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Blocking the Mineralocorticoid Receptor in Humans Prevents the Stress-Induced Enhancement of Centromedial Amygdala Connectivity with the Dorsal Striatum

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Two research lines argue for rapid stress-induced reallocations of neural network activity involving the amygdala. One focuses on the role of norepinephrine (NE) in mediating a shift towards the salience network and improving vigilance processing, whereas the other focuses on the role of cortisol in enhancing automatic, habitual responses. It has been suggested that the mineralocorticoid receptor (MR) is critical in shifting towards habitual responses, which are supported by the dorsal striatum. However, until now it remained unclear whether these two reallocations of neural recourses might be part of the same phenomenon and develop immediately after stress onset. We combined methods used in both approaches and hypothesized specifically that stress would lead to rapidly enhanced involvement of the striatum as assessed by amygala-striatal connectivity. Furthermore, we tested the hypothesis that this shift depends on cortisol interacting with the MR, by using a randomized, placebo-controlled, full-factorial, between-subjects design with the factors stress and MR-blockade (spironolactone). We investigated 101 young, healthy men using functional magnetic resonance imaging after stress induction, which led to increased negative mood, heart rate, and cortisol levels. We confirmed our hypothesis by revealing a stress-by-MR-blockade interaction on the functional connectivity between the centromedial amygdala (CMA) and the dorsal striatum. Stress rapidly enhanced CMA-striatal connectivity and this effect was correlated with the stress-induced cortisol response, but required MR availability. This finding might suggest that the stress-induced shift described by distinct research lines might capture different aspects of the same phenomenon, ie, a reallocation of neural resources coordinated by both NE and cortisol.
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INTRODUCTION

Encountering acute threat appears to shift neural network balance (Hermans *et al*, 2011). This reallocation of neural resources alters cognition and behavior, preferring sensory processing and fast responding over elaborate, flexible behavior. The amygdala is crucial in detecting threat and activates the locus coeruleus (LC), the major central source of norepinephrine (NE; Aston-Jones, 1985; Sara and Bouret, 2012; Valentino and Van Bockstaele, 2008). NE leads to an upregulation of the salience network and downregulation of the executive control network, improving vigilance at the cost of elaborative cognition (Hermans *et al*, 2014). Activation of this LC–NE system is supposed to enhance chances for survival by improving threat detection and reducing elaboration to enable fast responding.

Along intriguingly similar lines, researchers from the memory field have argued for a stress-induced shift in neural processing. This shift, again orchestrated by the amygdala, favors automatic, habitual responding mediated by the dorsal striatum at the expense of controlled, flexible responding mediated by hippocampus and prefrontal cortex (PFC; Kim *et al*, 2001; Packard and Teather, 1998; Schwabe and Wolf, 2013). This process is likewise supposed to promote survival by relying on well-learned reflexive behavior in the face of acute threat. The mechanistic focus of this research line is on the hypothalamus–pituitary–adrenal (HPA) axis. There is initial evidence that the mineralocorticoid receptor (MR), one receptor for cortisol, is crucial for this shift in humans (Schwabe *et al*, 2013b) and other species (Schwabe *et al*, 2010a).

Despite the striking similarities of these two reallocations of neural resources involving the amygdala, the research lines remained largely separate. According to their respective focus on either the fast-acting NE or the somewhat slow-acting cortisol, researchers also used different

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stress-induction procedures to either increase arousal and NE levels (eg, violent movies; Hermans *et al*, 2011) or cortisol levels (eg, social evaluation or cold pressure tasks; Schwabe *et al*, 2007). Moreover, these lines of research investigated stress-effects in different time domains. Research on NE focused on the immediate effects of stress to ensure high NE levels, but given the early time frame, this leads to relatively low cortisol levels. Research on cortisol effects, in contrast, usually took place at least 20 min after stress induction to ensure high cortisol levels. Finally, different tasks were used targeting either vigilance processing or memory formation.

Until now, it remained unclear whether these two stress-induced phenomena might be related reallocations of neural resources in the face of threat. With this background in mind, we aimed at better understanding fast effects of cortisol and designed an experiment crossing the borders between the two research lines described. We investigated whether the socially evaluated cold pressure test (Schwabe *et al*, 2008) leads to a rapid reallocation of neural resources to areas supporting habitual responses, the dorsal striatum, in a task probing vigilance processing. Furthermore, if such a shift would be present, we expected it to depend on MR availability. We focused on rapid glucocorticoid effects given recent findings, suggesting that the fast non-genomic effects, mediated primarily by the MR, lead to increased amygdala excitability and facilitation of adaptive behavior (Karst *et al*, 2010). Finally, we hypothesized that the amygdala would drive this reallocation of resources. However, the amygdala is not a homogenous structure, but consists of subnuclei with different functions and connectivity patterns (McDonald, 1998). Whereas the basolateral amygdala complex (BLA) is assumed to be the input structure and stores cue-threat associations (Johansen *et al*, 2011), the centromedial amygdala (CMA) constitutes the output structure, which is connected, among others, to the striatum, mediating habitual responses (Fudge *et al*, 2002; Han *et al*, 1997). This model was supported in humans using functional connectivity analyses on resting state data, demonstrating that the BLA is functionally connected to cortical regions, whereas the CMA is connected to more subcortical regions, including the dorsal striatum (Roy *et al*, 2009). We thus hypothesized that the stress-induced reallocation of neural resources to the striatum would be driven primarily by the CMA rather than the BLA. Therefore, we expected stronger connectivity between CMA and striatum during stress, depending on MR availability. To test this, we used a full-factorial design investigating the effects of acute stress and MR-blockade (spironolactone) on vigilance processing, task-related brain activity, and amygdala sub-region connectivity.

