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Corticosteroid Induced Decoupling of the Amygdala in Men

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The amygdala is a key regulator of vigilance and heightens attention toward threat. Its activity is boosted upon threat exposure and contributes to a neuroendocrine stress response via the hypothalamic–pituitary–adrenal (HPA) axis. Corticosteroids are known to control brain activity as well as HPA activity by providing negative feedback to the brain. However, it is unknown how corticosteroids affect the neural circuitry connected to the amygdala. Implementing a randomized, double-blind, placebo-controlled design, we here investigated the effects of 10-mg hydrocortisone on amygdala-centered functional connectivity patterns in men using resting state functional magnetic resonance imaging. Results showed generally decreased functional connectivity of the amygdala by corticosteroids. Hydrocortisone reduced "positive" functional coupling of the amygdala to brain regions involved in the initiation and maintenance of the stress response; the locus coeruleus, hypothalamus, and hippocampus. Furthermore, hydrocortisone reduced "negative" functional coupling of the amygdala to the middle frontal and temporal gyrus; brain regions known to be involved in executive control. A control analysis did not show significant corticosteroid modulation of visual cortex coupling, indicating that the amygdala decoupling was not reflecting a general reduction of network connectivity. These results suggest that corticosteroids may reduce amygdala's impact on brain processing in the aftermath of stress in men.

Keywords: amygdala, corticosteroids, fMRI, functional connectivity, resting state

Introduction

Corticosteroids are potent modulators of human cognitive function. The hormones are released in response to stress as the end product of the hypothalamic–pituitary–adrenal (HPA) axis and known to readily cross the blood-brain barrier to affect brain processing (McEwen 1979). Corticosteroids exert their actions upon binding of the mineralocorticoid (MR) and glucocorticoid receptor (GR), which are abundantly expressed in the brain (Sapolsky et al. 1983; Reul and de Kloet 1985; de Kloet 1991). By binding to these receptors, corticosteroids control the excitability of neuronal networks under rest, resulting in tonic inhibition of HPA axis activity (De Kloet and Reul 1987) but also during exposure to stress, contributing to behavioral adaptation (de Kloet et al. 1999). An additional prominent function of corticosteroids is to exert negative feedback on the HPA axis after stress exposure, which makes the hormones crucial for the limitation and termination of the stress response (De Kloet and Reul 1987). This negative feedback is primarily established by direct inhibition of the core structures of the HPA axis itself, the pituitary and the paraventricular nucleus (PVN) of the hypothalamus (Herman and Cullinan 1997), but the hippocampus has also been proposed to contribute to this negative feedback (de Kloet et al. 1993). Furthermore, corticosteroids are thought to provide both support and regulation of the sympatho-adrenomedullary (SAM) system, which subserves the initial "fight-or-flight" response to threat. Activation of the SAM system occurs immediately upon threat exposure and induces an elevation of central norepinephrine (NE) levels through increased tonic activity of the pontine locus coeruleus (LC) (Valentino and Van Bockstaele 2008). SAM activation thereby induces a hypervigilant state of processing that optimizes the detection and assessment of threats by prioritizing sensory processing (Henckens et al. 2009; van Marle et al. 2009; Shackman et al. 2011), while suppressing higher order executive function (Diamond et al. 2007; Arnsten 2009; Qin et al. 2009). Although this response serves a clear adaptive purpose, sustained activation of vigilance-related brain circuits may become maladaptive and culminate in mental diseases such as depression (Siever and Davis 1985), and proper regulation is of critical importance to human mental health.

One of the main targets of the SAM system and mediators of the initial surge in vigilance is the amygdala (de Kloet et al. 2005; Phelps and LeDoux 2005; van Marle et al. 2009). Its dense connectivity pattern places it at the center of the brain's emotional processing network as a physical hub linking numerous distant regions, allowing emotions to influence brain processing from the first stages of perception (Vuilleumier and Driver 2007) to the regulation of social behavior (Adolphs 2010). It is reciprocally connected to a frontal executive system, which is on the one hand involved in the control of this emotional processing state (Phillips et al. 2003) but on the other hand subjected to influences of this emotional state. Previous imaging studies have shown that acute or prolonged stress increase amygdala reactivity (van Marle et al. 2009; van Wingen et al. 2011a), impair higher executive function (Diamond et al. 2007; Arnsten 2009; Qin et al. 2009), and strengthen amygdala's connectivity to the other regions of the vigilance network, such as the LC (Seeley et al. 2007; van Marle et al. 2010; van Wingen et al. 2011a, 2011b). At the same time, animal and human studies have demonstrated that corticosteroids can reduce amygdala sensitivity (Henckens et al. 2010; Karst et al. 2010), suggesting that corticosteroids play a critical role in the restoration of homeostasis by normalizing/desensitizing brain processing following stress exposure (de Kloet et al. 2005). Corticosteroids might therefore also have an effect opposite to that of acute stress on amygdala connectivity, but this issue remains to be resolved.

Here, we investigated the effect of corticosteroids on amygdala-centered connectivity patterns in men during rest. We implemented a randomized, double-blind, placebo-controlled design, in which 48 male subjects received either placebo or 10-mg hydrocortisone prior to resting-state functional magnetic resonance imaging (fMRI). Functional connectivity was evaluated by exploring correlations in blood oxygen level–dependent (BOLD) signal fluctuations over time between brain areas, enabling us to map patterns of connectivity under rest. Given the key role of the amygdala in the stress response, we used a seed-region approach correlating fluctuations in amygdala activity over time to the rest of the brain, and tested the hypothesis that corticosteroids affect amygdala connectivity, especially to regions involved in the initiation and regulation of the stress response, including the LC, hypothalamus (PVN), hippocampus, and the frontal regions exerting executive control. To check the specificity of these effects, we included a control seed region analysis for the primary visual cortex, testing whether corticosteroids induced any general effects on network connectivity.

