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...
learning (9,17). The MR was formerly thought to have only a limited role in the stress response even giving 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 (fMRI). 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 to 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 (CSinterval, 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 CSinterval 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).
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 \( \alpha \) 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-available, 50% stress/MR-blocked, 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 (\( \rho < .001 \)) and higher cortisol levels (\( \rho < .05 \)) 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 CSinterval. To investigate a stress-induced shift in learning systems, we also directly compared CStrace and CSdelay, 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 CSinterval (3 seconds), for acquisition regressors modeling CS+ (delay, transient), CStrace, CS− (transient), CSdelay, CSinterval, CS− (transient), CS− (delay, transient), and six equivalent regressors for sustained activity (CS+ sustained, CS− (transient), CS− (delay, transient), CS− (transient). 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 CSinterval. 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

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control/MR-Available</th>
<th>Stress/MR-Available</th>
<th>Control/MR-Blocked</th>
<th>Stress/MR-Blocked</th>
<th>Overall Average</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>21.6 (2.2)</td>
<td>21.9 (4.0)</td>
<td>22.5 (2.8)</td>
<td>21.5 (2.4)</td>
<td>21.9 (2.9)</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>23.4 (2.4)</td>
<td>22.5 (1.9)</td>
<td>22.7 (2.4)</td>
<td>22.3 (2.5)</td>
<td>22.7 (2.3)</td>
</tr>
<tr>
<td>Trait Anxiety</td>
<td>28.4 (5.5)</td>
<td>29.0 (5.1)</td>
<td>28.5 (6.1)</td>
<td>29.5 (5.2)</td>
<td>29.0 (5.6)</td>
</tr>
<tr>
<td>Time in Water (sec)</td>
<td>180 (1)</td>
<td>135 (59)*</td>
<td>180 (2)</td>
<td>155 (51)*</td>
<td>163 (43)</td>
</tr>
</tbody>
</table>

*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.

* \( p < .001 \) compared with control subjects in the same drug group.

† \( p < .05 \) compared with control subjects in the same drug group.
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\textsubscript{+delay} than to CS\textsubscript{+trace} and CS\textsubscript{−} and stronger SCR to CS\textsubscript{+trace} than to CS\textsubscript{−}) (Figure 2). Also during the CS\textsubscript{interval} we found successful differentiation of CS types with stronger responses to CS\textsubscript{+trace-interval} and CS\textsubscript{+delay-interval} than to CS\textsubscript{−interval} but equally strong responses to CS\textsubscript{+trace-interval} and CS\textsubscript{+delay-interval}. The lack of a difference between the CS\textsubscript{+} intervals likely reflects the slow nature of SCR and a response to the omission of an expected shock after the unreinforced CS\textsubscript{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\textsubscript{+delay} and CS\textsubscript{+trace} directly. The groups did not differ in response to CS\textsubscript{−} or CS\textsubscript{−interval}. Potentially supporting our hypothesis of a stress-induced shift, we found a CS-type-by-stress interaction during CS\textsubscript{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 CS\textsubscript{−} or CS\textsubscript{−interval} (all p > .1). Error bars depict SEM. CS, conditioned stimulus; ITI, intertrial interval; MR, mineralocorticoid receptor.

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\textsubscript{1,163.6} = 30.531, p < .001). The SCR was greater for CS\textsubscript{+delay} than for CS\textsubscript{+trace} and CS\textsubscript{−} (both p < .001) and stronger for CS\textsubscript{+trace} than for CS\textsubscript{−} (p = .003). (Right) The SCR data for the trace interval also differed between CS types (F\textsubscript{1,89} = 20.972, p < .001), with stronger responses to CS\textsubscript{+trace-interval} and CS\textsubscript{+delay-interval} than to CS\textsubscript{−interval} (both p < .001). Although the stress/MR-available group showed numerically greater responses to the CS\textsubscript{+delay-interval} than the control group, the CS-type-by-stress interaction for the CS\textsubscript{interval} reached only trend-level significance (F\textsubscript{1,89} = 3.737, p = .056). The groups did not differ in their response to CS\textsubscript{−} or CS\textsubscript{−interval} (all p > .1). Error bars depict SEM. CS, conditioned stimulus; ITI, intertrial interval; 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\_delay,trace minus CS\_delay,trace) 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**

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-interval than for CS+\_delay-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 < 0.05, 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-interval (at p < 0.05, 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...
hhippocampus 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 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 posttraumatic stress disorder (49) and induced by heightened cortisol levels in healthy adults (60) and rodents (61).

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
Stress Shift From Trace to Delay Conditioning and MR

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 (17,50–62). 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 no 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

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

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