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A Stress-Induced Shift From Trace to Delay Conditioning Depends on the Mineralocorticoid Receptor

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ABSTRACT

BACKGROUND: Fear learning in stressful situations is highly adaptive for survival by steering behavior in subsequent situations, but fear learning can become disproportionate in vulnerable individuals. Despite the potential clinical significance, the mechanism by which stress modulates fear learning is poorly understood. Memory theories state that stress can cause a shift away from more controlled processing depending on the hippocampus toward more reflexive processing supported by the amygdala and striatum. This shift may be mediated by activation of the mineralocorticoid receptor (MR) for cortisol. We investigated how stress shifts processes underlying cognitively demanding learning versus less demanding fear learning using a combined trace and delay fear conditioning paradigm.

METHODS: In a pharmacological functional magnetic resonance imaging study, we tested 101 healthy men probing the effects of stress (socially evaluated cold pressor vs. control procedure) and MR-availability (400 mg spironolactone vs. placebo) in a randomized, placebo-controlled, full-factorial, between-subjects design.

RESULTS: Effective stress induction and successful conditioning were confirmed by subjective, physiologic, and somatic data. In line with a stress-induced shift, stress enhanced later recall of delay compared with trace conditioning in the MR-available groups as indexed by skin conductance responses. During learning, this was accompanied by a stress-induced reduction of learning-related hippocampal activity for trace conditioning. The stress-induced shift in fear and neural processing was absent in the MR-blocked groups.

CONCLUSIONS: Our results are in line with a stress-induced shift in fear learning, mediated by the MR, resulting in a dominance of cognitively less demanding amygdala-based learning, which might be particularly prominent in individuals with high MR sensitivity.

Keywords: Amygdala, Fear, Hippocampus, Memory systems, Mineralocorticoid receptor, Stress

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Fear learning in stressful situations is adaptive for survival by guiding subsequent behavior, but it is also a critical initiating factor for stress-related disorders (1). The neurobiology of fear learning has been studied extensively, implementing different fear conditioning paradigms. Most studies focused on delay conditioning, where a neutral stimulus co-occurs with an aversive unconditioned stimulus (US) and over time comes to elicit a fear response in itself (conditioned stimulus paired with the US, [CS+]). The neural basis of delay conditioning is well understood (2): the basolateral amygdala receives simultaneous sensory inputs from CS+ and US and stores this association, whereas the centromedial amygdala mediates autonomic and behavioral changes (2,3). In trace conditioning, a short interval is inserted between CS+ and US, changing the learning process and brain areas involved by preventing reflexive learning (4). Although less studied, trace conditioning is thought to be more cognitively demanding, to require higher level cognitive processes such as declarative memory (4,5), and to be perhaps relevant for fear learning in more complex real-life situations. The hippocampus seems to be necessary for trace conditioning, and the prefrontal cortex is involved in representing the temporal CS-US relationship (4,6,7).

In real-life situations, traumatic fear learning is often embedded in stressful life events. Despite the potential clinical significance, the interaction between stress and fear learning is not well understood. Stress in general appears to induce a reallocation of neural resources quickly causing a shift away from cognitively demanding to less demanding processing, for example, by impairing hippocampus-dependent but enhancing amygdala-dependent processing (8–11). These neural changes might differentially affect different types of fear learning (12–14). Initial evidence suggests that norepinephrine (15) and cortisol (9,16) are critical in this stress-induced shift, the latter via the mineralocorticoid receptor (MR). The involvement of this receptor was first discovered in the spatial memory domain where stress led to a shift from hippocampus-dependent to striatum-dependent

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learning (9,17). The MR was formerly thought to have only a limited role in the stress response given its high affinity leading to almost full occupation even at baseline. However, the discovery of a low-affinity, membrane-bound version acting via nongenomic pathways supports its importance in fast stress responses (18) and stress effects on memory formation (19). The MR is localized in brain regions important for fear learning (20,21) and involved in rodent fear conditioning (22,23); however, in humans the role of the MR in stress-induced changes in different fear learning systems has not yet been studied.

We set out to understand the role of the MR in the stress-related shift toward less cognitively demanding fear learning. This challenge required manipulating MR availability, inducing a state of stress, and administering a fear learning task that distinguished between cognitively demanding learning and less demanding fear learning while measuring neural correlates. We employed a randomized, placebo-controlled, full-factorial design (between-subjects factors stress, MR blockade) in healthy men undergoing a combined delay and trace conditioning paradigm while brain activity was measured using functional magnetic resonance imaging (MRI). We hypothesized that under MR availability, stress leads to a shift in fear learning such that delay conditioning comes to dominate over the more demanding hippocampus-dependent trace conditioning.

METHODS AND MATERIALS

The study was approved by the local ethical committee (NL37819.091.11) and registered in the Dutch trial registry (3595) and European trial registry (2011–003493–85).

Participants

Healthy right-handed male volunteers (N = 101) with normal weight (body mass index between 18.5 and 30) were included after general health screening; exclusion criteria are provided in Supplement 1. All participants provided written informed consent and were financially compensated.

General Procedure

Participants were randomly assigned to one of four groups: control/MR-available, stress/MR-available, control/MR-blocked, and stress/MR-blocked. Although the factor MR-availability was manipulated in a double-blind fashion, the factor stress was not.

Adaptation Phase Day 1. Testing took place in the afternoon to ensure stable endogenous levels of cortisol. After assessment of baseline cortisol, subjective mood, and vital signs (blood pressure, heart rate), participants were orally administered four capsules containing 100 mg of the MR antagonist spironolactone each (total of 400 mg, Teva Pharmachemie, Haarlem, The Netherlands) or placebo capsules. This dosage is in accordance with other studies (19,24). A delay of 80 minutes followed ensuring adaptation to the laboratory environment and drug absorption. Participants rested, and cortisol and vital signs were measured every 30 minutes.

Experimental Phase Day 1. Participants performed the fear conditioning paradigm in the MRI scanner immediately after the last part of either stress induction or a nonstressful control procedure (described subsequently). Two other tasks were performed before the last stress induction targeting amygdala reactivity (16) and spatial memory (S. Vogel, Dipl.-Psych., *et al.*, unpublished data, 2014). After an anatomic MRI scan, participants were debriefed about the stress induction procedure followed by a general assessment of well-being.

Recall Phase Day 2. Participants returned the next day (24 hour 32 minutes later, SD 105 minutes) for a recall session in a mock scanner, a reconstruction of an MRI scanner highly similar in appearance and sound.

Stress Induction

We adapted the socially evaluated cold pressor task (25) to an MRI scanner–compatible version (26). Participants were in a supine position on the scanner bench and immersed their right foot into ice water (0°–2°C) up to and including the ankle and held it there as long as possible (the task stopped after 3 minutes). During foot immersion, participants looked into a camera while being closely observed by two nonsupportive experimenters in white laboratory coats. To ensure sustained stress, a socially evaluated difficult mental arithmetic test was administered just before fear conditioning in the stress group (counting aloud backward from 2059 in steps of 17). For the control group, warm water was used (35°C–37°C), no camera was used, the arithmetic test was simple (counting forward in steps of 10), and the experimenter was friendly and casually dressed.

Stress Measurements

Negative mood, salivary cortisol levels, and vital signs were assessed repeatedly (Figure 1).

