

# Mineralocorticoid Receptor Blockade Prevents Stress-Induced Modulation of Multiple Memory Systems in the Human Brain

Lars Schwabe, Martin Tegenthoff, Oliver Höffken, and Oliver T. Wolf

**Background:** Accumulating evidence suggests that stress may orchestrate the engagement of multiple memory systems in the brain. In particular, stress is thought to favor dorsal striatum-dependent procedural over hippocampus-dependent declarative memory. However, the neuroendocrine mechanisms underlying these modulatory effects of stress remain elusive, especially in humans. Here, we targeted the role of the mineralocorticoid receptor (MR) in the stress-induced modulation of dorsal striatal and hippocampal memory systems in the human brain using a combination of event-related functional magnetic resonance imaging and pharmacologic blockade of the MR.

**Methods:** Eighty healthy participants received the MR antagonist spironolactone (300 mg) or a placebo and underwent a stressor or control manipulation before they performed, in the scanner, a classification task that can be supported by the hippocampus and the dorsal striatum.

**Results:** Stress after placebo did not affect learning performance but reduced explicit task knowledge and led to a relative increase in the use of more procedural learning strategies. At the neural level, stress promoted striatum-based learning at the expense of hippocampus-based learning. Functional connectivity analyses showed that this shift was associated with altered coupling of the amygdala with the hippocampus and dorsal striatum. Mineralocorticoid receptor blockade before stress prevented the stress-induced shift toward dorsal striatal procedural learning, same as the stress-induced alterations of amygdala connectivity with hippocampus and dorsal striatum, but resulted in significantly impaired performance.

**Conclusions:** Our findings indicate that the stress-induced shift from hippocampal to dorsal striatal memory systems is mediated by the amygdala, required to preserve performance after stress, and dependent on the MR.

**Key Words:** Dorsal striatum, glucocorticoids, hippocampus, memory systems, mineralocorticoid receptor, stress

**S**tress effects on learning and memory are well documented (1–3). Glucocorticoids, released from the adrenal cortex during stressful experiences, play a key role in these effects (4–6). Glucocorticoids can cross the blood-brain barrier and enter the brain, where they bind to glucocorticoid receptors (GR) and mineralocorticoid receptors (MR). Whereas GRs are widely distributed throughout the brain, MRs are predominantly expressed in limbic structures, such as the hippocampus and the amygdala (7,8). Animal studies suggest a role of GRs in memory consolidation and of MRs in the acquisition of cognitive tasks (9,10). Data on the role of GRs or MRs in human cognition, however, are largely missing.

Although stress effects on memory have been the topic of intense scientific inquiry for more than half a century, most research has focused on the impact of stress on a single memory system, mainly the hippocampus (11–15). Only rather recently, it has been shown that stress may not only affect the performance of a single memory system but may also orchestrate the contribution of multiple, anatomically and functionally distinct memory systems to learning [for reviews, see (16–18)]. In

particular, it has been demonstrated that stress and emotional arousal favor habit or procedural learning that is dependent on the dorsal striatum, at the expense of hippocampus-dependent cognitive or declarative learning (19–23). For example, in rats, stress favors the use of dorsal striatum-based stimulus-response learning over hippocampus-dependent spatial learning in a dual-solution water maze task (20). A similar preferential engagement of dorsal striatum-based learning in dual-solution tasks was observed in humans (19,24). This shift toward habit memory after stress may be highly relevant in the context of psychiatric disorders, such as depression, phobia, or posttraumatic stress disorder (PTSD) (17,25). For example, the stress-induced shift from cognitive to habit memory could (at least partly) explain the strong emotional responding to single trauma-related cues and the impaired integration of the traumatic event into autobiographical memory that can be observed in PTSD (26,27).

First evidence from rodents suggests that glucocorticoids act via the MR to promote the switch toward dorsal striatum-based habit learning (28). A recent study showed the stress-induced shift from hippocampal to striatal learning for the first time in the human brain (24). However, exactly how stress may coordinate hippocampus-based and dorsal striatum-based memory systems in the human brain remains unclear.

Here, we combined pharmacologic blockade of the MR with functional magnetic resonance imaging (fMRI) to examine the role of the MR in the stress-induced modulation of hippocampal and dorsal striatal learning in humans. Using a fully crossed, placebo-controlled, double-blind design, healthy participants received the MR antagonist spironolactone (aMR) or a placebo before they were exposed to a stressor (socially evaluated cold pressor test) (29) or a control manipulation. Shortly after the stressor, participants completed a probabilistic classification learning (PCL) task that can be supported by the hippocampus

From the Institute of Cognitive Neuroscience (LS, OTW), Department of Cognitive Psychology, and Department of Neurology (MT, OH), Ruhr-University Bochum, Bochum, Germany.

Address correspondence to Lars Schwabe, Ph.D., Ruhr-University Bochum, Department of Cognitive Psychology, Universitätsstrasse 150, Bochum 44780, Germany; E-mail: [Lars.Schwabe@ruhr-uni-bochum.de](mailto:Lars.Schwabe@ruhr-uni-bochum.de).

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and by the dorsal striatum (30–32) and a visual-motor control task in the scanner. To assess the engagement of hippocampus-dependent declarative and dorsal striatum-dependent procedural systems also at the behavioral level, we measured explicit task knowledge at the end of the experiment and analyzed the used learning strategies with mathematical modeling.