MATERIALS AND METHODS

The study was approved by the local ethical committee (NL37819.091.11), registered in the Dutch Trial Registry (3595), and the European Clinical Trials Database (2011-003493-85).

Participants

Healthy, right-handed male volunteers ($N = 101$) with a body mass index within normal range ($18.5 \leqslant \text{BMI} \leqslant 30$) were screened for the exclusion criteria described in the Supplementary Material. All participants gave written informed consent and were financially compensated. Two participants had to be excluded because of panic attacks during scanning and one participant did not comply with study instructions (participation in another drug study). This resulted in a final number of 98 participants (mean age 21.9 years (SD = 2.9), Table 1).

Study Design

We used a placebo-controlled, full-factorial between-subjects design with the factors stress (stress *vs* control) and MR-blockade (400 mg spironolactone *vs* placebo). Participants were randomly assigned to one of the four experimental groups. The factor MR-blockade was manipulated in a double-blind fashion. However, the subjects were informed about their assignment in terms of the stress factor before scanning (see below).

General Procedure

Adaptation phase. All testing took place in the afternoon. After baseline cortisol, subjective mood, and vital signs (blood pressure, heart rate) measurements, participants were administered either placebo or 400 mg spironolactone (tablets) orally in four capsules (Teva Pharmachemie, Haarlem, The Netherlands; half-life in plasma $\sim$1.5 h). This dosage is in accordance with other studies, investigating the MR in humans (Cornelisse *et al*, 2011; Rimmele *et al*, 2013). A waiting period of 80 min followed ensuring adaptation to the laboratory environment and absorption of the drug. Participants rested, cortisol and vital signs were measured every 30 min.

Table 1 General Characteristics of the Study Sample

Measure	Control/MR-available	Stress/MR-available	Control/MR-blocked	Stress/MR-blocked
N	24	24	26	24
Age	21.6 (2.2)	21.9 (4.0)	22.5 (2.8)	21.5 (2.4)
Body mass index	23.4 (2.4)	22.5 (1.9)	22.7 (2.4)	22.3 (2.5)
Time in water (s)	180 (1)	135 (59)[a]	180 (2)	155 (51)[b]

Values represent mean (SD).
[a]$p < 0.001$ compared with control in the same drug group.
[b]$p < 0.05$ compared with control in the same drug group.

Experimental phase. Participants were taken to the magnetic resonance imaging (MRI) room, and those assigned to the stress condition were informed that they would do the ice-water task. They were exposed to an MRI scanner compatible version of the socially evaluated cold-pressure task (SECPT; Schwabe *et al*, 2008) or a non-stressful control procedure. Immediately afterwards, a saliva sample and mood assessment were obtained before participants were instructed about and performed an emotional face-matching task assessing vigilance processing in the MRI scanner. The delay between stress onset and task onset was on average 9 min, 56 s (± 110 s; SD). Two other tasks followed, which will be reported elsewhere. Participants were debriefed about the stress induction procedure and could leave after a final assessment of well-being and vital signs.

Stress Induction

The SECPT is an established method to induce stress by asking participants to immerse one hand into ice water while being socially evaluated (Schwabe *et al*, 2008). We adapted it to an MRI compatible version to avoid changing the context between stress induction and task, and to minimize the delay in-between. Participants were placed in a supine position on the scanner bench, immersed the right foot into ice water (0–2 °C) up to and including the ankle, and held it there as long as possible. During foot immersion, participants looked into a camera while being closely observed by two experimenters in white laboratory coats (at least one female) acting neutral and non-supportive. In the control group, warm water was used (35–37 °C), there was no camera, and the experimenter was friendly and casually dressed. If participants did not remove their foot earlier, the experimenter stopped the task after 3 min. The stress group underwent a difficult mental arithmetic test (counting aloud backwards from 2059 in steps of 17) ~40 min after the initial stress induction to maintain heightened stress levels for subsequent tasks. Participants in the control condition did a simple control task (counting forwards in steps of 10).

Stress Measurements

Negative mood, cortisol levels, and vital signs were measured repeatedly as described in the Supplementary Materials and Methods.

Emotional Face-Matching Task

To assess vigilance processing, we applied a commonly used emotional face-matching task (Hariri *et al*, 2002; van Wingen *et al*, 2007), which contrasts an emotional condition with a visuomotor control condition, alternating in a blocked fashion. Each block lasted 30 s and consisted of six 5-s trials. Each trial comprised three simultaneously presented stimuli, either emotional faces (http://www.macbrain.org) or ellipses made of scrambled faces. A cue stimulus was presented above a target and a distractor stimulus. The participant had to indicate which of the latter two-matched the cue by pressing one of two buttons. In the emotion condition, participants had to identify the target face, which displayed the same emotional expression as the cue face (angry or fearful). In the visuomotor condition, the target was the ellipse with the same geometrical orientation as the cue (horizontal or vertical). Two emotion blocks were interleaved with three visuomotor blocks.