Materials and Methods

Participants

Forty-eight young (age range 19–28, median 21), right-handed, healthy male volunteers gave written informed consent to participate in the study. Women were excluded from participation for several reasons. First of all, functioning of the amygdala in women seems to differ from that in men; both amygdala responsivity (Cahill et al. 2004) and connectivity (Kilpatrick et al. 2006) are different between sexes. Furthermore, previous research has indicated that women respond differently to hydrocortisone than men, both in behavior (Andreano and Cahill 2006; Bohnke et al. 2010) and brain activation (Stark et al. 2006; Merz et al. 2010). We presently focused on men, allowing easier comparison with the results from an earlier study in which subjects were exposed to stress (Henckens et al. 2009), a situation that is known to induce more stable neuroendocrine response in men than in women (Kirschbaum et al. 1999; Kajantie and Phillips 2006; Bouma et al. 2009; Ossewaarde et al. 2010). Furthermore, individuals who met any of the following criteria were excluded from participation: history of head injury, autonomic failure, history of or current psychiatric, neurological, or endocrine disorders, current periodontitis, acute inflammatory disease, acute peptic or duodenal ulcers, regular use of corticosteroids, treatment with psychotropic medications, narcotics, beta-blockers, steroids, or any other medication that affects central nervous system or endocrine systems, medical illness within the 3 weeks prior to testing, self-reported mental or substance use disorder, daily tobacco or alcohol use (or experienced inconvenience in refraining from these activities for 3 days), exercising at the professional level, regular night shift work, or current stressful episode or major life event. Three participants were excluded from analyses because of unreliable cortisol manipulation (abnormal basal cortisol levels [1 × placebo] or no elevation in salivary cortisol level in response to hydrocortisone intake [2 × CORT]), and another 2 participants because of fMRI data dropout (2 × CORT). Thus, the results are based on data of 23 men in the placebo group and 20 in the hydrocortisone (CORT) group. The study was approved by the local ethics committee (CMO region Arnhem-Nijmegen, Netherlands) and executed in accordance with the declaration of Helsinki.

Study Design

Prior to Arrival

Prior to inclusion all eligible participants received an extensive information brochure, listing all in- and exclusion criteria and roughly explaining the setup of the experiment. If criteria were met (according to the participant's own insights), an appointment was made. To minimize differences in baseline cortisol levels, we instructed participants not to use any recreational drugs for 3 days and to refrain from drinking alcohol, exercising, and smoking for 24 h prior to the appointment. Furthermore, participants were requested not to brush their teeth, floss, or eat and drink anything but water for 1 h prior to the session enabling adequate saliva sampling for cortisol assessment. They were asked to take a light lunch and do so no later than 1 h before arrival; their lunch could not contain any citrus products, coffee, tea, milk, or sweets (Maheu et al. 2005). Throughout the entire study period, participants were only given water to drink, except for a scheduled lunch at $t = -120$ min.

Arrival

To minimize individual differences due to daily activities and to reduce the impact of diurnal variation in cortisol levels, all participants were invited to the laboratory in the early afternoon, between 12:00 and 13:00 h. Upon arrival, participants received an information brochure about the procedure, they gave written informed consent, and completed an intake questionnaire to ensure that in- and exclusion criteria were met. Furthermore, participants were asked to complete a first Profile of Mood States (POMS) questionnaire (Reddon et al. 1985; Wald and Mellenbergh 1990; de Groot 1992). During the entire waiting period (~3.5 h) prior to scanning, participants had to wait in a quiet room where they were free to conduct any activities except for anything potentially arousing. At specific time points, the experimenter entered to room to take a saliva sample. At 105 min prior to the resting-state scan (at $t = 0$), participants were asked to complete a second POMS questionnaire and received the drug capsule. Drug administration occurred in a randomized, double-blind, placebo-controlled manner in which participants received either 10-mg CORT or placebo (cellulose), depending on the group to which the participant was (randomly) assigned. Capsules were administered orally. This dose of hydrocortisone is known to elevate salivary cortisol levels to moderate-to-high stress levels (Kirschbaum et al. 1996; Groschl et al. 2002; Tops et al. 2003; Henckens et al. 2010).

At about 4.5 h after arrival, participants were taken to the scanner room. The resting-state run started 105 min after administration of the capsule and lasted for 8 min. Participants were asked to lay as still as possible, close their eyes, and think of nothing in particular. They were instructed to relax, but not fall asleep, which was checked by verbal debriefing immediately afterward. The session ended with a structural scan.

Physiological and Psychological Measures

Saliva Collection and Analysis

Cortisol levels were measured from saliva at 10 time points: 2 baseline measurements at the beginning of the experimental day ($t = -225$, -210 min), and 8 samples thereafter ($t = -180$, -150, -120, 0, 30, 60, 90, and 120 min) to assess cortisol changes throughout the experiment. Saliva was collected using a commercially available collection device (Salivette, Sarstedt, Germany). For each sample, the participant first placed the cotton swab provided in each Salivette tube in his mouth and chewed gently on it for 1 min to produce saliva. The swab was then placed back in the salivette tube, and the samples were stored in a freezer at −25 °C until assayed. Laboratory analyses were performed at the Department of Biopsychology, TU Dresden, Germany. After thawing, salivettes were centrifuged at 3000 rpm for 5 min, which resulted in a clear supernatant of low viscosity. Salivary free cortisol concentrations were subsequently measured using a commercially available chemiluminescence immunoassay with high sensitivity of 0.16 ng/mL (IBL, Hamburg, Germany).