## Methods and Materials

### Participants and Design

Eighty healthy, right-handed, nonsmoking university students with normal or corrected to normal vision and without any current medical conditions, medication intake, lifetime history of any neurological or psychiatric disorders, or any contraindications for magnetic resonance imaging participated in this experiment (age: mean = 24.6 years, SEM = .3 years). All participants provided written informed consent for participation in the study, which was approved by the ethics committee of the medical faculty of the Ruhr-University Bochum. Due to technical failure and noncompliance with instructions, five participants had to be excluded from analyses.

We used a placebo-controlled, double-blind, between-subjects design with the factors treatment (control vs. stress condition) and drug (placebo vs. 300 mg spironolactone), in which participants were randomly assigned to one of four experimental groups: control/placebo (10 men, 9 women), control/spironolactone (10 men, 8 women), stress/placebo (9 men, 10 women), and stress/spironolactone (stress-aMR; 10 men, 9 women).

### Procedure

Participants ingested a placebo pill or a spironolactone pill (300 mg; Ratiopharm, Ulm Germany), depending on the experimental group. This dosage of spironolactone was likely to result in effective MR blockade on the one hand (33) and to minimize potential discomfort on the side of the participants on the other hand. After a 90-minute break, participants underwent the Socially Evaluated Cold Pressor Test (SECPT), as described in detail elsewhere (29), or a control manipulation (Supplemental Methods and Materials in Supplement 1). To assess the effectiveness of the stress induction by the SECPT, blood pressure, salivary cortisol, and subjective feeling were measured at different time points across the experiment (for details, see Supplemental Methods and Materials in Supplement 1).

About 30 minutes after the stress/control manipulation and 120 minutes after pill intake, participants performed a PCL task, known as the weather prediction task (31,34), in the scanner. In this task, participants should learn how to classify card stimuli based on trial-by-trial feedback. In addition to the PCL task, participants performed a visual-motor control task. The procedure of the control task was exactly the same as in the PCL task, except that participants did not have to learn the probabilistic association between the cue patterns and outcomes (Supplemental Methods and Materials and Figure S1 in Supplement 1). Participants completed 100 PCL trials and 100 control trials, which were presented in random order.

After finishing the PCL task, participants completed (outside the scanner) a questionnaire containing 10 items that assessed explicit task knowledge. Moreover, the used learning strategy was assessed with a mathematical model in which the actual responses of a participant were compared with ideal responses if a participant was reliably using a particular strategy [for details, see (35,36)]. For the sake of simplicity and in line with previous

studies (24,37), we divided the strategies that participants may use to solve the PCL task into simple and complex strategies (for details, see Supplemental Methods and Materials in Supplement 1).

### Magnetic Resonance Imaging Acquisition and Data Analyses

Functional magnetic resonance images were acquired on a 3 T Philips Achieva scanner. Imaging data were analyzed with SPM8 (Wellcome Trust Center for Neuroimaging, University College London, London, United Kingdom), including standard preprocessing procedures (slice timing correction, spatial realignment, co-registration, segmentation, spatial normalization, and smoothing) and modeling the data by general linear models. We used explorative whole-brain analyses, as well as region of interest (ROI) analyses. A priori ROIs were the hippocampus, the caudate nucleus, the putamen, and the orbitofrontal cortex, as these structures were implicated in PCL in earlier studies (24,30,32). For the explorative whole-brain analyses, the significance threshold was set to  $p < .05$  on voxel level, corrected for multiple testing (family-wise error [FWE] correction), and a minimum cluster size of five voxels. Region of interest analyses were performed using the small volume correction options of SPM8 ( $p < .05$ ). For details, see Supplemental Methods and Materials in Supplement 1.

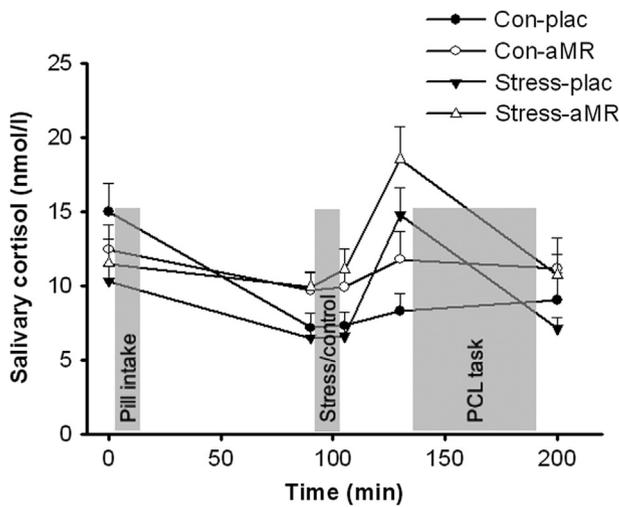
## Results

### Subjective and Physiological Measures

Changes in subjective feeling, blood pressure, and salivary cortisol verified the successful stress induction. Participants who underwent the SECPT rated the treatment as significantly more stressful, painful, and unpleasant than participants who underwent the control manipulation (all  $F > 120$ , all  $p < .001$ ). Moreover, systolic and diastolic blood pressure increased in response to the SECPT but not in response to the control manipulation (treatment  $\times$  time point of measurement interactions: both  $F_{3,213} > 49$ , both  $p < .001$ ; Table S1 in Supplement 1). Similarly, cortisol concentrations increased after the SECPT but not after the control manipulation (treatment  $\times$  time point of measurement interaction:  $F_{4,264} = 10.28$ ,  $p < .001$ ) and reached a maximum shortly before the PCL task started (Figure 1).