Combined Delay and Trace Fear Conditioning Procedure

To assess delay and trace conditioning in one task, we intermixed a CS+ that coterminated with the US ($CS+_{delay}$) (Figure 2), another CS+ that was followed by the US after an interval of 3 seconds ($CS+_{trace}$), and a third stimulus that was never reinforced (conditioned stimulus not paired with the US, CS-) (27). Three gray-scaled pictures of neutral male faces served as CS (28,29), and the assignment to CS type was counterbalanced across groups. During habituation, all CS were presented twice (4 seconds) capturing the orienting response, followed by a gray screen ($CS_{interval}$, 3 seconds) and an intertrial interval showing a fixation cross (11 seconds, 12 seconds, or 13 seconds). For acquisition, participants were instructed to find out whether there was a relationship between faces and shocks. Each CS was presented 26 times, and both CS+ were reinforced with a shock (US) (Supplement 1) in 50% of the trials. A short break was inserted after half of the trials to obtain a cortisol sample. On day 2, participants were again habituated and received the same instruction. All CS were presented six times during recall, always followed by $CS_{interval}$ and fixation cross but without reinforcement. Trial timing was similar throughout all experimental phases; trial order was pseudorandom with no more than two repetitions of the same cue. On both days, skin conductance response (SCR) was measured using silver/silver chloride electrodes on the left index and middle fingers (Supplement 1).

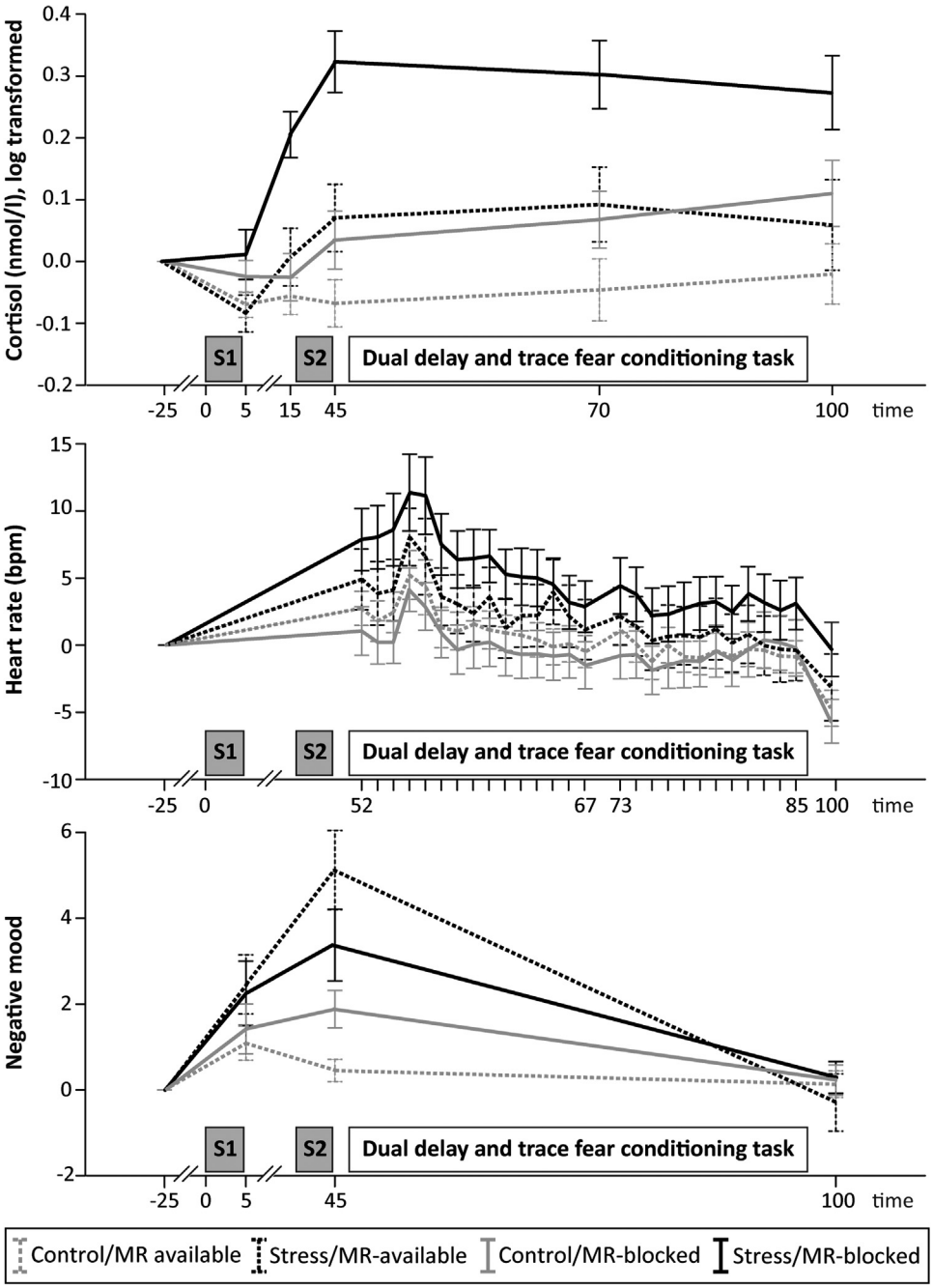

Figure 1. Cortisol levels (top), heart rate (middle), and negative mood (bottom) over the course of the experiment. Participants were randomly assigned to one of four groups: control/MR-available (gray dotted lines), stress/MR-available (black dotted), control/MR-blocked (gray solid), stress/MR-blocked (black solid). After pill ingestion and habituation to the laboratory environment, participants were brought to the magnetic resonance imaging room and underwent either the socially evaluated cold pressor (S1) and difficult mental arithmetic task (S2) or nonstressful control procedures. Afterward, all participants were fear conditioned (see text). Stress-related increases in negative mood (stress main effect [$F_{1,91}$ = 10.907, p = .001], time-by-stress interaction [$F_{2.4,218.4}$ = 9.812, p < .001]), cortisol (stress main effect [$F_{1,92}$ = 13.004, p = .001], time-by-stress interaction [$F_{2.5,229.5}$ = 8.927, p < .001]), and heart rate (time-by-stress interaction [$F_{6.8,569.7}$ = 3.096, p = .004]) showed successful stress induction in both drug groups (details are in Supplement 1). MR-blockade alone led to heightened cortisol levels (MR-blockade main effect [$F_{1,92}$ = 15.013, p < .001], time-by-MR-blockade interaction [$F_{2.5,229.5}$ = 6.217, p = .001]). There was a trend for MR-blockade to diminish the stress-induced increase in negative mood [$F_{2.4,218.4}$ = 2.692, p = .060]. Time is indicated in minutes after stress induction, and all measurements are baseline corrected to the last measurement during habituation (−25 min). Mean values are depicted, error bars represent 1 SEM. MR, mineralocorticoid receptor.

Behavioral and Physiologic Analysis

All behavioral and physiologic analyses were performed using IBM SPSS Statistics version 19 (IBM Corp, Armonk, New York). Univariate or repeated measures analysis of variance was implemented to analyze behavioral and physiologic data including the within-subject factors time and CS type (for SCR) and the between-subject factors stress and MR availability. The α level was set to .05 for all analyses (two-tailed), and Greenhouse-Geisser correction was applied when necessary. Because participants naïve to MRI scanning can show a stress response to the scanning procedure itself (30) and our experimental groups differed incidentally in their percentage of naïve participants (58% stress/MR-blocked, 50% stress/MR-available, 62% control/MR-blocked, 25% control/MR-available), we included scanner naïveté as a covariate of no interest in all of our analyses, including the functional MRI analyses. This approach was supported by the fact that naïve participants had higher heart rates (p < .001) and higher cortisol levels (p < .05) than nonnaïve participants.