Magnetic Resonance Imaging

MRI measurements were acquired using a 1.5 T Avanto Scanner (Siemens, Germany) equipped with a 32-channel head coil. Blood-oxygenation-level-dependent (BOLD) T2*-weighted multi-echo GRAPPA images (Poser *et al*, 2006) were obtained with the following parameters: repetition time (TR) = 2.14 s, echo times (TEs) = 9/21/33/44/56 ms, 34 transversal slices, ascending acquisition, distance factor 17%, effective voxel size = 3.3 × 3.3 × 3.0 mm, field of view (FOV) = 212 mm. We used this multiecho sequence for its improved BOLD sensitivity and lower susceptibility for artifacts, especially for ventral regions (Poser *et al*, 2006). In addition, we acquired high resolution T1-weighted anatomical image (TR = 2.73 s, TE = 2.95 ms, 176 sagittal slices, FOV = 256 mm, voxel size = 1.0 × 1.0 × 1.0 mm).

Behavioral and Physiological Analysis

All behavioral and physiological analyses were performed in SPSS 19 (Armonk, IBM Corp). The alpha level was set to 0.05 for all analyses (two-tailed) and Greenhouse–Geisser correction was applied when necessary. Missing cortisol data (seven non-adjacent measurements in six participants) were interpolated by averaging across the two neighboring measurements. For negative mood, missing items (six items in five participants) were replaced by the individual mean score. In line with previous work (Muehlhan *et al*, 2011), participants naive to the MRI scanner environment showed a stress response to the scanning procedure itself as witnessed by higher heart rate and cortisol levels than non-naive participants (both $p<0.05$). Given that our experimental groups had different percentages of naive participants (58% stress/MR-blocked, 50% stress/MR-available, 62% control/MR-blocked, 25% control/MR-available), we included scanner naivety as covariate of no interest in all of our analyses, including fMRI analyses.

Negative mood, cortisol, heart rate, blood pressure. To test for successful adaptation to the laboratory environment, the scores of these variables during adaptation were entered separately into repeated measures ANOVAs (rmANOVAs) with the within-subjects factor time and the between-subjects factors stress (yes, no), and MR-blockade (available, blocked). For the experiment phase, the scores were baseline corrected to the last measurement of the adaptation phase (− 25 min) and entered into rmANOVAs with the within-subjects factor time and the between-subjects factors as described above.

Performance in the emotional face-matching task. Reaction times and percentage hits were calculated per task condition (emotion, control) and entered separately in rmANOVAs with the within-subjects factor condition and the between-subjects factors stress and MR-blockade.

fMRI Analysis

Two participants (stress/MR-blocked, control/MR-blocked) were excluded for fMRI analyses due to technical failure and excessive head motion (>3.3 mm). Data were analyzed in SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK) using general linear modeling. Full preprocessing parameters can be found in the Supplementary Material. To assess the effects of stress and MR-blockade on neural responsivity, the task conditions (emotion, visuomotor) were modeled as 30 s box-car regressors and button presses were modeled as spikes, all convolved with the canonical HRF. In addition, six realignment parameters were included. Contrast images subtracting the visuomotor condition from the emotion condition were analyzed in a full-factorial design to test for group differences. For the explorative whole-brain analyses, we used a threshold of $p_{FWE}<0.05$ (cluster-level). To use small-volume correction (SVC) for our *a priori* regions of interest (ROIs), ie, amygdala sub-regions, we used an initial threshold of $p<0.005$, uncorrected, followed by FWE-correction ($p<0.05$) for multiple comparisons within the ROIs as implemented in SPM. Although our hypothesis primarily contrasted CMA and BLA, for completeness, we also included the superficial amygdala (SFA) as ROI. Masks of the bilateral sub-regions were taken from the Anatomy Toolbox for SPM (version 18, Institute of Neuroscience and Medicine, Jülich, Germany), which is based on probabilistic cytoarchitectonic maps derived from 10 post-mortem brains. The masks were thresholded at 50% (Amunts *et al*, 2005) to include only voxels with at least 50% probability to belong to each sub-region.

To investigate connectivity of amygdala sub-regions during the emotional face-matching task, we extracted the first eigenvariate of the time-courses of the bilateral sub-regions using the 'Physio/Psycho-Physiologic Interaction' tool as implemented in SPM8. Subsequently, we added each time-course separately as covariate of interest in addition to the first-level regressors. Correlating this time series to activity in the rest of the brain provides information on regions that show similar activation patterns and are therefore supposedly functionally connected. Global signal fluctuations were accounted for by extracting signal from individually defined white matter (WM) and cerebral spinal fluid (CSF) masks, and adding these two regressors to the model. A full-factorial design was used to investigate group differences in connectivity of the amygdala sub-regions. On the basis of our hypotheses, our ROI was the dorsal striatum (caudate nucleus, putamen). As these regions are not included in the Anatomy Toolbox, masks were defined using the automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer *et al*, 2002) as implemented in the Wake Forest University PickAtlas (version 2.4). Again, for SVC, we used an initial threshold of $p<0.005$, uncorrected, followed by FWE-correction ($p<0.05$). To control for the multiple testing problem inherent in testing three seed regions, only results with $p_{SVCcorr}<0.017$ will be considered significant.