Cortisol concentrations were also elevated, both in the stress and the control conditions, by the aMR (drug  $\times$  time point of measurement interaction:  $F_{4,264} = 2.50$ ,  $p < .05$ ). The MR is critically involved in the regulation of the hypothalamus-pituitary-adrenal (HPA) axis (8). For instance, blockade of hippocampal MR, which inhibits adrenocorticotrophic hormone secretion (38), may impair adrenocorticotrophic hormone-mediated negative feedback. Thus, increased cortisol concentrations after aMR intake were expected and verified the action of the drug (33,39). Importantly, however, the aMR did not affect the cortisol response to the SECPT (treatment  $\times$  drug  $\times$  time point of measurement interaction:  $F_{4,264} = .24$ ,  $p = .92$ ); also, when the area under the curve with respect to the cortisol increase (40) was analyzed, there was no modulatory effect of the aMR on the cortisol response to the stressor (treatment  $\times$  drug interaction:  $F_{1,70} = .61$ ,  $p = .44$ ).

Although animal data suggest reduced sympathetic activity after MR blockade (41), we did not find an influence of the aMR on the blood pressure response to the stressor or subjective feeling (all  $p > .10$ ), in line with other human data (33,39). Participants' sex did not affect the physiological response to the stressor or the aMR (all  $F < 2.81$ , all  $p > .08$ ).



**Figure 1.** Salivary cortisol concentrations across the experiment. Both spironolactone intake and the exposure to the socially evaluated cold pressor test led to elevated cortisol concentrations. Error bars represent SEM. con-aMR, control/spironolactone; con-plac, control/placebo; PCL, probabilistic classification learning; stress-aMR, stress/spironolactone; stress-plac, stress/placebo.

**Classification Learning**

During fMRI scanning at 3T, about 30 minutes after the stressor and about 2 hours after drug intake, participants performed the PCL task and the visual-motor control task. A treatment (control vs. stress) × drug (placebo vs. aMR) × learning block analysis of variance on the percentage of correct responses yielded a significant effect of learning block ( $F_{9,630} = 15.96, p < .001$ ) and a significant treatment × drug interaction ( $F_{1,70} = 3.99, p < .05$ ; all other main or interaction effects:  $p > .10$ ). Overall, classification performance in the PCL task increased from 41% to 65% correct responses across the learning session. As shown in Figure 2A, stress after placebo did not alter learning performance, nor did MR blockade alone (i.e., without subsequent stress) alter learning performance. However, if participants were administered the aMR before the stressor exposure, stress impaired performance significantly (stress-aMR group vs. each other group: all  $p < .05$ , least

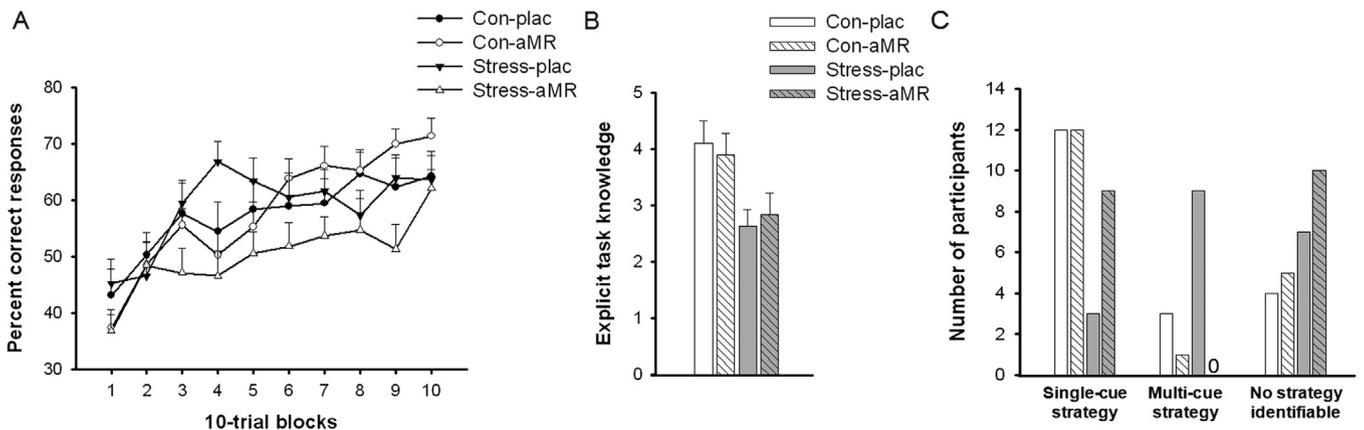
significant difference post hoc tests). Interestingly, the differences between groups remained when we subjected our data to an analysis of covariance with the cortisol area under the curve with respect to the cortisol increase as a covariate ( $F_{1,70} = 3.81, p = .05$ ), suggesting that differences in cortisol concentrations between groups could not explain the impact of stress after aMR administration on learning performance.

In addition, stress before PCL reduced explicit task knowledge ( $F_{1,71} = 11.84, p = .001$ ; Figure 2B), irrespective of whether participants had received the placebo or the aMR before the stressor (drug and treatment × drug effects: both  $p > .56$ ); overall, the explicit task knowledge scores were very similar to those observed in our previous study after stress (24). Moreover, analysis of learning strategies with the help of mathematical modeling (for details, see Supplemental Methods and Materials in Supplement 1) revealed significant group differences in the engaged strategy ( $\chi^2_6 = 23.37, p = .001$ ; Figure 2C). Participants that had ingested a placebo before stressor exposure used multi-cue strategies that are related to procedural learning (24,42) significantly more often and single-cue strategies associated with declarative learning (24,42) significantly less often compared with the other three groups (all  $p < .01$ ). After MR blockade, however, stressed participants showed the same preference for single-cue learning as the control groups; although, for about 50% of the stress-aMR group, no strategy could be identified due to the impaired performance of this group (Supplemental Methods and Materials in Supplement 1).