Scores for negative mood, cortisol, heart rate, and blood pressure during the experimental phase were baseline corrected to the last measurement of the adaptation phase (−25 minutes). For SCR, we analyzed baseline-to-peak responses in nonreinforced trials (Supplement 1) for CS and $CS_{interval}$. To investigate a stress-induced shift in learning systems, we also directly compared $CS+_{trace}$ and $CS+_{delay}$, subtracting the CS− from both CS+ and analyzing the resulting composite scores. Because we were primarily interested in effects on differential delay and trace conditioning, we focused on main effects and interactions involving the factor CS type.

MRI Analysis

All functional MRI data were analyzed using SPM8 (Wellcome Trust Centre for Neuroimaging, London, United Kingdom); Supplement 1 contains details of acquisition and preprocessing. In line with earlier studies, we focused on transient, learning-related activity (31–33), which is supposed to decrease as soon as the associations are learned (31–34) and the US is reliably predicted (35–37). We expected learning-related activity in the amygdala for delay conditioning and in the hippocampus for trace conditioning. For completeness, we also analyzed sustained activity related to the expression of fear, which varies little over the course of the task and is found in the anterior insula, anterior cingulate cortex, dorsomedial prefrontal cortex (dmPFC), and midbrain (32) and sometimes in the amygdala (38). The first-level models contained the following predictors: for habituation regressors representing CS (4 seconds) and $CS_{interval}$ (3 seconds), for acquisition regressors modeling $CS_{transient}$ ($CS+_{delay,transient}$, $CS+_{trace,transient}$, $CS-_{transient}$), $CS_{interval,transient}$ ($CS+_{delay\text{-}interval,transient}$, $CS+_{trace\text{-}interval,transient}$, $CS-_{interval,transient}$), and six equivalent regressors for sustained activity ($CS_{sustained}$, $CS_{interval,sustained}$). The transient predictors were constructed by multiplying each sustained regressor with a linear decaying function (32). We added regressors for instructions, shocks (.2 second), six realignment parameters, and a constant. Because the administration of shocks can lead to large and fast signal fluctuations (39), we included a regressor with the mean signal intensity per volume.

Similar to our behavioral analysis, we first tested for brain regions differentiating between the three CS types during CS and $CS_{interval}$. We tested a possible stress-induced shift, directly comparing delay and trace conditioning. To identify brain regions showing stronger learning-related activation to the $CS+_{delay}$ than the $CS+_{trace}$, we computed a contrast subtracting $CS+_{trace,transient}$ from $CS+_{delay,transient}$. Analogous contrasts were computed for the $CS_{interval}$ and sustained activity. For exploratory whole-brain analyses, the significance threshold was set to $p < .05$, familywise error correction (cluster-level). For regions included in our a priori hypotheses (bilateral amygdala, hippocampus, insula, dmPFC), we implemented small volume correction, using an initial threshold of $p < .005$, uncorrected, followed by familywise error correction ($p < .05$) for multiple comparisons within regions of interest. The amygdala mask was obtained similar to another study (40) based on the overlap of the contrast US greater than baseline at $p < .05$, familywise error correction and an anatomic mask (Automated Anatomical Labeling atlas, in Wake Forest University PickAtlas version 2.4) (41). The amygdala reacts strongly to electrical shocks (39), and by using a functional amygdala mask independent of our task effects, we hoped to enhance sensitivity. Anatomic masks for hippocampus, insula, and dmPFC were taken from the Automated Anatomical Labeling atlas (for dmPFC, we combined supplementary motor area and median cingulate).

RESULTS

The experimental groups did not differ significantly in age, body mass index, or trait anxiety (Table 1). The stress group immersed their foot in water for a shorter duration than the control group ($F_{1,93} = 20.123$, $df = 1$, $p < .001$), but there was no influence of MR availability (no main effect or interaction).

Stress Measures Adaptation Phase

Decreases throughout the adaptation phase in negative mood, cortisol levels, heart rate, and blood pressure indicated successful adaptation to the laboratory environment (all main effects of time $p < .001$). MR blockade led to higher cortisol levels 25 minutes before stress onset (time-by-MR-availability interaction [$F_{1.7,155.4} = 13.333$, $p < .001$; $t_{96} = 3.126$, $p = .002$]) in line with a regulatory role of the MR on hypothalamic-pituitary-adrenal axis activity (42). Within medication groups, there was no significant difference between stress and control in any measure before stress induction (all $p > .1$).

Stress Measures Experiment Phase

Stress-related increases in negative mood, cortisol, and heart rate showed successful stress induction in both medication groups (Figure 1). Detailed statistics and further analyses can be

Table 1. General Characteristics of Study Sample

Measure	Control/MR-Available	Stress/MR-Available	Control/MR-Blocked	Stress/MR-Blocked	Overall Average
Age	21.6 (2.2)	21.9 (4.0)	22.5 (2.8)	21.5 (2.4)	21.9 (2.9)
Body Mass Index	23.4 (2.4)	22.5 (1.9)	22.7 (2.4)	22.3 (2.5)	22.7 (2.3)
Trait Anxiety	28.4 (5.5)	29.0 (5.1)	28.5 (6.1)	29.5 (5.2)	29.0 (5.8)
Time in Water (sec)	180 (1)	135 (59)[a]	180 (2)	155 (51)[b]	163 (43)

Trait anxiety was assessed using the Dutch translation of the Spielberger State Trait Anxiety Inventory (73). Trait anxiety and body mass index were assessed during screening. Values represent mean (SD).

MR, mineralocorticoid receptor.

[a] $p < .001$ compared with control subjects in the same drug group.

[b] $p < .05$ compared with control subjects in the same drug group.