Heart Rate

Cardiac rhythm of the participants was measured during scanning using a pulse oximeter placed on their left index finger. Participants were instructed to keep their hands as still as possible during the measurement. After the completion of scanning, in-house software was used for offline artifact correction and the analysis of heart rate signal, calculating heart rate frequency (HRF) and heart rate variability (HRV). The HRF was calculated as 60/mean interbeat interval and HRV as the root mean squares of successive differences between successive

interbeat intervals. This method assesses high-frequency variability in HR, which is thought to result from parasympathetic action mainly and is expected to show a decrease as a function of stress (Berntson et al. 1997; Goedhart et al. 2007).

Mood State

To exclude potential psychological side-effects of hydrocortisone administration, mood state was assessed using the POMS questionnaire (Reddon et al. 1985; Wald and Mellenbergh 1990; de Groot 1992) at 3 time points: at the beginning of the experiment (t = −225 min), just prior to the intake of the capsule (t = 0 min), and at the end of the experiment (t = 120 min).

Physiological and Psychological Statistical Analysis

Behavioral and physiological data were analyzed in SPSS 15.0 (SPSS, Inc., Chicago, IL) using repeated measured analyses of variance (ANOVAs) with drug condition (placebo vs. CORT) as between subject factor. Due to the high levels of skewness and kurtosis of the POMS questionnaire (Reddon et al. 1985; Wald and Mellenbergh 1990; de Groot 1992), mood data were analyzed using nonparametric tests. Changes over time in mood state were assessed by Friedman tests, and Mann–Whitney U tests were used to assess potential drug effects on mood. Alpha was set at 0.05 throughout.

MRI Acquisition

Participants were scanned by a Siemens (Erlangen, Germany) MAGNETOM Avanto 1.5 Tesla MRI scanner equipped with an 8-channel head coil. A series of 265 BOLD T_2^*-weighted gradient-echo echo planar imaging (EPI) images (≈ 8 min) was acquired with the following parameters: time repetition = 1870 ms, time echo (TE) = 35 ms, FA = 80°, 39 axial slices approximately aligned with AC-PC plane, slice matrix size = 64 × 64, slice thickness = 3 mm, slice gap = 0.35 mm, field of view = 224 × 224 mm^2. Owing to its relatively short TE, this sequence yields optimal contrast-to-noise ratio in the medial temporal lobes (Stocker et al. 2006).

fMRI Data Analysis

Data were analyzed using Statistical Parametric Mapping software (SPM5; UCL, London). The first 5 EPI volumes were discarded to allow for T_1 equilibration. Prior to analysis, the images were motion corrected using rigid body transformations and least sum of squares minimization. Subsequently, they were temporally adjusted to account for differences in sampling times across different slices. All functional images were then coregistered with the high-resolution T_1 weighted structural image using normalized mutual information maximization. The anatomical image was subsequently used to normalize all scans into MNI152 (Montreal Neurological Institute) space. All functional images were resampled to a voxel size of 2 mm isotropic. Finally, all images were smoothed with an isotropic 8-mm full-width at half-maximum Gaussian kernel in order to accommodate residual functional/anatomical variance between subjects.

Next, we extracted the amygdala time course using an anatomical mask that was created based on the locus of previously observed corticosteroid effects on amygdala responsivity (4-mm sphere around the peak coordinates [x = −28, y = −4, z = −12] and [x = 26, y = −4, z = −12]) (Henckens et al. 2010). Second, the first eigenvariate of the set of time courses from voxels comprising the amygdala was calculated for each subject. The resulting time series was used as a covariate of interest in a whole-brain, linear regression, statistical parametric analysis. Correlating this pattern of activity (the time series) to that observed in the rest of the brain provides information on regions that are "coupled" in activity and supposedly functionally connected. Besides this regressor, the amygdala time course, the realignment parameters, consisting of 6 parameter rigid body transformations (3 translations and 3 rotations) used for motion correction, were additionally included to model potential movement artifacts. Furthermore, global fluctuations, originating presumably from such systemic effects as respiration and cardiac-induced pulsations (Macey et al. 2004; Birn et al. 2006), were accounted for by extracting signal from individually defined white matter, gray matter, and cerebrospinal fluid (CSF) masks and including these in the model. Masks were generated by segmenting the high-resolution structural images in SPM5 and downsampling the obtained white matter and CSF masks to the same resolution as the functional data. Contrast parameter images for the seed region covariate generated at the single subject level were then submitted to second-level random effects analysis.

To evaluate whether any corticosteroid effects on amygdala connectivity were related to general corticosteroid effects on brain connectivity, we performed the same analyses on a control seed region consisting of the primary visual cortex defined by a 4 mm sphere (similar to the amygdala) around the probabilistic cytoarchitectonic center of Brodmann area 17, the occipital cortex (hOC1) ([x = −10, y = −89, z = 8] and [x = 15, y = −85, z = 8]) (Amunts et al. 2000).