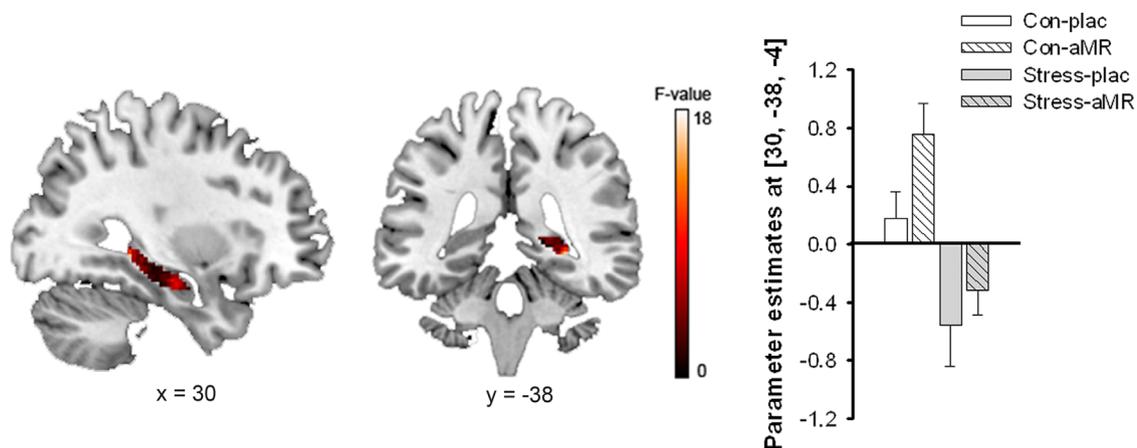
Performance in the visual-motor control task was, as expected, close to ceiling (mean percent correct: 94%) and comparable in the four experimental groups (all  $p > .16$ ). Men and women did not differ in their learning performance, the used strategy, or the explicit task knowledge, nor did participants’ sex modulate the influence of stress or aMR on these parameters (all  $F < 1.50$ , all  $p > .22$ ).

**Imaging Data**

In line with previous studies on the neural basis of PCL (24,30,32), our fMRI data showed that, compared with the control task, performance of the PCL task was associated with activation in a broad network of frontal, temporal, and parietal areas, including the hippocampus, the caudate nucleus, and the putamen (Table S2 in Supplement 1).



**Figure 2.** Performance in the probabilistic classification learning task. (A) Percent correct classification increased across training in all groups, yet the group that had received the mineralocorticoid receptor antagonist spironolactone (aMR) before stress was impaired relative to the other groups. (B) Stress decreased explicit knowledge of the probabilistic classification learning task, irrespective of whether participants had ingested a placebo or the aMR before. (C) Stress after placebo led to a relative shift from single-cue learning to more multi-cue learning. This stress effect on the engaged learning strategy was abolished in participants that received the aMR before the stressor. Error bars represent SEM. con-aMR, control/spironolactone; con-plac, control/placebo; stress-aMR, stress/spironolactone; stress-plac, stress/placebo.



**Figure 3.** Impact of stress on successful classification learning-related activation in the hippocampus. During correct probabilistic classification learning trials, the hippocampus was significantly less activated in participants that were exposed to the stressor before learning. Sagittal and coronal sections are shown, superimposed on a T1-template image. Shown is the activation of the hippocampus as a predefined region of interest. Right: parameter estimates of the peak voxel in the four groups. Error bars represent SEM. con-aMR, control/spironolactone; con-plac, control/placebo; stress-aMR, stress/spironolactone; stress-plac, stress/placebo.

To assess whether stress and/or MR blockade altered brain activation associated with successful PCL, we subjected activation in the contrast correct minus incorrect PCL to a full factorial model with the factors treatment (control vs. stress) and drug (placebo vs. aMR). This analysis showed no effect of drug and no treatment  $\times$  drug interaction but a significant main effect of treatment: as shown in Figure 3, stress reduced activation in the hippocampus ( $x = 30, y = -38, z = -4, Z = 3.74, p = .027$ , FWE corrected, 363 voxels). In a next step, we analyzed the contribution of the hippocampus and the dorsal striatum to successful PCL in the four groups. Therefore, we correlated activation in the contrast correct minus incorrect PCL with performance (expressed as percentage correct responses) in the PCL task. We obtained a positive correlation between hippocampal activation and classification performance both in control participants that had received a placebo ( $x = 20, y = -14, z = -18, Z = 3.95, p = .019$ , FWE corrected, 204 voxels) and in control participants that had received the aMR ( $x = 20, y = -18, z = -20, Z = 3.76, p = .032$ , FWE corrected, 71 voxels). In the stress-placebo group, however, PCL performance correlated positively with activation of the caudate nucleus ( $x = -18, y = 16, z = 10, Z = 3.45, p = .06$ , FWE corrected,  $p_{\text{uncorrected}} < .001$ , 200 voxels). Furthermore and in sharp contrast to the control groups, hippocampal activation was negatively correlated with PCL performance in the stress-placebo group ( $x = 26, y = -10, z = -26, Z = 3.85, p = .023$ , FWE corrected, 104 voxels; Figure 4A–C). Thus, stress after placebo reduced hippocampal activation and changed the hippocampal contribution to performance, although the exact location of these stress-related changes in hippocampal activation appears to be different (Figures 3 and 4). In stressed participants that were administered the aMR, there were no significant associations between brain activation and PCL performance (all  $p > .80$ ).