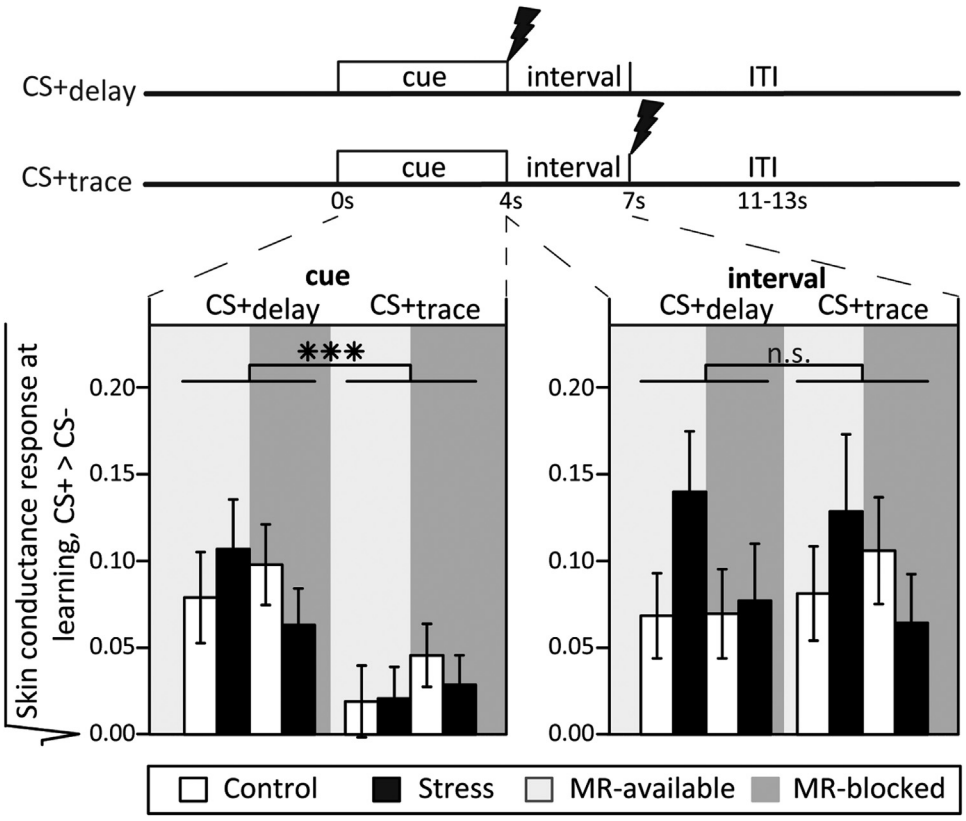

Figure 2. (Top) Schematic overview of delay and trace conditioning. (Bottom) Skin conductance responses (SCRs) revealed successful acquisition of delay and trace conditioning. (Left) The SCR data for the cue period showed successful distinction between CS types ($F_{1.8,163.6} = 30.531$, $p < .001$). The SCR was greater for $CS+_{delay}$ than for $CS+_{trace}$ and CS− (both $p < .001$) and stronger for $CS+_{trace}$ than for CS− ($p = .003$). (Right) The SCR data for the trace interval also differed between CS types ($F_{1.5,135.9} = 20.972$, $p < .001$), with stronger responses to $CS+_{trace\text{-}interval}$ and $CS+_{delay\text{-}interval}$ than to $CS-_{interval}$ (both $p < .001$). Although the stress/MR-available group showed numerically greater responses to the $CS+_{delay\text{-}interval}$ than the control group, the CS-type-by-stress interaction for the $CS_{interval}$ reached only trend-level significance ($F_{1,89} = 3.737$, $p = .056$). The groups did not differ in their response to CS− or $CS-_{interval}$ (all $p > .1$). Error bars depict SEM. CS, conditioned stimulus; ITI, intertrial interval; MR, mineralocorticoid receptor.

found in the figures and Supplement 1. As expected, MR blockade led to heightened cortisol levels (42). Stress-related increases were comparable in both medication groups, although there was a trend for MR blockade to reduce stress-induced negative mood. To conclude, stress induction was successful leaving stress levels elevated during fear conditioning.

Successful Acquisition of Delay and Trace Conditioning

The SCR data for the cue revealed successful differentiation between CS types (i.e., greater SCR to $CS+_{delay}$ than to $CS+_{trace}$ and CS− and stronger SCR to $CS+_{trace}$ than to CS−) (Figure 2). Also during the $CS_{interval}$ we found successful differentiation of CS types with stronger responses to $CS+_{trace\text{-}interval}$ and $CS+_{delay\text{-}interval}$ than to $CS-_{interval}$, but equally strong responses to $CS+_{trace\text{-}interval}$ and $CS+_{delay\text{-}interval}$. The lack of a difference between the CS+ intervals likely reflects the slow nature of SCR and a response to the omission of an expected shock after the unreinforced $CS+_{delay}$ (43). To summarize, participants successfully acquired trace and delay conditioned fear responses.

Subsequently, we tested whether stress affected fear expression on day 1 using the composite score to contrast $CS+_{delay}$ and $CS+_{trace}$ directly. The groups did not differ in response to CS− or $CS-_{interval}$. Potentially supporting our hypothesis of a stress-induced shift, we found a CS-type-by-stress interaction during $CS_{interval}$ presentation at trend level. However, no post hoc test reached significance, and no influence of MR availability was found.

Stress-Induced Shift on Recall of Delay and Trace Conditioning

On day 2, we did not find any significant group difference in cortisol, negative mood, heart rate, or blood pressure (all $p > .05$) (Supplement 1), supporting full drug washout and an absence of residual stress effects. Possible group differences in SCR data during recall can be readily interpreted as stress or MR availability effects on learning and consolidation.

The SCR data during cue presentation at recall were influenced by MR availability and, at trend level, by stress, although we found no significant differentiation of CS types overall. The groups did not differ in response to the CS− or $CS-_{interval}$. To elucidate the group differences further, we analyzed the composite scores (CS+ minus CS−), confirming that these differences were present in the differential response to $CS+_{delay}$ versus $CS+_{trace}$. When directly contrasting both CS+, we found stronger early recall (first three trials) of the $CS+_{delay}$ compared with the $CS+_{trace}$ after stress in the MR-available groups, but no such difference in the MR-blocked groups (Figure 3). Additionally, we found stronger early and late recall of the $CS+_{delay}$ compared

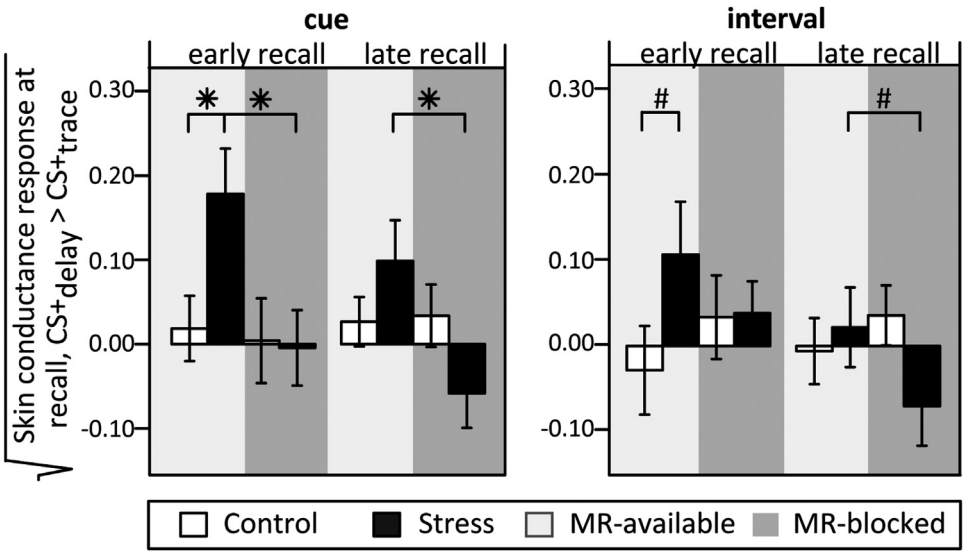

Figure 3. Skin conductance responses (SCRs) during recall on day 2. Data are plotted as $CS+_{delay}$ over $CS+_{trace}$ with SCR to the CS− and $CS-_{interval}$ being subtracted from CS+ and $CS+_{interval}$, respectively. The groups did not differ in response to the CS− or $CS-_{interval}$ (all $p > .1$). Individual variance was high leading to weak differential recall (Figure S1 in Supplement 1). (Left) The SCRs during cue presentation were affected by the factors MR availability and, at trend level, stress (CS-type-by-MR-availability interaction [$F_{1.9,169.6} = 3.352$, $p = .039$]; CS-type-by-stress-by-MR-availability interaction [$F_{1.9,169.6} = 2.827$, $p = .063$]). Analyzing the composite score confirmed these effects (CS-type-by-MR-availability interaction [$F_{1,87} = 5.087$, $p = .027$], CS-type-by-stress-by-MR-availability interaction [$F_{1,87} = 5.144$, $p = .026$]). When directly comparing $CS+_{delay}$ and $CS+_{trace}$, we found stronger SCRs during early recall to the $CS+_{delay}$ compared with the $CS+_{trace}$ after stress in the MR-available groups ($p = .020$), but no such difference in the MR-blocked groups. The SCRs during recall to the $CS+_{delay}$ compared with the $CS+_{trace}$ were stronger in the stress/MR-available group than the stress/MR-blocked group (early, $p = .012$; late, $p = .017$). (Right) The SCRs during interval presentation showed no differentiation between CS types in the general analysis of variance. However, the composite scores revealed a trend-level influence of stress and MR availability (CS-type-by-time-by-stress interaction [$F_{1,87} = 3.624$, $p = .060$], CS-type-by-stress-by-MR-availability interaction [$F_{1,87} = 3.217$, $p = .076$]), showing a similar, although weaker, pattern as the analysis on the cue period. Figure S1 in Supplement 1 illustrates the responses relative to CS−. Error bars depict SEM. CS, conditioned stimulus; MR, mineralocorticoid receptor.

with the $CS+_{trace}$ in the stress/MR-available group than the stress/MR-blocked group. This pattern of results supports our hypothesis of a stress-induced, MR-dependent shift toward a dominance of reflexive delay conditioning.