Statistical parametric maps were created within SPM5 using a two sample t-test contrasting the CORT group versus the placebo group. Statistical tests were family wise error (FWE) rate corrected (P < 0.05) for multiple comparisons at the voxel level for main effects of amygdala coupling across drug conditions and on the cluster level using a height threshold of P < 0.005 to assess cortisol effects. Correction for multiple comparisons was done across the entire brain or for the search volume for regions of interest (ROIs) using a small volume correction. Given our a priori hypotheses on corticosteroid modulation, the LC, hypothalamus (PVN), hippocampus, and frontal cortex were targeted as ROI's in our analysis of amygdala coupling. Specifically, we implemented a reduced spherical search volume (5-mm radius) around anatomically defined center coordinates for the LC (Astafiev et al. 2010) and hypothalamus, which was centered on the PVN (Baroncini et al. 2012). Data concerning the hippocampus and prefrontal cortex were corrected for reduced search regions through anatomical masks as defined by the WFU PickAtlas Tool (version 2.4) (Maldjian et al. 2003). In analyzing potential effects of corticosteroids on primary visual cortex coupling, 4 other ROI's were selected, based on their known dense connectivity pattern of these regions to the primary visual cortex and their role in visual processing (McIntosh et al. 1994; Lowe et al. 1998). Data concerning the cuneus, calcarine, lingual gyrus, and fusiform gyrus were corrected for reduced search regions through anatomical masks as defined by the WFU PickAtlas Tool (version 2.4) (Maldjian et al. 2003). Furthermore, the targeted ROI's for amygdala coupling, that is, the LC, hypothalamus (PVN), hippocampus and prefrontal cortex, were included into this analysis.

Visualizations of activations were created in SPM5 by superimposing statistical parametric maps thresholded at P < 0.001 uncorrected (unless specified otherwise) onto a canonical T_1-weighted image in a standard MNI 152 space.

Results

Physiological and Psychological Measures

As expected, oral administration of 10-mg hydrocortisone increased salivary cortisol levels to those observed during moderate to high levels of stress (Schommer et al. 1999; Morgan et al. 2000) (Table 1), which was evidenced by a significant main effect of group ($F_{1,41}$ = 55.34, P < 0.001) and a time × group interaction ($F_{9,33}$ = 16.46, P < 0.001). Groups did not differ on baseline cortisol levels ($F_{1,41}$ = 1.50, n.s). Increased levels were observed from 30-min postadministration onward (t = 30 min), and the levels remained similarly elevated for at least 90 min (t = 120 min). Thus, during resting state fMRI scanning (at t = 105 min), the CORT group displayed significantly higher cortisol levels than the control group.

Postexperiment debriefing showed that participants were unable to identify the substance received. As expected, hydrocortisone administration did not affect autonomic measures of HRF (main effect of drug: t_{39} = −1.73, n.s.) and HRV (t_{39} = 1.24, n.s.) (Supplementary Table 1). Furthermore, drug administration did not affect mood as assessed 3 times during the

Table 1
Cortisol manipulation

Time	Salivary cortisol (nmol/L)	
	Placebo	Hydrocortisone
t = −225 min	9.58 (0.77)	11.80 (1.40)
t = −210 min	8.49 (0.74)	9.91 (1.32)
t = −180 min	6.29 (0.43)	7.74 (1.05)
t = −150 min	6.07 (0.60)	7.38 (0.90)
t = −120 min	5.50 (0.59)	6.14 (0.72)
t = 0 min	7.87 (0.75)	5.85 (0.45)
t = 30 min	7.06 (0.64)	32.64 (6.51)***
t = 60 min	7.48 (0.84)	25.71 (2.21)***
t = 90 min	6.47 (0.69)	25.62 (1.96)***
t = 120 min	5.60 (0.65)	24.51 (1.90)***

Note: The resting state scan was recorded at t = 105 min. Mean values (standard error of the mean) ***: $P < 0.001$.

experiment using the POMS questionnaire (Reddon et al. 1985; Wald and Mellenbergh 1990; de Groot 1992) (Supplementary Table 1). Although significant reductions in levels of anger scores ($\chi^2(2) = 11.22$, $P = 0.004$), vigor scores ($\chi^2(2) = 45.72$, $P < 0.001$), tension scores ($\chi^2(2) = 13.27$, $P < 0.001$), and close to significant reductions in depression scores (Friedman's ANOVA; $\chi^2(2) = 5.78$, $P = 0.056$) were observed over the course of the experiment and levels of fatigue increased ($\chi^2(2) = 36.01$, $P < 0.001$), none of these factors was affected by drug administration (all Ps > 0.05). Groups did also not differ on baseline levels of these mood measures at intake (all Ps > 0.05). Hence, differences in brain activity found between drug conditions cannot readily be explained by any physiological or psychological side effects of hydrocortisone nor because of initial group differences in physiological or psychological traits.

Amygdala Coupling

To investigate the effect of corticosteroids on amygdala connectivity, we analyzed resting-state data using a seed region approach. First, brain regions were identified that were functionally coupled, that is, displaying significantly correlated patterns of activity, to the amygdala across both experimental groups (taking the CORT and control groups together). Spontaneous activity in the amygdala positively predicted spontaneous activity in a large activation cluster covering the bilateral amygdala itself, the brain stem (including the LC), hippocampus, hypothalamus, parahippocampal gyrus, temporal pole, pallidum, putamen, insula, and inferior frontal cortex. Other regions positively predicted by amygdala activity included the fusiform, ACC, middle orbitofrontal cortex, and regions within the cerebellum ($P_{corr} < 0.05$, see Table 2, Fig. 1*A*). Conversely, amygdala activity was negatively associated with activity in frontal and posterior regions such as the middle frontal gyrus, medial superior frontal gyrus, superior frontal gyrus, middle temporal gyrus, cuneus, brain stem, and cerebellum (Table 2, Fig. 1*B*). Overall, these patterns of functional connectivity are in line with previous studies (Roy et al. 2009; van Marle et al. 2010) and support models of emotion processing that suggest reciprocal ventral and dorsal systems (Phillips et al. 2003).