Based on the idea that the amygdala mediates stress effects on memory processes in other brain areas (3,43), we hypothesized that the amygdala may also play a part in the coordination of hippocampal and dorsal striatal learning after stress. To test this hypothesis, we performed a functional connectivity analysis that identified brain regions showing stronger coupling with the amygdala during successful PCL (for details, see Supplemental Methods and Materials in Supplement 1). Overall, this analysis revealed amygdala connectivity with both the hippocampus ( $x =$

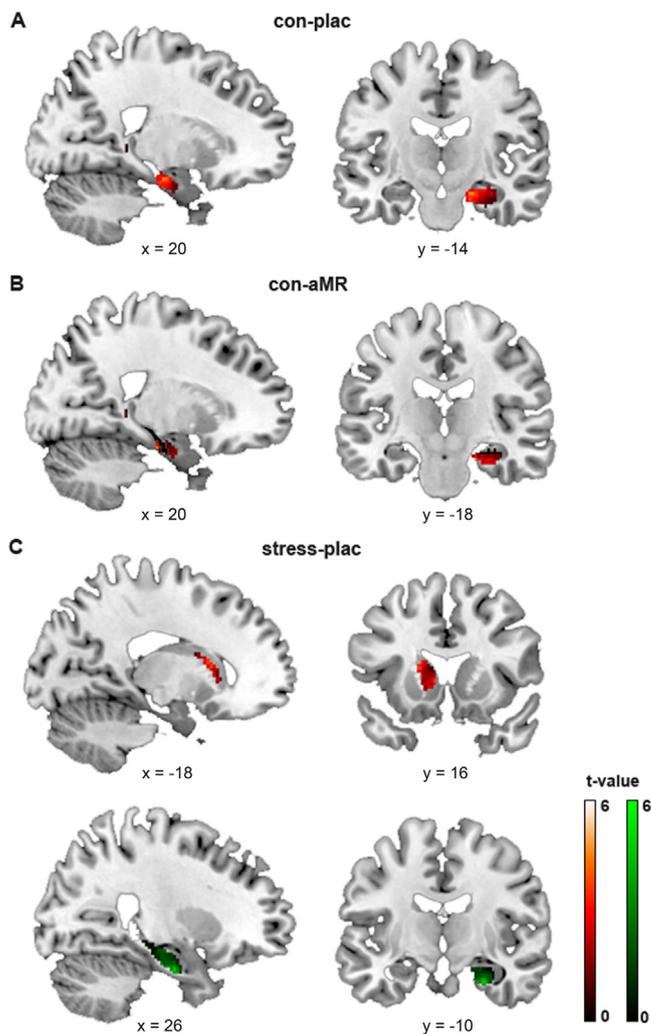
$24, y = -12, z = -18, Z = 3.64, p = .034$ , FWE corrected, 169 voxels) and the dorsal striatum (putamen:  $x = -28, y = -18, z = 8, Z = 3.52, p = .054$ , FWE corrected, 172 voxels). Most interestingly, stress (vs. control) after placebo decreased amygdala connectivity with the hippocampus ( $x = -24, y = -40, z = -2, Z = 3.65, p = .03$ , FWE corrected, 84 voxels) and increased amygdala connectivity with the dorsal striatum, in particular the putamen ( $x = -30, y = -2, z = -2, Z = 3.42, p = .08$ , FWE corrected,  $p_{\text{uncorrected}} < .001$ , 65 voxels; Figure 5). However, after MR blockade by spironolactone, stress did not alter amygdala coupling with the hippocampus and dorsal striatum compared with the referring control groups (all  $p > .70$ , FWE corrected; all  $p_{\text{uncorrected}} > .01$ ).

In addition to the reported activations in the predefined ROIs, we did not find group differences in the activation of the orbitofrontal cortex and the exploratory whole-brain analyses did not reveal any further significant activation. Moreover, we did not obtain any significant sex differences in the reported activations.

## Discussion

The present findings show for the first time in humans the critical role of the MR in the modulatory effect of stress on the engagement of multiple memory systems. Our results confirm previous studies suggesting a shift from hippocampus-dependent declarative to dorsal striatum-dependent procedural learning after stress (19,20,28). Stress (after placebo) reduced declarative task knowledge and increased the use of procedural multi-cue strategies. In addition, dorsal striatal activation correlated with PCL performance after stress, whereas hippocampal activation correlated with classification performance in control participants. Mineralocorticoid receptor blockade by spironolactone prevented the stress-induced shift toward dorsal striatal control of memory and stressed participants that were administered the aMR showed still a preference for declarative single-cue strategies. Mineralocorticoid receptor blockade alone, however, did not change the engagement of memory systems during classification learning.

Although the MR is relatively well characterized in rodents (44,45), there are only very few studies on the role of the MR in



**Figure 4.** Correlations between probabilistic classification learning performance and brain activation. **(A)** In the control-placebo group (con-plac) and **(B)** the control-spirolactone group (con-aMR), performance correlated positively with hippocampal activation. **(C)** In the stress-placebo group (stress-plac), however, caudate activation correlated positively and hippocampal activation correlated negatively with performance. Sagittal and coronal sections are shown, superimposed on a T1-template image. Shown are the activation of the hippocampus and the caudate nucleus as predefined regions of interest. Activations associated with positive correlations are shown in red/yellow; activations associated with negative correlations are shown in green.