The analysis on the SCR composite scores during $CS_{interval}$ at recall ($CS+_{interval}$ minus $CS-_{interval}$) discerned a CS-type-by-time interaction and, at trend level, CS-type-by-time-by-stress and CS-type-by-stress-by-MR-availability interactions in the same direction as for the cue. These results support again, although being statistically weaker, an MR-dependent stress-induced shift toward better recall of delay than trace conditioning (Figure 3).

Neural Mechanisms Underlying Learning

Brain Regions Showing Transient Activation to $CS+_{delay}$. After successful fear learning was confirmed at the physiologic level, we investigated brain regions involved in learning by modeling a response decaying over time. We observed a transient bilateral amygdala response to the $CS+_{delay}$ (Figure 4). However, we did not find the hypothesized differential transient amygdala activity when testing for regions differentiating CS types. This might be explained by the fact that our stimuli were faces, which intrinsically activate the amygdala (as opposed to geometric shapes) (32), possibly making it more difficult to find differences in transient responses to the CS types.

Brain Regions Showing Transient Activation to $CS+_{trace\text{-}interval}$. We found that activity in bilateral medial temporal clusters overlapping with the hippocampus differed between $CS_{interval}$ types. When testing specifically for regions showing a stronger transient response for $CS+_{trace\text{-}interval}$ than for $CS+_{delay\text{-}interval}$, we again found extended hippocampal clusters (Figure 4).

Together, our findings confirm a transient role for the hippocampus during the trace interval in trace conditioning (31). Our findings also support evidence that the amygdala is involved in encoding the cue for delay conditioning (32).

Stress-by-MR-Availability Effects on Fear Learning–Related Brain Activity

We extracted data from the bilateral amygdala reactions to the $CS+_{delay,transient}$ (at $p < .005$, uncorrected), but the analysis on the parameter estimates for $CS+_{delay,transient}$ revealed no effect of stress or MR availability (Figure 4). However, a similar analysis on the bilateral medial temporal cluster responding to the $CS+_{trace\text{-}interval}$ (at $p < .05$, familywise error correction) revealed a stress-by-MR-availability interaction (Figure 4). Stress decreased the transient response in the MR-available groups indicative of less learning-related activity, but not in the MR-blocked groups. In line with the SCR results at recall, this finding suggests an MR-dependent stress-induced impairment of trace conditioning resulting in a relative dominance of reflexive forms of fear learning.

Neural Mechanisms Underlying Expression of Fear

In line with previous studies, we found sustained activity in a set of brain regions overlapping with a network consistently activated during the expression of conditioned fear—the so-called salience network (Figure 5; Supplement 1) including the amygdala for delay and both the amygdala and the

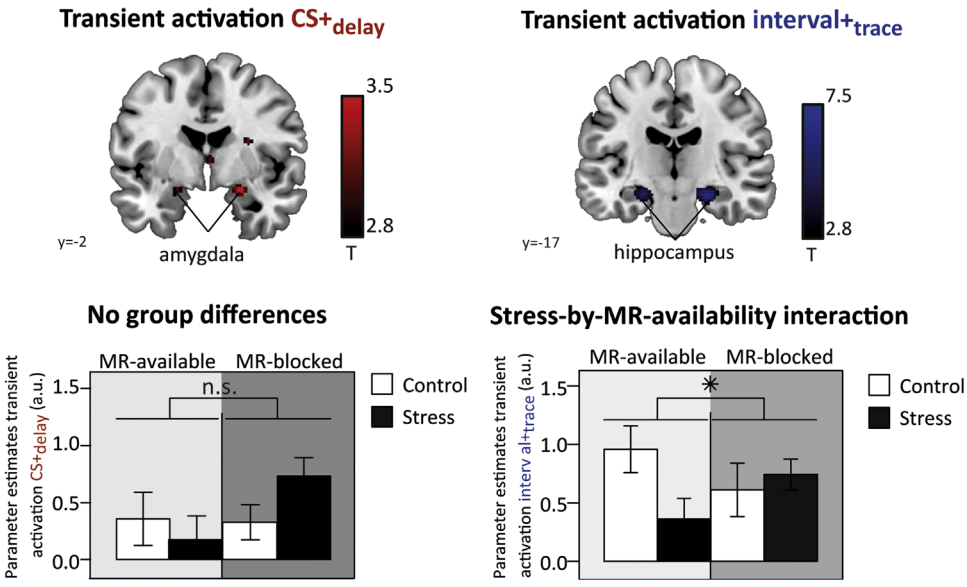

Figure 4. (Top left) Transient activation to the CS+$_{delay}$ in the bilateral amygdala (left, p = .024, small volume correction, k = 37, T = 3.24; right, p = .016, small volume correction, k = 40, T = 3.58). For illustrative purposes, this image is thresholded at $p < .005$, uncorrected. (Top right) Transient activation to the trace interval was found only in the bilateral hippocampus ($p < .05$, familywise error correction). Similar medial temporal lobe activations were found when testing for regions differentiating the three CS+$_{interval}$-types (left, p = .001, small volume correction, k = 89, F = 14.68, p = .063, small volume correction, k = 27, F = 9.10; right, $p < .001$, small volume correction, k = 95, F = 19.41, p = .005, small volume correction, k = 65, F = 12.26) or for regions showing a stronger transient response to CS+$_{trace\text{-}interval}$ than CS+$_{delay\text{-}interval}$ (right, $p < .05$, familywise error correction; left, $p <$ .008, small volume correction, k = 75, T = 4.41). (Bottom) Parameter estimates for the contrast CS+$_{delay,transient}$ (left) and CS+$_{trace\text{-}interval,transient}$ (right). Parameter estimates were extracted from the cluster shown on top. We found a stress-by-MR-availability interaction on the parameter estimates for CS+$_{trace\text{-}interval,transient}$ ($F_{1,83}$ = 4.573, p = .035), but not CS+$_{delay,transient}$. Stress decreased learning-related activity to the trace interval (p = .031), but only if the MR was available. This effect was prevented in the MR-blocked groups. Error bars depict SEM. CS, conditioned stimulus; MR, mineralocorticoid receptor.

hippocampus for trace conditioning. This activity was not significantly affected by stress or MR availability.

DISCUSSION

We present results supporting a dominance of cognitively less demanding fear learning under stress, depending on cortisol interacting with the MR. In line with our hypothesis, stress led to a dominance of delay conditioning over trace conditioning in SCR at recall, paralleled by an MR-dependent, stress-induced impairment of hippocampal fear learning during acquisition. Previous studies investigating stress effects on fear learning led to equivocal results possibly secondary to differences in design, stress induction method, time interval between cortisol increase and fear conditioning, and outcome measures (27). Nevertheless, as of yet no study investigated the effect of stress induction just before a combined delay and trace paradigm including a later recall test, by which we could reveal a stress-induced dominance shift in fear learning systems.