Second, when contrasting the CORT and the control group, we found reduced correlated activity of the amygdala with 2 regions critically involved in the initiation of the stress response; the LC ([x = −8, y = −36, z = −22], t_{41} = 3.61, P_{SVC} = 0.047) and the hypothalamus ([x = 0, y = 0, z = −14], t_{41} = 2.99, P_{SVC} = 0.044, Table 2, Fig. 1*C*). The peak coordinates of the cluster observed in the hypothalamus seemed to colocalize with the location of the PVN (x = 2, y = 1, z = −12) (Baroncini et al. 2012), which is the expected target for corticosteroid-mediated negative feedback (Herman and Cullinan 1997).

Moreover, significantly reduced correlated activity was observed between the amygdala and hippocampus ([x = 24, y = −22, z = −14] t_{41} = 4.15, P_{corr} = 0.025). Extraction of the parameter estimates of these regions showed that their activity was positively correlated with activity of the amygdala in the placebo conditions and that hydrocortisone reduced this positive coupling (Fig. 1*C*).

Looking at the opposite contrast, that is, CORT-increased correlations in activity patterns of the amygdala, revealed bilateral clusters in the middle frontal cortex ([x = 32, y = 34, z = 24] t_{41} = 4.45, $P_{corr} < 0.001$, [x = −28, y = 30, z = 24], t_{41} = 4.42, $P_{corr} < 0.001$) and a region within the middle temporal gyrus [x = 58, y = −66, z = 20], t_{41} = 4.16, P_{corr} = 0.032, Table 2, Fig. 1*D*). Further data analyses showed that this "increased" correlation between activity in the amygdala and these frontal and temporal regions in fact reflected "reduced negatively" correlated activity between these regions; whereas activity in these regions was negatively related to amygdala activity in the placebo condition, this negative relationship was nonexistent in the hydrocortisone condition (Fig. 1*D*). Thus, corticosteroids seem to "decouple," or disconnect, the amygdala from the rest of the brain by reducing functional connectivity to regions that are positively as well as negatively correlated with its activity.

Visual Cortex Coupling

To evaluate whether the observed corticosteroid-induced decoupling within the amygdala network was due to a general corticosteroid-induced network decoupling, we performed the same analyses on a control seed region in the primary visual cortex. The overall connectivity analysis showed a pattern of positive coupling in an extended visual processing network covering the occipital lobe, cuneus, calcarine, lingual gyrus, and fusiform gyrus, which is in line with previous studies (McIntosh et al. 1994; Lowe et al. 1998). Negative coupling of the primary visual cortex was observed for the cerebellum and a region within the brain stem (Supplementary Table 2). Importantly, this connectivity pattern was not significantly affected by corticosteroid administration, neither within the visual processing network itself nor to the regions observed for which altered amygdala coupling was observed (minimum P_{corr} = 0.565). These findings suggest that the observed corticosteroid-induced amygdala decoupling did not reflect a general reduction of network connectivity.

Discussion

The present study aimed at investigating how corticosteroids influence the functional amygdala network. The amygdala showed decreased "positive" coupling in response to corticosteroids to brain areas implicated in the initiation and regulation of the stress response: the LC, hypothalamus, and hippocampus. Diminished negative amygdala coupling due to corticosteroids was observed in executive control areas: the middle frontal gyrus and middle temporal gyrus. No strengthening of any connections was observed. Analysis of the connectivity patterns of a control seed region, the primary visual cortex, revealed no such corticosteroid modulation, indicating that these alterations

Table 2

Peak voxels and corresponding *T* values of significantly activated clusters that show functional coupling with the bilateral amygdala for both groups combined and for main effects of hydrocortisone

Region	Cluster size	MNI coordinates			*T* value
		x	*y*	*z*	
Positive coupling amygdala					
Extended cluster covering the bilateral amygdala, brain stem (including LC), hippocampus, hypothalamus, parahippocampal gyrus, temporal pole, pallidum, putamen, insula, and inferior frontal cortex	6323***	28	−6	−12	22.30
Putamen, L	11**	−26	10	8	6.22
R	5**	36	6	14	6.16
Fusiform, L	1*	−34	−36	−14	5.82
Anterior cingulate cortex	13***	−6	50	0	6.20
	12***	−4	30	−6	5.98
Middle orbitofrontal gyrus	110***	4	48	2	7.52
Superior frontal cortex, R	1*	16	70	12	5.83
Cerebellum 6, L	7**	−32	−52	−26	6.18
R	52***	32	−50	−26	6.50
Cerebellum 8, L	62***	−4	−68	−30	7.33
Cerebellum 9, L	3*	−6	−56	−32	6.04
R	3*	10	−58	−34	5.98
Cerebellum Crus1, L	3**	−42	−50	−30	5.86
R	2*	46	−50	−34	5.74
Negative coupling amygdala					
Cuneus	30***	0	−94	16	6.69
Medial superior frontal gyrus	148***	8	52	44	7.28
Middle frontal gyrus, R	74***	48	18	42	6.72
Superior orbitofrontal cortex, R	4**	12	46	−22	6.05
Cerebellum, L	13***	−12	−50	−24	6.91
R	1*	20	−34	−46	6.00
Brain stem	24***	−2	−18	−4	6.60
Negative main effect of hydrocortisone					
Hypothalamus	$5^{\#}$	0	0	−14	2.99
Hippocampus, R	335^{+}	24	−22	−14	4.15
Locus Coeruleus (LC)	$4^{\#}$	−8	−36	−22	3.13
Positive main effect of hydrocortisone					
Middle frontal gyrus, L	574^{+++}	−28	30	24	4.42
R	1142^{+++}	32	34	24	4.45
Middle temporal gyrus, R	320^{+}	58	−66	20	4.16

Note: MNI, Montreal Neurological Institute; R, right; L, left. All effects are analyzed using cluster-level statistics. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$ (whole brain corrected, height threshold at $P < 0.05$ FWE corrected at voxel level); $^{+++}P < 0.001$; $^{+}P < 0.05$ (whole brain corrected, height threshold at $P < 0.005$ uncorrected at voxel level); $^{\#}P < 0.05$ (small-volume corrected for ROI, height threshold at $P < 0.005$ uncorrected at voxel level).

did not reflect a general reduction of network connectivity. Thus, in men, corticosteroids appear to "decouple" rather specifically the amygdala from the rest of the brain.