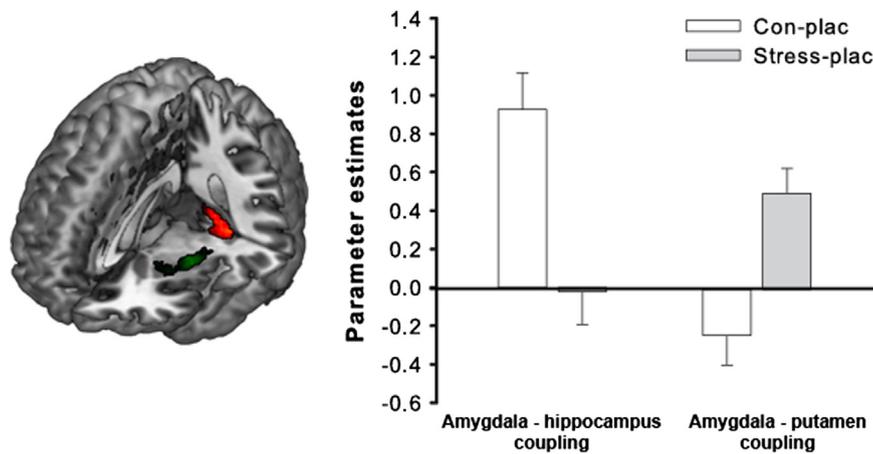
humans, in particular in relation to human cognition. Several studies demonstrated—same as the present study—reduced inhibitory control of the HPA axis after MR blockade (33,39,46). Furthermore, it has been shown that MR blockade impairs working memory and selective attention in humans (33,39). Such effects, however, cannot account for our results because MR blockade (without stress) did not affect PCL performance in the present study. This study is, to the best of our knowledge, the first to show that a stress effect on human memory (systems) can be prevented by MR blockade and is thus MR dependent. The specific involvement of the MR in the stress-induced modulation of hippocampal and dorsal striatal learning is in line with recent data in rodents (28).

Rodent studies suggest that the amygdala might mediate the impact of stress on the relative use of multiple memory systems. There is a large body of evidence indicating that stress hormone

effects converge in the amygdala, in particular in its basolateral part, which then modulates performance in other memory systems such as the hippocampus (3,47). More specifically, injections of anxiogenic drugs into the amygdala resulted in a shift from hippocampus-dependent to dorsal striatum-dependent memory in rats (21). Our functional connectivity data showed that stress reduced amygdala-hippocampus coupling and, at the same time, increased amygdala-putamen coupling, thus suggesting that the amygdala may operate as a switch between hippocampal and dorsal striatal memory systems in the human brain. Stress effects on single memory systems necessitate concurrent glucocorticoid and noradrenergic activity in the amygdala (43,48). In line with this idea, glucocorticoid elevations after MR blockade (in the control condition, i.e., without the SECPT-induced increase in sympathetic activation) did not reduce hippocampal activation or explicit task knowledge in the present study. However, whether noradrenergic arousal is, in addition to glucocorticoids, also required for the shift from hippocampal to dorsal striatal memory needs to be tested in future studies.

So how does stress modulate the engagement of hippocampal and dorsal striatal memory systems? Previous studies suggest that stress disrupts the hippocampal system, thus allowing the dorsal striatum to dominate behavior (20,24,28). The present data suggest a more complex picture that involves (at least) two distinct mechanisms that operate in tandem. First, stress impairs the hippocampus-dependent system, as reflected in reduced hippocampal activation and explicit task knowledge in the present study. This impairment, however, appears to be independent of the MR because MR blockade neither prevented the reduced hippocampal activation nor the impairment in task knowledge after stress, which is in line with recent data from MR forebrain knockout mice (49). Instead, these effects may be dependent on the GR, which has recently been shown to exert rapid, nongenomic actions in structures such as the hippocampus (9,50). Given the competitive interactions between the hippocampal and dorsal striatal memory systems that have been suggested in previous studies (32,51), impaired hippocampus-dependent learning may be one mechanism that facilitates striatal learning. Second, stress enhances amygdala connectivity with the dorsal striatum and disrupts amygdala connectivity with the hippocampus. Because the amygdala is known to facilitate memory processes in the hippocampus (52,53) and presumably also in the dorsal striatum (54), it is reasonable to assume that the observed changes in amygdala connectivity with the hippocampus and dorsal striatum further promote a shift from hippocampal to striatal control of learning. In contrast to the stress effect on the hippocampus, the changes in amygdala connectivity seem to be dependent on the MR. After MR blockade, stress did not alter the connectivity of the amygdala with the hippocampus and dorsal striatum. Because the basolateral part of the amygdala is critical for stress effects on memory (48), is involved in the modulation of multiple memory systems (21), and expresses membrane-bound MR that mediates rapid glucocorticoid effects (55), this area is a likely locus of the stress-induced shift from hippocampal to striatal systems. The finding that the aMR blocked both stress effects on amygdala connectivity and the shift toward dorsal striatal memory suggests that the proposed modulatory influence of the (basolateral) amygdala is necessary for stress effects on the engagement of multiple memory systems.