Stress-induced changes are brought about by different waves of neuromodulators (44). Initially, norepinephrine leads to activation of a neural salience network and enhanced vigilance (15). Conversely, activation of the hypothalamic-pituitary-adrenal axis results in slower action of cortisol at glucocorticoid receptor (GR) and MR. Both receptors mediate rapid, nongenomic and slow, genomic effects (45). It is assumed that rapid MR-mediated effects are permissive, facilitating adaptive behavior in stressful situations, whereas slow, mostly GR-mediated effects reinstall homeostasis (46,47). We extend findings that the MR, presumably via nongenomic pathways, is implicated in stress-induced shifts between multiple memory systems (9,16,17) by showing its crucial role in inducing a shift in fear learning. However, genomic effects might have contributed in later stages of acquisition or consolidation. GR and MR have been implicated in fear learning before in rodents (22,23), but these studies did not include a comparison between stressful and nonstressful conditions, allowing no conclusion about rapid or genomic effects. Studies manipulating the timing between stress and task (27) would provide a better mechanistic understanding of stress effects on memory formation.

Although there are reports on impaired hippocampal functioning (9–11) and memory retrieval under stress (26,48), stress often enhances encoding of declarative (item) memory. In apparent contrast, we found impaired hippocampus-dependent fear learning under stress. Fear conditioning differs substantially from standard declarative memory tasks in that the same few stimuli are repeated, resulting in recurring encoding-retrieval cycles. It is suggested that when such encoding-retrieval cycles occur under stress, hippocampal impairment of the retrieval component may disrupt stabilization of more complex associations. This resembles the impaired contextualization of emotional memories associated with post-traumatic stress disorder (49) and induced by heightened cortisol levels in healthy adults (50) and rodents (51).

Our findings support the hypothesis that trace conditioning poses additional demands (e.g., working memory) and engages brain regions beyond those needed for delay conditioning (7,31,52,53). Although it is unclear how these regions interact while encoding temporal CS-US relationships, trace conditioning seems to require additional resources to encode the more complex stimulus contingencies. In contrast, the simpler stimulus-shock associations and concurrent sensory inputs in delay conditioning can be encoded by the amygdala

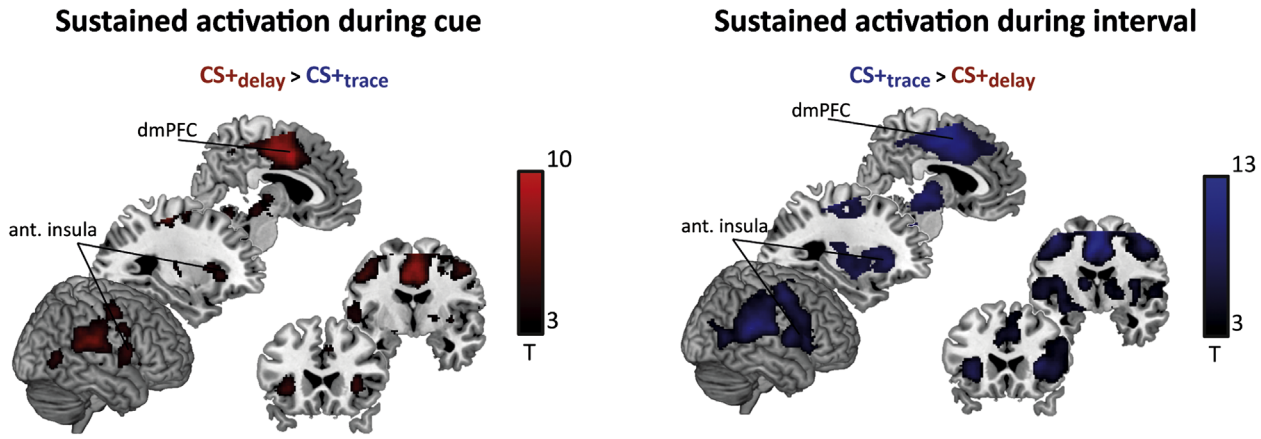

Figure 5. (Left) Brain regions showing stronger activity to the $CS+_{delay}$ than the $CS+_{trace}$ during presentation of the cue. Activation of the dorsomedial prefrontal cortex, midbrain, and anterior insula, which are regions of the salience network (all $p < .05$, familywise error correction), and the left amygdala ($p = .021$, small volume correction). (Right) Brain regions showing stronger activation to the trace interval than the interval after the $CS+_{delay}$. Activation of the dorsomedial prefrontal cortex, midbrain, and anterior insula and the left hippocampus (all $p < .05$, familywise error correction). ant. insula, anterior insula; CS, conditioned stimulus; dmPFC, dorsomedial prefrontal cortex.

even without the hippocampus (54). Testing stress effects on cognitively demanding forms of fear learning might provide additional insight because they rely on different brain structures and may be relevant for complex real-life situations.

Despite the strengths of this study, such as the large sample size, its full-factorial design, a task combining delay and trace conditioning, and a pharmacologic manipulation enabling us to investigate effects of stress depending on MR availability, some limitations should be considered. Overall recall on day 2 in SCR was weak, possibly as a result of strong interindividual differences and the complexity of the task. Nevertheless, we were able to observe meaningful group differences related to stress and MR availability.

As spironolactone affects hypothalamic-pituitary-adrenal axis regulation (42), it might affect cortisol and corticotropin-releasing factor levels. More cortisol becomes available for GR binding, shifting the balance between MR and GR activation. Although most rapid effects so far have been ascribed to the MR (45,46), GR activation might have played a role, too, especially in later trials. Finally, spironolactone can affect other receptors (e.g., progesterone receptors) (55).

It is important to note that we investigated male participants only. A recent study (9) demonstrated the stress-induced shift in both sexes, suggesting that our finding might hold for female participants as well. However, other studies found sex differences in stress effects when investigating fear memory formation (56). Also, the prevalence of anxiety disorders is higher in women (57), suggesting sex-specific effects in stress and anxiety. Finally, stress effects might vary across the menstrual cycle (58,59). Although our choice for testing male subjects only was deliberate given practical constraints to our sample size, a follow-up study deciphering the mechanism of a stress-induced shift in female subjects is needed to ensure the generalizability of our findings.

Finally, we emphasize a difference between the task we implemented and the paradigms employed by earlier studies investigating a stress-induced shift between systems supporting different types of spatial memory (17,60–62). In earlier studies, a test trial was used to identify which memory system dominated behavior, and it was assumed that these systems act competitively. However, in our recall task, participants could demonstrate good performance in delay and trace conditioning (i.e., there was not competition between the two systems). We cannot readily conclude that the stress-induced increase in delay conditioning is directly linked to a decrease in trace conditioning. Nonetheless, we observed a relative shift in the activity balance between the two fear learning systems supporting a comparative increase in cognitively less demanding fear learning. More research is needed to gain a deeper understanding of the precise interactions of different fear memory systems in humans.

Studies in other domains support that individuals under stress or directly after stress shift toward cognitively less demanding systems. Under stress, more reflexive behavior (63,64) and less strategic decisions are made (65). Also, in reinforcement learning, stress reduces complex, model-based contributions to behavior (66). Together, these studies suggest that stress leads to a rapid shift in neural processing, resulting in a dominance of less demanding systems in a broad range of cognitive domains.