The amygdala is the key modulator of vigilance and emotional processing in the brain (Phelps and LeDoux 2005). Functional connectivity studies have indicated that it is part of a ventral emotional processing system, comprising the insula, ventral striatum, and ventral regions of the ACC and prefrontal cortex. This network of regions is known to be involved in the identification of the emotional significance of a stimulus and the production of an affective state (Phillips et al. 2003; Roy et al. 2009). It is reciprocally connected to a dorsal control system that includes the hippocampus and dorsal regions of the anterior cingulate and prefrontal cortex, which is responsible for the regulation of this affective state (Phillips et al. 2003). Our findings on overall amygdala connectivity patterns, displaying positively and negatively correlated activity with ventral and dorsal regions, respectively, are in line with these networks and previous literature on amygdala connectivity during rest (Stein et al. 2007; Roy et al. 2009).

Corticosteroids reduced positive coupling between the amygdala and the hypothalamus and hippocampus, which may have consequences for cognitive functioning and control of the HPA axis under rest and possibly also after stress. For instance, both the hypothalamus and hippocampus exert a tonic inhibitory influence over HPA axis activity under rest via MR activation, while negative feedback after stress may take place via GR activation (De Kloet and Reul 1987). By contrast, activation of amygdala's GRs is thought to stimulate the HPA axis (Herman et al. 2003). If cortisol would act in a similar way in the aftermath of stress as presently observed in nonstressed subjects, it might promote normalization of the HPA axis by decoupling of the amygdala from these regions. The peak coordinates of the changes observed in the hypothalamus seemed to colocalize with the location of the PVN (Baroncini et al. 2012), which is the expected target for corticosteroid-mediated negative feedback (Herman and Cullinan 1997). However, fMRI lacks the spatial resolution to pinpoint signal activation or coactivation to anatomically minute structures such as distinct hypothalamic nuclei, thereby limiting our conclusions to altered connectivity of the amygdala to the entire hypothalamus.

Interestingly, hydrocortisone also reduced the positive coupling of the amygdala to the LC, the forebrain's main source of NE and activator of the SAM system (Sara 2009). During the initiation of the stress response, the amygdala relies heavily on its reciprocal connections to both the LC and hypothalamus (Silverman et al. 1981; Van Bockstaele et al. 2001; Valentino and Van Bockstaele 2008). Functional coupling between these stress regions increases during acute stress (van Marle et al. 2010), LC firing increases (Valentino and Van Bockstaele 2008), and hypothalamic CRH release is elevated (Gray 1991; Feldman et al. 1995), resulting in elevated levels of arousal (Aston-Jones et al. 1991; Valentino and Van Bockstaele 2008; Joels and Baram