RESULTS

The experimental groups did not differ significantly in age or BMI (Table 1). Participants in the stress condition kept their foot in the ice water for over 2 min on average, which was nevertheless shorter than participants in the control procedure ($F_{(1,93)}=20.123$, df = 1, $p<0.001$). Importantly, there was no influence of MR-blockade on the time in water.

Stress Measures in the Adaptation Phase Indicate Adaptation to the Laboratory Environment

Decreases throughout the adaptation phase in negative mood ratings, cortisol levels, heart rate, and blood pressure indicate successful adaptation to the laboratory environment (all main effects of time $p<0.001$). Furthermore, within drug groups there was no difference between stress and control groups in any measure prior to stress induction (all $p>0.1$). In line with a role of the MR in negative feedback of the HPA axis, the MR-blocked groups had higher cortisol levels 25 min before stress than the MR-available groups ($t_{96}=3.126$, $p=0.002$).

Stress Measures in the Experiment Phase Indicate Successful Stress Induction

Stress-related increases in negative mood, cortisol, and heart rate evidenced successful stress induction in both drug groups (Figure 1, negative mood: main effect stress ($F_{(1,91)}=10.907$, $p=0.001$), time-by-stress interaction ($F_{(2.4,218.4)}=9.812$, $p<0.001$); cortisol: main effect stress ($F_{(1,92)}=13.004$, $p=0.001$), time-by-stress interaction ($F_{(2.5,229.5)}=8.927$, $p<0.001$); heart rate: main effect stress ($F_{(1,88)}=4.665$, $p=0.033$); for details see Supplementary Results). MR-blockade led to heightened cortisol levels (main effect MR-blockade ($F_{(1,92)}=15.013$, $p<0.001$), time-by-MR-blockade interaction ($F_{(2.5,229.5)}=6.217$, $p=0.001$)) without affecting heart rate or blood pressure.

Behavioral Measures are not Affected by Stress or MR-Blockade

As expected, participants displayed almost perfect performance in the emotional face-matching task with no significant difference between conditions (mean hit rate emotion: 91.9% (SD: 15.85), visuomotor: 92.1% (SD: 15.6)). In terms of reaction times, participants were faster in matching orientations of ellipses than emotional expressions of faces (mean reaction time emotion 1.89 s (0.47), visuomotor 1.08 s (0.33), $F_{(1,93)}=165.210$, $p<0.001$). However, neither stress nor MR-blockade significantly affected hit rate or reaction time.

Task-Related Brain Activity is not Influenced by Stress or MR-Blockade

Task-related brain activation. When comparing emotion *vs* visuomotor blocks, we found a bilateral cluster of activation including the inferior frontal gyrus and the ventral visual stream reaching into the fusiform gyrus, hippocampus, putamen, caudate, and amygdala (all $p_{FWE}<0.05$, Figure 2, Supplementary Table S1). Importantly, this activation was highly significant in all three amygdala sub-regions (bilateral, all $p_{FWE}<0.05$). The opposite contrast (control > emotion) revealed activations in regions considered to be part of the default mode network (Fox and Raichle, 2007):