In conclusion, this stress-induced shift might prove relevant for any disorder involving well-learned maladaptive behaviors or cognitive rigidity. For example, anxiety or stress can lead to relapse in drug addiction (67,68), and it is conceivable that this might hold for obsessive-compulsive disorder, too. The shift might also have implications for posttraumatic stress disorder, which is assumed to result from excessive fear learning under stress and is characterized by impaired hippocampal functioning (49). No studies have been conducted as of yet specifically to target a stress-induced cognitive shift in patient populations. However, if our findings hold in patient samples, we suggest that the shift is dependent on MR-activation and might be prevented by short-term administration of MR antagonists. Related to this suggestion, more recent studies associated genetic variants in the gene encoding the MR with interindividual differences in risk for psychopathology (69–72). Our findings could have mechanistic implications for understanding of the impact of stress on daily life and mental

well-being, which might be particularly prominent in individuals with high MR sensitivity.

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REFERENCES

1. Mineka S, Zinbarg R (2006): A contemporary learning theory perspective on the etiology of anxiety disorders: It's not what you thought it was. Am Psychol 61:10–26.
2. LeDoux JE, Cicchetti P, Xagoraris A, Romanski LM (1990): The lateral amygdaloid nucleus: Sensory interface of the amygdala in fear conditioning. J Neurosci 10:1062–1069.
3. Nader K, Majidishad P, Amorapanth P, LeDoux JE (2001): Damage to the lateral and central, but not other, amygdaloid nuclei prevents the acquisition of auditory fear conditioning. Learn Mem 8:156–163.
4. Clark RE, Squire LR (1998): Classical conditioning and brain systems: The role of awareness. Science 280:77–81.
5. Weike AI, Schupp HT, Hamm AO (2007): Fear acquisition requires awareness in trace but not delay conditioning. Psychophysiology 44: 170–180.
6. Kronforst-Collins MA, Disterhoft JF (1998): Lesions of the caudal area of rabbit medial prefrontal cortex impair trace eyeblink conditioning. Neurobiol Learn Mem 69:147–162.
7. Knight DC, Cheng DT, Smith CN, Stein EA, Helmstetter FJ (2004): Neural substrates mediating human delay and trace fear conditioning. J Neurosci 24:218–228.
8. van Marle HJF, Hermans EJ, Qin SZ, Fernandez G (2009): From specificity to sensitivity: How acute stress affects amygdala processing of biologically salient stimuli. Biol Psychiatry 66:649–655.
9. Schwabe L, Tegenthoff M, Höffken O, Wolf OT (2013): Mineralocorticoid receptor blockade prevents stress-induced modulation of multiple memory systems in the human brain. Biol Psychiatry 74:801–808.
10. Cousijn H, Rijpkema M, Qin S, van Wingen GA, Fernández G (2012): Phasic deactivation of the medial temporal lobe enables working memory processing under stress. Neuroimage 59:1161–1167.
11. Pruessner JC, Declovic K, Khalili-Mahani N, Engert V, Pruessner M, Buss C, *et al*. (2008): Deactivation of the limbic system during acute psychosocial stress: Evidence from positron emission tomography and functional magnetic resonance imaging studies. Biol Psychiatry 63:234–240.
12. Schwabe L, Wolf OT (2013): Stress and multiple memory systems: From 'thinking' to 'doing'. Trends Cogn Sci 17:60–68.
13. Hermans EJ, Henckens MJAG, Joëls M, Fernández G (2014): Dynamic adaptation of large-scale brain networks in response to acute stressors. Trends Neurosci 37:304–314.
14. Packard MG, Teather LA (1998): Amygdala modulation of multiple memory systems: Hippocampus and caudate-putamen. Neurobiol Learn Mem 69:163–203.
15. Hermans EJ, van Marle HJF, Ossewaarde L, Henckens MJAG, Qin S, van Kesteren MTR, *et al*. (2011): Stress-related noradrenergic activity prompts large-scale neural network reconfiguration. Science 334: 1151–1153.
16. Vogel S, Klumpers F, Krugers HJ, Fang Z, Oplaat KT, Oitzl MS, *et al*. (2015): Blocking the mineralocorticoid receptor in humans prevents the stress-induced enhancement of centromedial amygdala connectivity with the dorsal striatum. Neuropsychopharmacology 40:947–956.
17. Schwabe L, Schachinger H, de Kloet ER, Oitzl MS (2010): Corticosteroids operate as a switch between memory systems. J Cogn Neurosci 22:1362–1372.
18. Joëls M, Karst H, DeRijk R, de Kloet ER (2008): The coming out of the brain mineralocorticoid receptor. Trends Neurosci 31:1–7.
19. Cornelisse S, Joels M, Smeets T (2011): A randomized trial on mineralocorticoid receptor blockade in men: Effects on stress responses, selective attention, and memory. Neuropsychopharmacology 36:2720–2728.
20. Patel PD, Lopez JF, Lyons DM, Burke S, Wallace M, Schatzberg AF (2000): Glucocorticoid and mineralocorticoid receptor mRNA expression in squirrel monkey brain. J Psychiatr Res 34:383–392.
21. Klok MD, Alt SR, Irurzun Lafitte AJM, Turner JD, Lakke EAJF, Huitinga I, *et al*. (2011): Decreased expression of mineralocorticoid receptor mRNA and its splice variants in postmortem brain regions of patients with major depressive disorder. J Psychiatr Res 45:871–878.
22. Zhou M, Bakker EHM, Velzing EH, Berger S, Oitzl M, Joels M, *et al*. (2010): Both mineralocorticoid and glucocorticoid receptors regulate emotional memory in mice. Neurobiol Learn Mem 94:530–537.
23. Zhou M, Kindt M, Joëls M, Krugers HJ (2011): Blocking mineralocorticoid receptors prior to retrieval reduces contextual fear memory in mice. PLoS One 6:e26220.
24. Rimmele U, Besedovsky L, Lange T, Born J (2013): Blocking mineralocorticoid receptors impairs, blocking glucocorticoid receptors enhances memory retrieval in humans. Neuropsychopharmacology 38:884–894.
25. Schwabe L, Haddad L, Schachinger H (2008): HPA axis activation by a socially evaluated cold-pressor test. Psychoneuroendocrinology 33: 890–895.
26. Schwabe L, Joëls M, Roozendaal B, Wolf OT, Oitzl MS (2012): Stress effects on memory: An update and integration. Neurosci Biobehav Rev 36:1740–1749.
27. Cornelisse S, van Ast VA, Joëls M, Kindt M (2014): Delayed effects of cortisol enhance fear memory of trace conditioning. Psychoneuroendocrinology 40:257–268.
28. Lundqvist D, Flykt A, Ohman A (1998): Karolinska Directed Emotional Faces [Database of standardized facial images]. Psychology Section, Department of Clinical Neuroscience, Karolinska Hospital, Stockholm, Sweden.
29. Oosterhof NN, Todorov A (2008): The functional basis of face evaluation. Proc Natl Acad Sci U S A 105:11087–11092.
30. Muehlhan M, Lueken U, Wittchen H-U, Kirschbaum C (2011): The scanner as a stressor: Evidence from subjective and neuroendocrine stress parameters in the time course of a functional magnetic resonance imaging session. Int J Psychophysiol 79:118–126.
31. Buchel C, Dolan RJ, Armony JL, Friston KJ (1999): Amygdala-hippocampal involvement in human aversive trace conditioning revealed through event-related functional magnetic resonance imaging. J Neurosci 19:10869–10876.
32. Marschner A, Kalisch R, Vervliet B, Vansteenwegen D, Büchel C (2008): Dissociable roles for the hippocampus and the amygdala in human cued versus context fear conditioning. J Neurosci 28:9030–9036.
33. Büchel C, Morris J, Dolan RJ, Friston KJ (1998): Brain systems mediating aversive conditioning: An event-related fMRI study. Neuron 20:947–957.
34. Tabbert K, Stark R, Kirsch P, Vaitl D (2005): Hemodynamic responses of the amygdala, the orbitofrontal cortex and the visual cortex during a fear conditioning paradigm. Int J Psychophysiol 57:15–23.

