., 2000). Thus, the increase in RSFC between the amygdala and mPFC could very well be mediated by binding of cortisol to glucocorticoid receptors in this region.

The pgACC has been described extensively as an important region in exerting top-down inhibitory control over the amygdala (Phillips et al., 2003a; Pezawas et al., 2005; Quirk

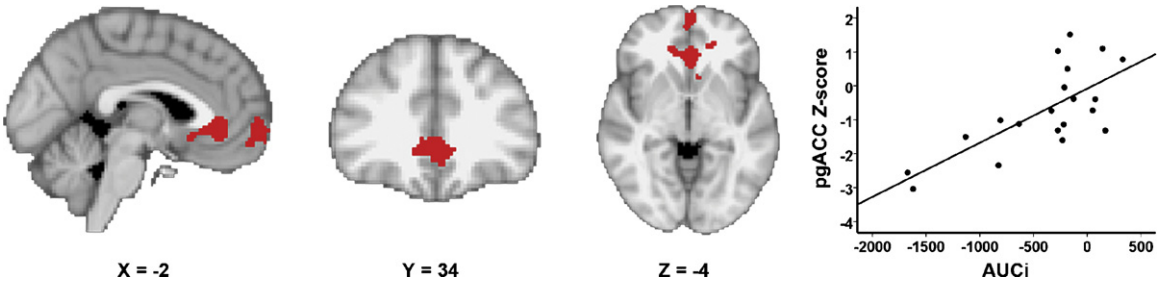

Figure 2 Results ($Z > 2.3$, $p < .05$, cluster corrected for multiple comparisons) overlaid on the 2 mm MNI standard space template. The left side of the brain corresponds to the right hemisphere and vice versa. The scatter plot illustrates the correlation between cortisol AUCi and strength of amygdala RSFC with the pgACC.

and Beer, 2006; Pessoa, 2008), thereby contributing to adaptive emotion regulation. This is supported by the direct anatomical connections between the two regions (Ghashghaei and Barbas, 2002; Ghashghaei et al., 2007). As such, the pgACC also provides a good candidate for adjusting the stress response. Accordingly, studies in rodents ascribe this function to the dorsal prelimbic cortex, commonly considered a homologue of the human pgACC: lesions within this region have been found to cause diminished regulation and thereby disinhibition of the stress response (Diorio et al., 1993; Boyle et al., 2005; Furay et al., 2008; Ulrich-Lai and Herman, 2009). Additionally, in humans decoupling of the pgACC and amygdala has been well-documented in relation to disturbed emotion regulation in stress-related psychiatric disorders (Phillips et al., 2003b; Heinz et al., 2005; Shin et al., 2006; Johnstone et al., 2007; Veer et al., 2010), a feature that might also underlie the aberrant HPA-axis activity so often found to accompany these disorders (McEwen, 2005; Liberzon et al., 2007; MacKenzie et al., 2007). Interestingly, recent studies indicate that glucocorticoid administration might be effective in treating posttraumatic stress disorder and phobias (de Quervain and Margraf, 2008), potentially impacting the pgACC. The putative role of stress agents in pgACC function is furthermore underscored in a recent study showing diminished decreased activity in the pgACC when viewing emotional faces after administration of vasopressin (Zink et al., 2010).

The association of cortisol with the connection between the amygdala and the medial frontal pole (BA10) does resemble one of the effects found in the group of participants that did receive stress (Veer et al., 2011). The current results thus suggest that participants who showed a lesser decrease or even a small increase in endogenous cortisol over the course of the experiment demonstrate a connectivity pattern similar to what is found in participants who had been exposed to stress. In the stress group, however, this effect was irrespective of the cortisol response to the stressor, possibly due to a ceiling effect in their physiological response or a more complex interaction between neuroendocrine responses to the stressor. On the other hand, using FDG-PET imaging Kern et al. (2008) did show that stress-induced cortisol was related to decreased glucose metabolism in BA10, albeit such a finding is often difficult to relate to RSFC measures as obtained with fMRI. Since BA10 is hypothesized to be involved in stimulus oriented behavior (Burgess et al., 2007a,b), the increased RSFC of BA10 with the amygdala found in our study might indicate that an increase in cortisol promotes more vigilance towards threatening stimuli in our surroundings.

Importantly, we found that baseline cortisol showed a strong inverse association with AUCi dynamics. That is, higher cortisol levels at baseline were indicative of larger cortisol decreases over the course of the experiment, whereas participants with lower baseline cortisol levels tended to demonstrate either a flattened AUCi or a small increase. Nevertheless, Urry et al. (2006) demonstrate that steeper (i.e., more normative) diurnal cortisol curves are related to higher vmPFC and lower amygdala activity and better performance during affect regulation, which could pertain to the results found in the current study: Participants demonstrating large AUCi decreases also showed strong negative functional connectivity between the amygdala and mPFC. This might indicate how dynamical behavior of diurnal cortisol aids successful regulation of stress- and, more general, emotional responses.