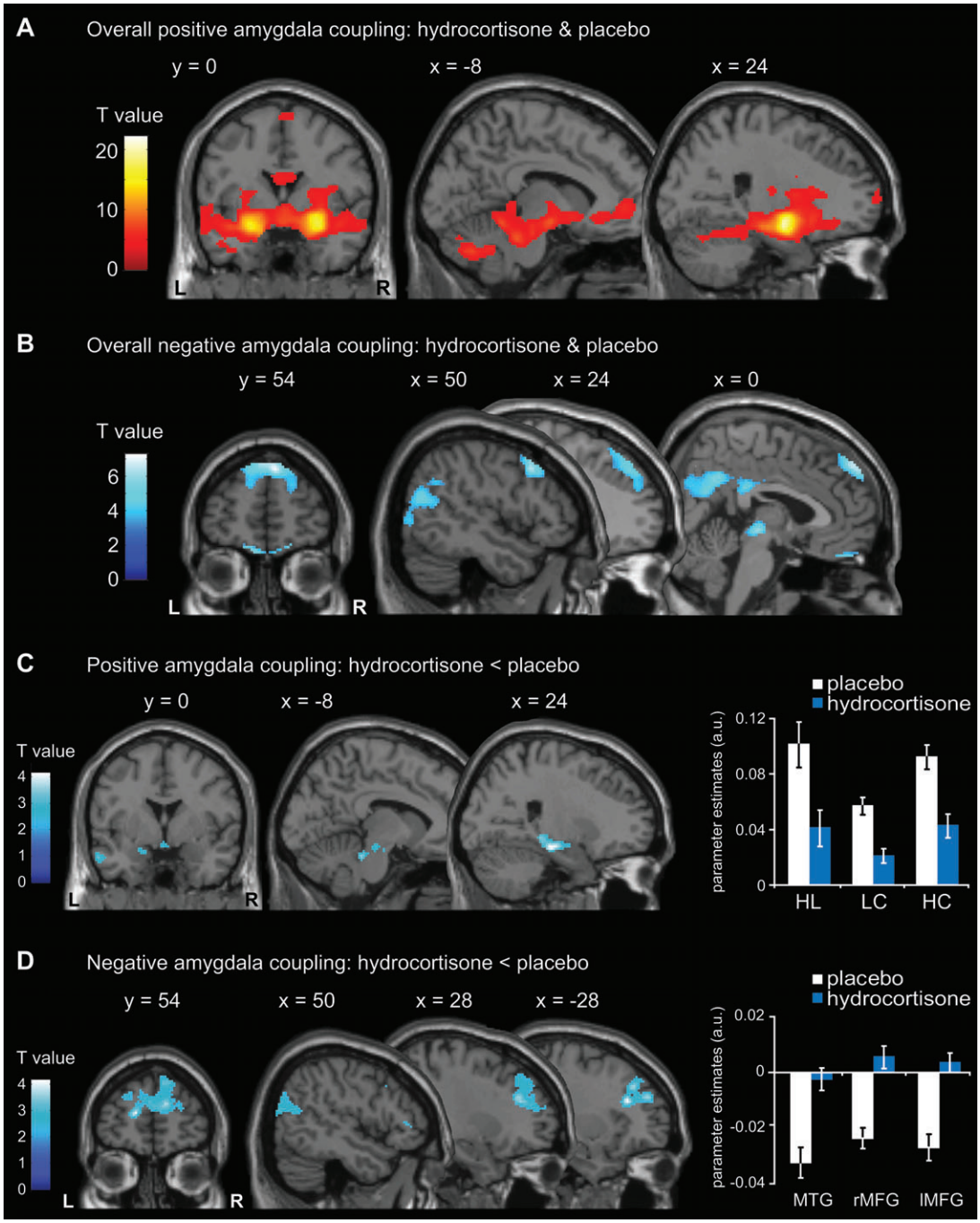

Figure 1. Functional connectivity pattern of the bilateral amygdala during resting state and its modulation by corticosteroids. (*A*) Amygdala's spontaneous activity was positively correlated to an extended network of emotional processing regions, encompassing the brain stem (including the LC), hypothalamus, medial temporal lobe (including the hippocampus), insula, inferior frontal cortex, and the ACC. (*B*) Regions in frontal and posterior brain areas exerting executive control, such as the middle and superior frontal gyrus, displayed negative correlations with activity patterns of the amygdala during rest. (*C*) Hydrocortisone intake attenuated the positive coupling between the amygdala and the hypothalamus (HL), LC, and hippocampus (HC) (all $P_{corr} < 0.05$). (*D*) The negative coupling between the amygdala and regions within middle temporal gyrus (MTG) and the bilateral middle frontal gyrus (rMFG, right; lMFG, left) was also reduced after intake of hydrocortisone (all $P_{corr} < 0.05$). Error bars represent standard error of mean. See Table 2 for exact statistical tests. For visualization purposes, the statistical parametric maps of Figure 1*A*,*B* are thresholded at $P < 0.05$ FWE whole brain corrected at voxel level. Figure 1*C*,*D* are thresholded at $P < 0.01$ uncorrected with a minimal cluster size of 25 voxels (small-volume corrected clusters; Fig. 1*C*) and 250 voxels (whole-brain corrected clusters; Fig. 1*D*), respectively.

2009). Thereby, the brain is shifted into a hypervigilant state of processing in which limbic pathways prevail over prefrontal cortical pathways in the control of affect (Diamond et al. 2007; Arnsten 2009). Corticosteroids reduce the coupling between the amygdala and LC and thereby could prevent subsequent activation of the SAM system. We furthermore speculate that corticosteroids may act similarly in the aftermath of stress exposure, which could curtail prior activation of the LC and thereby normalize the hypervigilant state.

Besides reducing positive functional coupling of the amygdala, corticosteroids also reduced amygdala's negative coupling to the middle frontal gyrus and middle temporal

gyrus. Activity in these regions was negatively correlated with activity in the amygdala under control conditions, but this coupling was diminished under conditions of high cortisol. The observed clusters are part of the so-called executive control network (Seeley et al. 2007), which enables an organism to sustain attention and supports working memory (Curtis and D'Esposito 2003) and response selection (Lau et al. 2006). Activity in this network ensures response flexibility, by directing attention to pertinent stimuli as behavioral choices are weighed against shifting conditions, background homeostatic demands, and context (Seeley et al. 2007). Animal research has already shown that the induction of long-term potentiation of the amygdala–prefrontal cortex pathway by stimulation of the amygdala was impaired in the aftermath of stress (Maroun and Richter-Levin 2003). This, together with our findings, suggests that corticosteroids reduce amygdala's influence on executive function. Such reduction might aid cognitive control processes in the aftermath of stress and contribute to the return to homeostasis.

Some limitations to this design should be mentioned. First of all, recent research has pointed out that corticosteroids are capable in inducing distinct rapid and slow effects by activating non-genomic and genomic cascades, respectively (Joels et al. 2006). Whereas the rapid effects can occur within minutes after brain exposure to corticosteroids (Karst et al. 2005), the typical slow genomic effects take several hours to develop and can last for days (Pavlides et al. 1995; Wiegert et al. 2005). Here, we assessed the effects of corticosteroids ~105 min after hydrocortisone intake. This design ensured elevated corticosteroid levels during fMRI scanning, but maximal rapid effects of corticosteroids might have occurred earlier. Moreover, the rather long delay permitted genomic effects to occur as well, which makes that the corticosteroid effects as reported here are most likely the result of a mixture of both non-genomic and genomic effects on amygdala's functional connectivity patterns, and future studies will be necessary to disentangle both effects.