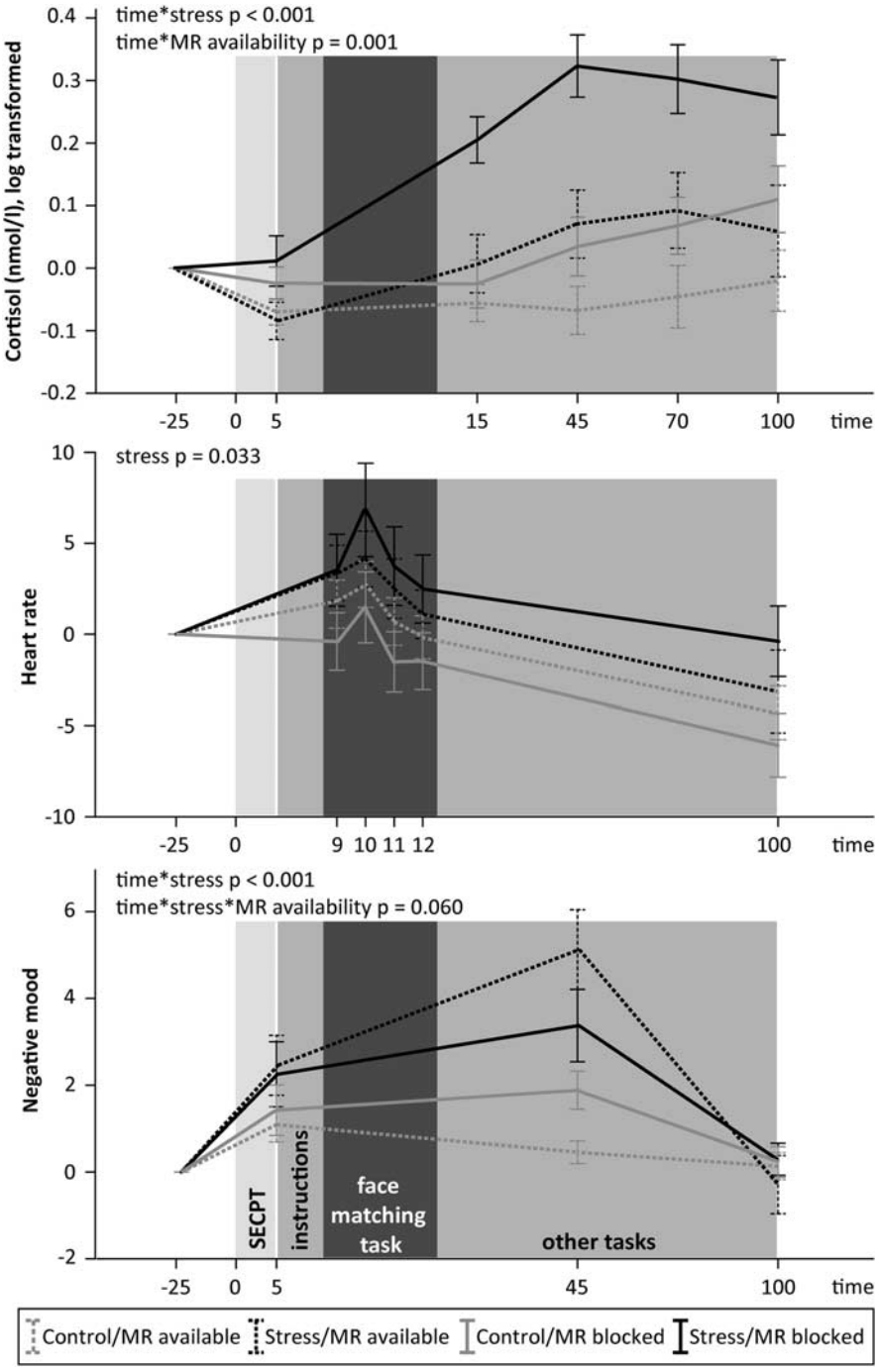

Figure 1 Stress measurements over the course of the experiment. Participants arrived and were randomly assigned to one of four groups: control/MR-available, stress/MR-available, control/MR-blocked, stress/MR-blocked. After drug or placebo ingestion and habituation to the laboratory environment, participants entered the MRI room and underwent either a stress induction or a control procedure. This was immediately followed by the emotional face-matching task during, which fMRI data were acquired for subsequent connectivity analyses. The figure shows cortisol levels (top), heart rate (middle), and negative mood (bottom) for all experimental groups over the course of the experiment. Time is indicated in minutes after stress induction. All measurements were baseline corrected to the last measurement during habituation (− 25 min). Light gray-shaded areas indicate stress induction (or non-stressful control procedure), intermediate gray-shaded areas indicate the time of fMRI scanning, dark gray-shaded area indicates the emotional face-matching task. *SECPT,* socially evaluated cold pressure task. Mean values are depicted, error bars represent 1 SEM.

medial PFC, posterior cingulate, and parietal cortex ($p_{FWE} < 0.05$). Neither the whole brain nor the ROI analyses (SVC) of activity led to reliable main effects of stress or stress-by-MR-blockade interactions. However, when extracting the parameter estimates for the contrast emotion over control from critical ROIs of the salience network (bilateral insula, dorsal ACC, defined using the AAL atlas), we found that stress led to stronger activity in the bilateral insula ($F_{(1,91)} = 4.05$, $p = 0.047$). This effect was not influenced by MR-availability.

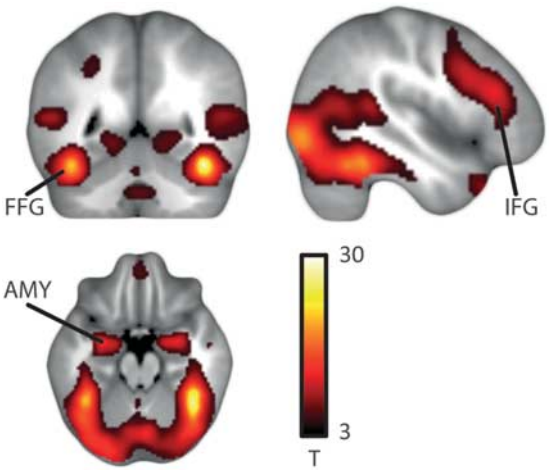

Figure 2 Brain areas activated by processing emotional faces *vs* ellipses plotted on the average anatomical scan of all participants. Bilateral activation was found in visual areas, the fusiform gyrus (FFG), amygdala (AMY), and inferior frontal gyrus (IFG). All $p<0.05$, FWE corrected (cluster level).

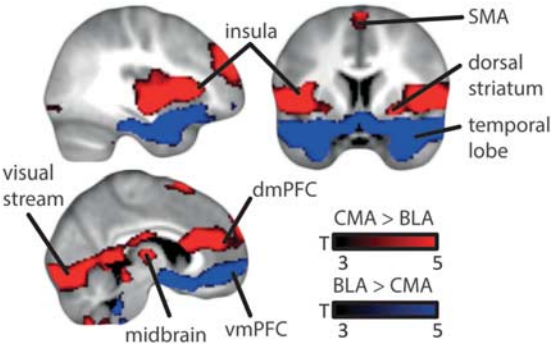

Figure 3 Brain areas showing differential connectivity to either the centro-medial (CMA) or the basolateral amygdala (BLA) during an emotional face-matching task, plotted on the average anatomical scan of all participants. SMA, supplementary motor area; dmPFC, dorso-medial prefrontal cortex; vmPFC, ventro-medial prefrontal cortex. All $p<0.05$, FWE corrected (cluster level).