35. McNally GP, Johansen JP, Blair HT (2011): Placing prediction into the fear circuit. Trends Neurosci 34:283–292.
36. Boll S, Gamer M, Gluth S, Finsterbusch J, Büchel C (2013): Separate amygdala subregions signal surprise and predictiveness during associative fear learning in humans. Eur J Neurosci 37:758–767.
37. Li J, Schiller D, Schoenbaum G, Phelps EA, Daw ND (2011): Differential roles of human striatum and amygdala in associative learning. Nat Neurosci 14:1250–1252.
38. Sehlmeyer C, Schöning S, Zwitserlood P, Pfleiderer B, Kircher T, Arolt V, *et al*. (2009): Human fear conditioning and extinction in neuroimaging: A systematic review. PLoS One 4:e5865.
39. Klumpers F, Raemaekers MAHL, Ruigrok ANV, Hermans EJ, Kenemans JL, Baas JMP (2010): Prefrontal mechanisms of fear reduction after threat offset. Biol Psychiatry 68:1031–1038.
40. Schiller D, Kanen JW, LeDoux JE, Monfils M-H, Phelps EA (2013): Extinction during reconsolidation of threat memory diminishes prefrontal cortex involvement. Proc Natl Acad Sci 110:20040–20045.
41. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, *et al*. (2002): Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15:273–289.
42. de Kloet ER, Joels M, Holsboer F (2005): Stress and the brain: From adaptation to disease. Nat Rev Neurosci 6:463–475.
43. Dunsmoor JE, LaBar KS (2012): Brain activity associated with omission of an aversive event reveals the effects of fear learning and generalization. Neurobiol Learn Mem 97:301–312.
44. Joëls M, Baram TZ (2009): The neuro-symphony of stress. Nat Rev Neurosci 10:459–466.
45. Joëls M, Sarabdjitsingh RA, Karst H (2012): Unraveling the time domains of corticosteroid hormone influences on brain activity: Rapid, slow, and chronic modes. Pharmacol Rev 64:901–938.
46. Groeneweg FL, Karst H, de Kloet ER, Joëls M (2011): Rapid non-genomic effects of corticosteroids and their role in the central stress response. J Endocrinol 209:153–167.
47. de Kloet ER, Karst H, Joels M (2008): Corticosteroid hormones in the central stress response: Quick-and-slow. Front Neuroendocrinol 29: 268–272.
48. Roozendaal B, Okuda S, De Quervain DJF, McGaligh JL (2006): Glucocorticoids interact with emotion-induced noradrenergic activation in influencing different memory functions. Neuroscience 138: 901–910.
49. Acheson DT, Gresack JE, Risbrough VB (2012): Hippocampal dysfunction effects on context memory: Possible etiology for posttraumatic stress disorder. Neuropharmacology 62:674–685.
50. van Ast VA, Cornelisse S, Meeter M, Joëls M, Kindt M (2013): Time-dependent effects of cortisol on the contextualization of emotional memories. Biol Psychiatry 74:809–816.
51. Kaouane N, Porte Y, Vallée M, Brayda-Bruno L, Mons N, Calandreau L, *et al*. (2012): Glucocorticoids can induce PTSD-like memory impairments in mice. Science 335:1510–1513.
52. Raybuck JD, Lattal KM (2014): Bridging the interval: Theory and neurobiology of trace conditioning. Behav Processes 101: 103–111.
53. Gilmartin MR, Balderston NL, Helmstetter FJ (2014): Prefrontal cortical regulation of fear learning. Trends Neurosci 37:455–464.
54. Bechara A, Tranel D, Damasio H, Adolphs R, Rockland C, Damasio AR (1995): Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. Science 269: 1115–1118.
55. Schane HP, Potts GO (1978): Oral progestational activity of spironolactone. J Clin Endocrinol Metab 47:691–694.
56. Merz CJ, Wolf OT, Schweckendiek J, Klucken T, Vaitl D, Stark R (2013): Stress differentially affects fear conditioning in men and women. Psychoneuroendocrinology 38:2529–2541.
57. Kessler R, Berglund P, Demler O, Jin R, Merikangas K, Walters E (2005): Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. Arch Gen Psychiatry 62:593–602.
58. Duchesne A, Tessera E, Dedovic K, Engert V, Pruessner JC (2012): Effects of panel sex composition on the physiological stress responses to psychosocial stress in healthy young men and women. Biol Psychol 89:99–106.
59. Ossewaarde L, Hermans EJ, van Wingen GA, Kooijman SC, Johansson I-M, Bäckström T, *et al*. (2010): Neural mechanisms underlying changes in stress-sensitivity across the menstrual cycle. Psychoneuroendocrinology 35:47–55.
60. Packard MG, Wingard JC (2004): Amygdala and "emotional" modulation of the relative use of multiple memory systems. Neurobiol Learn Mem 82:243–252.
61. Wingard JC, Packard MG (2008): The amygdala and emotional modulation of competition between cognitive and habit memory. Behav Brain Res 193:126–131.
62. Schwabe L, Oitzl MS, Philippsen C, Richter S, Bohringer A, Wippich W, *et al*. (2007): Stress modulates the use of spatial versus stimulus-response learning strategies in humans. Learn Mem 14:109–116.
63. Schwabe L, Höffken O, Tegenthoff M, Wolf OT (2011): Preventing the stress-induced shift from goal-directed to habit action with a β-adrenergic antagonist. J Neurosci 31:17317–17325.
64. Porcelli AJ, Delgado MR (2009): Acute stress modulates risk taking in financial decision making. Psychol Sci 20:278–283.
65. Leder J, Häusser JA, Mojzisch A (2013): Stress and strategic decision-making in the beauty contest game. Psychoneuroendocrinology 38: 1503–1511.
66. Otto AR, Raio CM, Chiang A, Phelps EA, Daw ND (2013): Working-memory capacity protects model-based learning from stress. Proc Natl Acad Sci 110:20941–20946.
67. Herman CP, Polivy J (1975): Anxiety, restraint, and eating behavior. J Abnorm Psychol 84:666–672.
68. Weiss F, Ciccocioppo R, Parsons LH, Katner S, Liu XIU, Zorrilla EP, *et al*. (2001): Compulsive drug-seeking behavior and relapse. Ann N Y Acad Sci 937:1–26.
69. DeRijk RH, de Kloet ER, Zitman FG, van Leeuwen N (2011): Mineralocorticoid receptor gene variants as determinants of HPA axis regulation and behavior. Endocr Dev 20:137–148.
70. Klok MD, Giltay EJ, Van der Does AJW, Geleijnse JM, Antypa N, Penninx BWJH, *et al*. (2011): A common and functional mineralocorticoid receptor haplotype enhances optimism and protects against depression in females. Transl Psychiatry 1:e62.
71. Bogdan R, Williamson DE, Hariri AR (2012): Mineralocorticoid receptor Iso/Val (rs5522) genotype moderates the association between previous childhood emotional neglect and amygdala reactivity. Am J Psychiatry 169:515–522.
72. Vogel S, Gerritsen L, van Oostrom I, Arias-Vásquez A, Rijpkema M, Joëls M, *et al*. (2014): Linking genetic variants of the mineralocorticoid receptor and negative memory bias: Interaction with prior life adversity. Psychoneuroendocrinology 40:181–190.
73. Spielberger CD, Gorsuch RL, Lushene R, Vagg PR, Jacobs GA (1983): Manual for the State-Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologists Press.