The shift from hippocampus-dependent to dorsal striatum-dependent memory after stress rescues learning performance (22,28). Corroborating earlier findings (24), the obtained negative correlation between hippocampal activation and performance in



**Figure 5.** Influence of stress on amygdala coupling with the hippocampus and dorsal striatum. Psychophysiological interaction analyses showed that stress after placebo intake reduced amygdala connectivity with the hippocampus (green) but increased amygdala connectivity with the putamen (red). Right: parameter estimates of the peak voxel in the hippocampus and putamen, respectively, for the groups that had received a placebo before the stress/control manipulation. Shown are the activation of the hippocampus and the putamen as predefined regions of interest. Error bars represent SEM. con-plac, control/placebo; stress-plac, stress/placebo.

stressed participants who had received a placebo suggests that attempts to recruit the declarative system after stress leads to impaired learning, whereas the engagement of striatum-based procedural learning in this group was generally paralleled by intact classification performance. If the use of dorsal striatum-based learning after stress is prevented by MR blockade (presumably in the amygdala), neither of the two memory systems is capable of controlling performance (as reflected in the lack of any brain behavior correlations in the stress-aMR group); individuals are forced to rely on the impaired hippocampus-dependent system, which results in impaired performance.

Even though the engagement of dorsal striatal memory can be beneficial for learning performance, it may contribute to psychiatric disorders, such as phobia, addiction, or posttraumatic stress disorder, that are characterized by altered stress responses and aberrant memory processes (56). For example, the overly strong trauma memory in PTSD is often seen as the result of an overconsolidation due to the action of stress hormones released during the traumatic event (5,57). In addition, however, the extreme stress during the traumatic experience might also result in the predominant engagement of the dorsal striatum-based memory system, which could explain the strong responses of PTSD patients to trauma-related cues, as well as their difficulties in integrating the traumatic event into their autobiographical memory (17,58).

Although the present data point, in line with previous data (28), to a critical involvement of the MR in stress effects on the engagement of multiple memory systems, the GR might also play a part in these effects. The higher glucocorticoid concentrations after MR blockade act primarily at GR, which are also critically involved in HPA axis regulation (59,60) and stress effects on cognition (10,61). Both HPA axis functioning and stress effects on cognition depend on balanced MR and GR activation (62). Future studies are required to further elucidate the role of the GR in the modulation of multiple memory systems.

At last, some potential limitations of this study should be noted. First, the sample size of this study was moderate and might have prevented the identification of potential gender differences, which were suggested recently in mice (63). Although studies combining pharmacologic manipulations with fMRI are costly, future studies should aim for larger sample sizes. Second,

we administered the same spironolactone dosage to all participants. Future studies are required to administer drug dosages in relation to body weight and to test for potential dose-dependent effects of spironolactone. Finally, we focused here on the contrast correct minus incorrect PCL because we were interested in the brain areas involved in successful learning. However, because participants most likely attempted to learn also during incorrect trials, this contrast rather neglected such unsuccessful learning attempts.

In summary, we examined here the neuroendocrine mechanisms involved in the stress-induced modulation of multiple memory systems in the human brain. Our findings suggest that stress impairs the hippocampal system, that the amygdala orchestrates the shift from hippocampal declarative to striatal procedural learning after stress, and that this shift is dependent on MR activation.

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# **Mineralocorticoid Receptor Blockade Prevents Stress-Induced Modulation of Multiple Memory Systems in the Human Brain**

## **Supplemental Information**

### **Supplemental Methods and Materials**

**Figure S1.** Probabilistic classification learning (PCL) task and control task.

**Table S1.** Subjective and blood pressure response to the stress and control manipulation.

**Table S2.** Significant brain activation during the PCL task compared to the control task.

### **Supplemental References**

## Supplemental Methods and Materials

### Experimental Procedure

In order to control for diurnal variations of cortisol, all testing took place between 1 pm and 6:30 pm. Upon arrival, participants gave a first saliva sample (see below) and a first blood pressure measurement was taken. Next, participants ingested a placebo pill or a spironolactone pill (300 mg; Ratiopharm), depending on the experimental group. After a 90-minute break during which participants were allowed to read, participants underwent the stress or control manipulation.

*Stress induction and physiological measurements.* In the stress condition, participants were exposed to the socially evaluated cold pressor test (SECPT) as described in detail elsewhere (1). Briefly, participants were asked to immerse their right hand up to and including the wrist for 3 minutes into ice water (0-2°C). During hand immersion, they were videotaped and monitored by a rather cold and non-reinforcing experimenter. Participants in the control condition submerged their right hand up to and including the wrist for 3 minutes into warm water (35-37°C); they were neither monitored by an unsociable experimenter nor videotaped. In order to assess the efficacy of the SECPT, we measured subjective and physiological stress responses at several time points across the experiment. Participants rated immediately after the stress/control manipulation on a scale from 0 (“not at all”) to 100 (“very”) how stressful, painful, and unpleasant they had experienced the previous treatment. In addition, we measured participants’ blood pressure after their arrival as well as before, during, and immediately after the stress/control manipulation. Moreover, participants collected saliva samples with the help of salivette collection devices (Sarstedt, Germany) after the arrival at the lab, immediately before and after the stress/control manipulation as well as immediately before and after the probabilistic

classification learning (PCL) task in the scanner. Saliva samples were stored at -20°C until analysis. From saliva we analyzed cortisol concentrations using an immunoassay (IBL; inter- and intraassay coefficients of variance < 10 percent).

*PCL and control task.* About 30 minutes after the stress/control manipulation and 120 minutes after pill intake, participants performed a PCL task known as the ‘weather prediction task’ (2, 3), in the scanner. Before the task started, participants were instructed that they would see different cards and that they should learn to predict the weather based on the presented cards. Between one and three (out of four) cards appeared on each trial, yielding 14 different cue patterns. These cue patterns were associated with two possible outcomes (sun and rain) in a probabilistic manner such that a particular cue was associated with the outcome ‘sun’ with a probability of 75.6, 57.5, 42.5, or 24.4 percent across 100 trials; these probabilities were same as in previous studies using this task (2-4). A response was counted as correct if it matched the outcome that was associated most strongly with the referring cue pattern. Participants completed 100 PCL trials. On each trial, they saw one of the 14 cue patterns and had 2.5 sec to respond ‘sun’ with a right button press or ‘rain’ with a left button press. After a short fixation period (1.5 to 6 sec), they received feedback about the actual weather by presenting the word ‘sun’ or ‘rain’ for 1.5 sec (see Figure S1). We varied the interval between response and feedback to exclude any potentially biasing effects of feedback expectation. The interval between feedback offset and the onset of the next trial varied between 8 and 12 sec.