Stress Enhances Connectivity between CMA and Dorsal Striatum Depending on the Availability of MRs

Brain connectivity. First, we investigated differential connectivity of both CMA and BLA (see Supplementary Figure S1 for connectivity of the sub-regions separately). As illustrated by Figure 3, regions that showed stronger connectivity to the CMA than the BLA included frontal regions, the dorsal striatum, bilateral insula, dorso-medial PFC, the ventral visual stream, the midbrain, and the supplementary motor area (all $p_{FWE}<0.05$). Regions showing stronger connectivity with the BLA included the ventro-medial and ventro-lateral PFC, and large parts of the temporal lobe. This analysis confirms differential connectivity of amygdala sub-regions, and connectivity between CMA and dorsal striatum. Interestingly, we found that the CMA was also coupled to parts of the salience network.

Next, we investigated main effects of stress on connectivity between the amygdala sub-regions and the rest of the brain, but no significant effects emerged. However, we found a stress-by-MR-blockade interaction in the connectivity between the CMA and the dorsal striatum, more specifically the caudate nucleus ($k=21$, peak T-value 4.58, $p_{SVCcorr}=0.005$, Figure 4). Post hoc tests revealed that stress in the MR-available group was associated with stronger connectivity between the CMA and the caudate, extending to the putamen, ($k=39$, peak T-value 5.08, $p_{SVCcorr}=0.001$). This stress-induced increase in connectivity was absent in the MR-blocked groups. Importantly, this cluster showed stronger connectivity to the CMA as compared with the BLA ($p_{SVC}<0.05$, corrected for the anatomical caudate mask). No other regions showed significant stress-by-MR-blockade interactions (whole-brain or SVC in our ROIs) in connectivity to the CMA. Also, no significant stress-by-MR-blockade interactions on functional connectivity from the other two seed regions, BLA and SFA, were found.

Finally, we investigated whether inter-individual differences in stress-induced increases in cortisol levels were associated with stress-related changes in connectivity. To this end, we calculated the area under the curve with respect to the increase in cortisol during the experimental phase (AUCi; Pruessner *et al*, 2003) and correlated this to the parameter estimates extracted from the cluster shown in Figure 4 (threshold $p<0.005$, uncorrected). We found a positive correlation in the stress/MR-available group ($r=0.448$, $p=0.028$, Figure 5), indicating that participants with higher cortisol reactivity showed stronger connectivity between the CMA and the dorsal striatum when the MR was available. We did not find this association in any other group (all $p>0.4$), and the correlation in the stress/MR-available group was significantly stronger than in the control/MR-available group (Fisher's $z=-2.05$, $p=0.040$), but not in comparison with the other groups. Interestingly, this association in the stress/MR-available was already present at the first measurement after stress induction ($\rho=0.417$, $p=0.042$), ie, not driven purely by cortisol increases after the task and supporting a role for the MR in determining the threshold for HPA axis activation (Pace and Spencer, 2005).

DISCUSSION

Our results reveal a stress-induced shift in amygdala connectivity with regions supporting automatic, habitual behavior. Stress enhanced connectivity between the CMA and the dorsal striatum (tested independent of task condition) and the strength of this effect was correlated with the strength of the stress-induced cortisol response across subjects when the MR was available. Moreover, we demonstrate a rapid onset of this shift within few minutes after stress induction, when NE should still be active and cortisol levels are rising. This finding might suggest that the two shifts in brain networks during stress attributed to NE (Hermans *et al*, 2014) and cortisol (Schwabe *et al*, 2013b), might be related, coordinating reallocations of neural resources involving amygdala processing.

Stress-induced changes are brought about by different waves of neuromodulators (Joels and Baram, 2009).

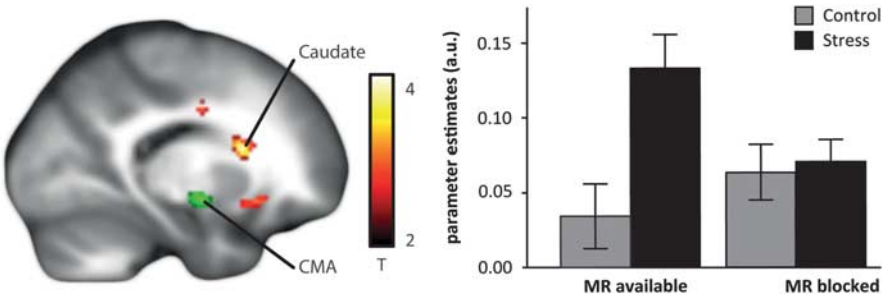

Figure 4 Stress-by-MR-blockade interaction on the connectivity between the centromedial amygdala and the caudate nucleus. Left: analysis of the stress-by-MR-blockade interaction revealed increased connectivity between the centromedial amygdala seed (CMA, indicated in green) and the caudate nucleus during stress in the MR-available group (displayed using tangential slice). However, this effect was abolished in the MR-blocked groups. Right: for all groups, we extracted the data from the cluster showing significantly stronger connectivity with the CMA in the stress/MR-available group than the control/MR-available group. Error bars represent SEM. For visualization, the statistical parametric map is plotted on the average anatomical scan of all participants and thresholded at $p < 0.005$, uncorrected.