In the current study setup, however, we cannot infer whether baseline cortisol alone or its interaction with time as is measured with the AUCi is driving our effects. However, our analyses strongly suggest that baseline cortisol alone is predictive of functional coupling between the amygdala and mPFC. Baseline cortisol was measured almost 90 min before RS data acquisition, yet was still associated with the strength of functional coupling of the amygdala. This might be indicative of a slow acting effect of cortisol, which has previously been related to altered functional coupling between the amygdala and mPFC during an emotional task paradigm (Henckens et al., 2010), and homeostatic processes in the aftermath of stress in general (Sapolsky et al., 2000).

Since our effects are based on correlations, it must be noted that we cannot make any inference on causality. That is, effects could be interpreted as either bottom-up or top-down in the case of amygdala-mPFC connectivity, or either as cause or consequence in the case of cortisol levels. Nevertheless, our interpretation of mPFC mediated top-down regulation of the amygdala does seem plausible given the number of studies reporting such a causal relationship between the pgACC and amygdala (Pezawas et al., 2005; Quirk & Beer, 2006; Stein et al., 2007). Furthermore, an mPFC dependent regulation of HPA-axis activity has been well established in animal research, pointing to a facilitating role of cortisol in this circuit (Boyle et al., 2005; Diorio et al., 1993; Radley et al., 2006; Furay et al., 2008; Ulrich-Lai and Herman, 2009). A second limitation of the method pertains to network specificity when studying cortisol. Although the

amygdala and its connections are heavily implicated in the brain's stress circuitry, employing a seed-based connectivity analysis renders us blind to any effects of cortisol on other resting-state functional connectivity networks. Thirdly, our results might have been influenced by the emotional working memory task that preceded the resting-state scan. Although there was a 20-min interval in between the two scans, we cannot rule out such an effect, especially since perceived stress was mildly elevated directly after the task. Nonetheless, we did not find a relation between perceived stress and the functional connectivity patterns observed, nor was there an association with cortisol either measured as AUCi, at baseline, or during resting-state acquisition.

Our participants were not exposed to a stress paradigm, so the nature of the difference in endogenous cortisol fluctuations remains speculative, though several explanations can be proposed: (1) although not intended, (the anticipation of) lying inside the MRI scanner might have induced stress in some of our participants. Mild increases in cortisol levels have been called "scanner-induced stress" recently (Muehlhan et al., 2011), a scenario that is especially plausible when including scanner naïve participants, as was the case in our study. In addition, the increase in perceived stress inside the scanner argues in favor of such scanner-induced stress. (2) Related to the previous point, anticipation of the experiment might already have caused elevated cortisol levels in some participants prior to arrival, while tension could have decreased after intake and instructions. (3) A flattened cortisol curve, as was observed in several participants, also could have been related to stressful life circumstances rather than being induced by the experimental context (Polk et al., 2005). However, participants were specifically required to score low on psychoneuroticism, anxiety, and depressive symptoms to be included in this study, which renders it unlikely that a recent stressful life event would have caused flattening of the cortisol morning curve. (4) Another explanation could lie in the time of arrival, in spite of counterbalancing within the group, because subjects arriving early in the morning might demonstrate higher cortisol baseline levels and therefore a steeper decrease over the course of the experiment. However, no difference in either AUCi or baseline cortisol was found between the early and late arrival participants. (5) We did not, however, obtain information on the time participants woke up on the morning of the experiment. Therefore, we cannot exclude that some baseline cortisol levels were higher due to a shorter time frame between wakening and participation in the experiment. (6) Lastly, differences in genetic makeup (e.g., expression of cortisol receptors throughout the brain) potentially could explain the individual differences in HPA-axis activity in our sample (Wüst et al., 2000; Ouellet-Morin et al., 2008).

In sum, here we show that the strength of RSFC between the amygdala and mPFC can be related to individual differences in endogenous cortisol under relatively stress-free circumstances. Although tentative, this finding could be indicative of a cortisol-mediated regulatory circuit served to adaptively adjust stress- and, more generally, emotional responses. This hypothesis should be further tested, however, using a controlled manipulation of cortisol levels, for example by dose—response experiments in which several dosages of hydrocortisone are administered. Although the current analysis was carried out on a group of participants that was not intentionally exposed to stress, our results might explain how this feedback mechanism may cause cessation of a stress response, pointing to the putative role of glucocorticoids in reaching homeostasis after a stressful event (McEwen, 2005). The current results also might provide an important link to the pathophysiology of stress-related psychiatric disorders, in which such feedback seems to fail. For the first time in humans, our results show a link between endogenous cortisol and functional connectivity between the amygdala and pgACC, which might further establish the role of cortisol in adaptive emotion regulation.

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Conflict of interest

All authors declare not to have any financial interests or potential conflicts of interest in having the results of this study being published.

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