Secondly, results are not based on a randomly selected population-based sample and are therefore by definition not representative for the entire population. We opted to recruit participants with the most stable response to corticosteroids, making that they had to meet rather strict in- and exclusion criteria in order to be enrolled in this study. Most important, we only included men as participants. Women were excluded because amygdala functioning appears to differ between sexes; both amygdala's responsivity (Cahill et al. 2004) and connectivity (Kilpatrick et al. 2006) have been shown to be different in men and women. Furthermore, women are known to respond differently to hydrocortisone than men, both in behavior (Andreano and Cahill 2006; Bohnke et al. 2010) and brain activation (Stark et al. 2006; Merz et al. 2010). Therefore, we restricted this study to men only. Obviously, because of these in- and exclusion criteria, the results cannot be readily generalized. Future studies will be needed to test whether corticosteroids exert similar effects in women.

Another factor to investigate in future studies is the effect of corticosteroids on the psychological state the participants are in. Mood state is known to modulate amygdala's functional connectivity patterns (Harrison et al. 2008) and could thereby be related to the observed effects in amygdala connectivity. We assessed this state using the POMS questionnaire (Reddon et al. 1985; Wald and Mellenbergh 1990; de Groot 1992) and did not observe a significant difference between groups. However, this lack of significance could be due to too low statistical power to detect any changes in behavioral output. Therefore, future studies implementing larger sample sizes should determine whether hydrocortisone administration induces any effect on mood, potentially related to our findings of altered amygdala connectivity.

Furthermore, the pharmacological model for the effects of corticosteroids used in this study obviously does not capture all aspects of the complex stress response. Real-life cortisol release in response to stress is accompanied by the release of many other neuromodulators, such as NE, CRH, dopamine, and serotonin (Joels and Baram 2009), with which corticosteroids could potentially interact. Because we did not induce stress, the generalization from our results to stressful situations remains speculative. Nevertheless, mere administration of hydrocortisone reveals a cleaner mechanistic account for the corticosteroid effect, which was the aim of this study.

Moreover, we did not check for all environmental factors known to modulate amygdala function and HPA axis dynamics. Although participants with any history of or current psychiatric illness, any past or current use of antidepressants or anxiolytics, or participants currently in a stressful period or undergoing a major life event were excluded from participation, we did not check for stress during early life (e.g., childhood trauma). Early life adversity is known to constitute a major risk factor for psychiatric disorders and has been associated with structural and functional brain alterations (Kessler et al. 1997; Cohen et al. 2006; Frodl et al. 2010), as well as alterations in HPA axis functioning (Bremner 2003; Gillespie et al. 2009). Moreover, also recent trauma can trigger the onset of psychiatric disorders (e.g., post-traumatic stress disorder) and cause long lasting changes in amygdala functioning (van Wingen et al. 2011b). We obtained this data from the majority of subjects (n = 23; 12 × placebo, 11 × CORT) retrospectively, using an adapted version of the List of Threatening Life Events developed by Brugha et al. (1985). This inventory encompasses life events, which are likely to occur relatively frequently and score relatively high on long-term threat. Groups did not differ in the occurrence of overall nor severe life events as assessed by this questionnaire, neither during childhood nor later during life (all P's > 0.05). Therefore, the obtained results cannot be readily explained by differences in early life stress.

Lastly, one should note that although findings on the hypothalamus and LC were corrected for all comparisons done over the specific ROI, that is, FWE rate corrected, these findings would not remain significant after correction for testing of multiple ROIs. Future studies should aim to replicate these findings.

In conclusion, corticosteroids inhibit amygdala connectivity to several regions in the male brain. Amygdala's positive connectivity patterns to the stress-related structures including the LC, hypothalamus, and hippocampus were reduced, as well as its negative connectivity patterns to executive control regions including the middle frontal gyri and middle temporal gyrus. These effects of cortisol on amygdala connectivity in men appear to be opposite to the effects of rapidly acting stress hormones. Acute stress has been shown to strengthen amygdala's connectivity to other regions of the salience network, to boost emotional processing (van Marle et al. 2009), and impair executive control (Arnsten 2009; Qin et al. 2009), resulting in a state of hypervigilance. If corticosteroids would act in a similar way after stress exposure as observed in the current study, the hormones

might play a critical role in the restoration of homeostasis following stress exposure by desensitizing and normalizing brain processing (de Kloet et al. 2005). In line with this hypothesis, it was recently shown that corticosteroids suppress amygdala responsivity (Henckens et al. 2010) and boost executive control function (Henckens et al. 2011). Here, we provide additional evidence by showing that corticosteroids reduce amygdala's influence on brain processing by weakening its connectivity patterns. "Disconnecting" the amygdala in the aftermath of stress might contribute to a curtailed neuroendocrine stress response and minimized stress influence on executive control function, which suggests an essential role of corticosteroids in normalizing brain function in the aftermath of stress. Such normalization may be compromised in individuals liable to PTSD that are thought to have only brief exposure to cortisol after stress due to a stronger negative feedback mechanism (Yehuda et al. 1993). Moreover, our results suggest that the low ambient cortisol levels as observed in PTSD (Rohleder et al. 2004) might contribute to the increased amygdala connectivity detected in these patients (Gilboa et al. 2004; Osuch et al. 2008; Lanius et al. 2010). Conversely, continuously elevated cortisol levels such as observed in major depression (Parker et al. 2003) might result in a more chronic state of amygdala decoupling, as was indeed described for depressed individuals (Moses-Kolko et al. 2010; Veer et al. 2011). In sum, these observations underline the critical role of corticosteroids in the regulation of amygdala's influence on the brain.

Supplementary Material

Supplementary material can be found at http://www.cercor.oxfordjournals.org/.

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Notes

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