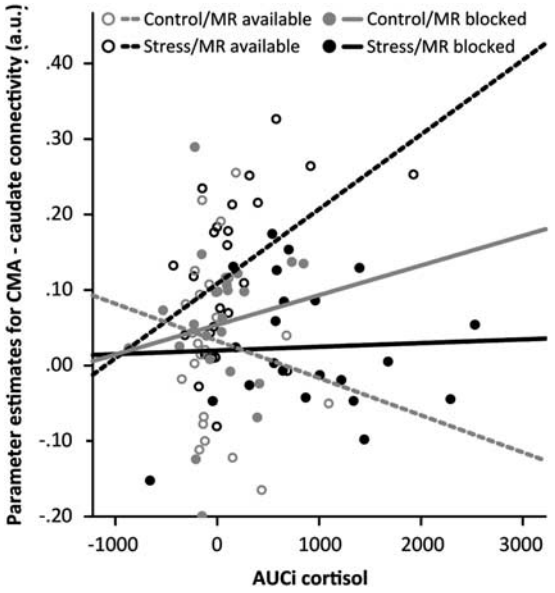

Figure 5 Correlation between cortisol reactivity (area under the curve with respect to the increase, AUCi) and functional connectivity between the centromedial amygdala (CMA) and the striatal cluster showing a stress-by-MR-availability interaction in Figure 4.

Activation of the NE-LC system together with the peripheral sympatho-adrenomedullary system leads to the rapid release of catecholamines, exciting the salience network in humans (Hermans *et al*, 2011) and enhancing vigilance. Activation of the HPA axis, conversely, results in a somewhat slower action of corticosteroids, which bind to two receptors in the brain, the glucocorticoid receptor (GR) and the MR. Both receptors have two modes of action, a rapid non-genomic mode and a slow genomic mode, mediated by receptors residing at the membrane or in the cytoplasm, respectively, as demonstrated mostly in rodents or *in vitro* (Joëls *et al*, 2012). Whereas the slow genomic effects promote reinstalling homeostasis in humans and rodents (presumably GR-mediated, Henckens *et al*, 2011; Herman *et al*, 2012), rapid non-genomic (MR-mediated) effects seem to enhance catecholaminergic effects within minutes, for example, by enhancing excitability of the amygdala *in vitro* (Karst *et al*, 2010). Studies investigating emotional memories in rodents have already demonstrated that both stress-systems have synergistic effects in the amygdala (eg, Roozendaal *et al*, 2006). Importantly, we did not measure NE levels directly. However, considering the stress-induced increase in heart rate during the task, an activation of the LC–NE system is strongly suggested. Thus, our findings support the idea of interactive effects of NE and cortisol, potentially affecting resource allocation to different brain networks during an acutely stressful experience. More specifically, our data support that rapid, non-genomic effects of cortisol mediated by the MR are involved in changing functional connectivity.

A similar stress-induced shift in brain connectivity between amygdala and the right caudate was observed earlier in a probabilistic classification-learning task (Schwabe *et al*, 2013b). However, while the previous study showed this effect 40 min after stress induction, here we demonstrate its appearance even within a few minutes after stress induction. Considering that such a shift should help individuals to handle stressful situations and spare resources by relying on automatic, well-learned behavior, it seems plausible that this would happen immediately. Furthermore, we could show the stress-induced shift depending on MR-blockade in a task probing emotional vigilance. This leads us to carefully conclude that the stress-induced reallocation of neural resources might be more general in nature, activating the salience network, increasing connectivity between the salience network and the dorsal striatum, and at the same time it might inhibit prefrontal processing. Finally, we could refine the earlier result (Schwabe *et al*, 2013b), by showing that a particular sub-region of the amygdala, the CMA, orchestrates the stress-induced shift in brain connectivity.

Only few studies have investigated differential connectivity of amygdala sub-regions in humans. Roy *et al*, 2009 revealed that the CMA preferentially connects to subcortical brain regions, whereas the BLA preferentially connects to cortical regions in healthy adults. A study in social anxiety disorder patients showed increased gray matter volume in the CMA and less distinct connectivity patterns of the sub-regions (Etkin *et al*, 2009) compared with healthy controls.

In another study, post-traumatic stress disorder patients showed enhanced connectivity between BLA and regions of the salience network (Brown *et al*, 2014). These studies highlight a potential clinical relevance of these distinct regional amygdala circuits. It is important to note that our definition of the amygdala sub-regions was based on probability maps and due to inherent methodological limitations, we cannot be certain that we have optimally mapped these structures in each individual participants. However, the distinct connectivity patterns we found, indicate our ability to separate signals coming from different sub-regions.

Our study supports the role of the MR in stress-related changes in cognition and behavior. Specifically, the MR appears to mediate a shift to more striatal control over behavior, favoring well-learned habit-like responses and stimulus-response learning over controlled, flexible behavior guided by long-term goals (Schwabe *et al*, 2010b). This might have implications for our understanding of stress-related mental disorders. For example, the shift might be relevant in preventing relapse in addiction, or the re-appearance of maladaptive behavior in anxiety disorders. A stress-induced shift towards habitual behavior and short-term outcomes, together with impaired control mechanisms, might facilitate these symptoms (Arnsten, 2009). If this model would hold, one may speculate that the MR might serve as a drug target affecting, for example, amygdala-striatum interactions.

The findings of this study should be viewed within its strengths and limitations. Strong points are the large sample size, its full-factorial design, and a pharmacological manipulation, enabling us to investigate the effects of stress depending on current MR-availability. However, one should keep in mind that our measure of functional connectivity is correlational in nature and provides no information on causality. Accordingly, it cannot be concluded that the CMA 'drives' the stronger connectivity to the caudate. Nevertheless, animal studies point to a causal role of the amygdala in the stress-induced shift towards striatal control over behavior (Packard and Wingard, 2004). Interestingly, while the CMA is the critical output structure of the amygdala when it comes to fear memory and its modulatory effects of the amygdala on behavior and autonomic responses (LeDoux *et al*, 1988), in the animal literature, the BLA was suggested to be critical in modulating different memory systems in the spatial domain (Packard and Teather, 1998; Packard and Wingard, 2004). Although our results point to a critical role of the CMA, the BLA might also be involved in shifting brain networks under stress.

Interestingly, we did not find main effects of stress on amygdala reactivity, which is in contrast to studies reporting enhanced amygdala activity under stress (van Marle *et al*, 2009). However, this latter study tested female participants only and there is initial evidence for sex-specific effects of stress-related neuromodulators on face processing in the amygdala (Schwabe *et al*, 2013a). Furthermore, the study by van Marle used a different stress induction procedure (violent movies), which has strong effects on arousal and the NE system, but is less effective in activating the HPA axis. One might also speculate why we did not find a stress-induced decrease in connectivity between amygdala and the hippocampus as shown previously (Schwabe *et al*, 2013b), which might be related to differences in timing and task. Whereas the latter study had a delay of 40 min between stress induction and testing, we tested immediately after stress-induction, and without changing the context. Possibly, the decrease of amygdala-hippocampus connectivity needs more time to develop. Furthermore, the emotional face-matching task does not contain a direct explicit memory component. Although we did find that stress increased task-related activity in the insula, we did not observe an increase in salience network activity in general, which is in contrast to earlier reports (eg, Hermans *et al*, 2011). This might be explained by differences in the design (movie watching *vs* specific task) and differences in delay between stress induction and data acquisition (during the first 2 min of stress induction as opposed to during a task starting 10 min after stress induction onset). Future studies directly targeting amygdala-LC interactions would be important to better understand the mechanisms underlying stress-induced changes in neural networks.

The stress-induced increase of connectivity between CMA and caudate and its blockage by spironolactone might be partially due to other, indirect endocrine effects of the drug. The administration of spironolactone led to a rise in cortisol levels, both in the control and in the stress group. This is in line with previous studies (Cornelisse *et al*, 2011; Otte *et al*, 2007; Schwabe *et al*, 2013b) and can be interpreted as verification of drug action. The heightened cortisol levels most likely resulted from blocked negative HPA-axis feedback, where the MR is a critical regulator (de Kloet *et al*, 2005). Importantly, however, MR-blockade did not affect the cortisol response to stress (no time-by-stress-by-MR-blockade interaction, $p > 0.3$). This was reported before (Cornelisse *et al*, 2011; Schwabe *et al*, 2013b) and is not due to our baseline correction, as the interaction was also absent using uncorrected, raw values ($p > 0.2$). Spironolactone might also change levels of adrenocorticotropic hormone (ACTH) and corticotropin-releasing factor (CRF). Interestingly, no adjustment of ACTH levels was found after spironolactone application in recent human studies (Otte *et al*, 2007; Rimmele *et al*, 2013). Nevertheless, possible differences in CRF release could have potentially affected the stress response (Sajdyk *et al*, 1999). Furthermore, after MR blockade, more cortisol will be available for binding to GRs, and GR-activation or the ratio between MR and GR activation might thus have contributed to our effects. Therefore, our results may be interpreted in terms of a decrease in the relative balance between MR and GR activation rather than the consequence of MR-blockade only. Finally, spironolactone primarily binds to MRs, but can also affect other receptors, for example progesterone receptors (Schane and Potts, 1978). Animal experiments with more specific drugs might help here in further specifying the role of the MR in the stress-induced shift.

Regardless of these limitations, our results are in line with a model in which stress induces a rapid reallocation of neural resources towards vigilance processing, which enhances CMA connectivity with the striatum. Furthermore, we could show that this shift depends critically on the availability of MRs in the early stages of stress. Most importantly, we suggest that the two stress-induced shifts in brain networks involving amygdala processing might be part of the same underlying process, ie, a coordinated

reallocation of neural resources preferring the salience network and the striatum at the expense of the executive control network. Future studies are needed to obtain a more comprehensive picture of the different neuromodulators and neural networks involved in the stress-induced shift, their correlates in cognition and behavior, and the precise neural mechanisms associated.

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