In addition to the PCL task, participants performed a visual-motor control task, in which they were presented between one and three identical cards and asked to indicate by left or right button press whether  $< 2$  or  $\geq 2$  cards are shown. Same as in the PCL task, 14 different cue patterns were used, one pattern was presented per trial, participants had 2.5 sec to respond, after

a 1.5 to 6 sec fixation period the correct answer was presented ('Less than two' or 'Two or more') for 1.5 sec, and the next trial started 8 to 12 sec after feedback offset. Thus, the procedure of the control task was exactly the same as in the PCL task, except that participants had not to learn the probabilistic association between the cue patterns and outcomes. Participants completed also 100 trials of the control task. PCL and control trials were presented in random order.

*Explicit task knowledge test.* After the scanning session, participants completed a questionnaire containing 10 items that assessed explicit knowledge of the PCL task. For example, participants were asked how many cards were presented per trial or which card was most strongly associated with the outcome 'sun'. One point was given for each correct answer, i.e., participants could reach up to 10 points in the explicit knowledge test.

*Learning strategy analysis.* The used learning strategy was assessed with a mathematical model in which the actual responses of a participant across the whole PCL task were compared to ideal responses if a participant was reliably using a particular strategy. We constructed ideal data defined as the pattern of results that was expected across the 100 trials if a participant was reliably using a certain strategy. A least-means-squared estimate indicated the fit between the ideal data (for each strategy) and the participants' actual responses. This estimate resulted in a score between 0 and 1, with 0.0 indicating a perfect fit between the ideal data and participants' actual responses. Comparing across all strategies examined, the strategy associated with the lowest score was defined as the best-fit for that participant. If the best-fit score was higher than 0.1, participants' strategy was classified as 'non-identifiable' (5). For the sake of simplicity and in line with previous studies (6, 7), we divided the strategies that participants may use to solve the PCL task into 'single-cue' (use of single cues) and 'multi-cue' (use of multiple cues) strategies. A more detailed description of the strategy analysis can be found elsewhere (5, 8).

## **MRI Acquisition**

Imaging was performed with a 3 Tesla Philips Achieva scanner. For each participant, a high-resolution T1-weighted anatomical scan was acquired (slice thickness 1 mm; 220 sagittal slices). For functional imaging, 1620 T2-weighted echoplanar images (EPI) were acquired parallel to the AC-PC plane (30 slices; slice thickness 3 mm; repetition time (TR) = 2.0 sec; echo time (TE) = 30 ms; 64 × 64 matrix; 2 mm × 2 mm pixel size; 200 mm FOV). The first 3 images were discarded to allow T1 equilibration.

## **Data Analyses**

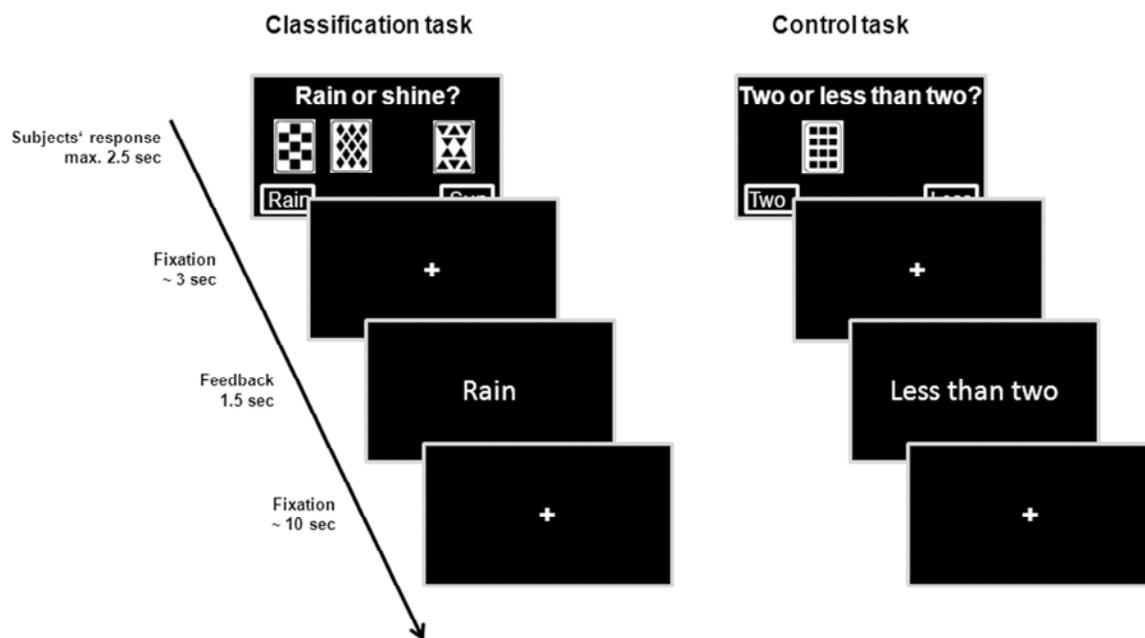
Physiological data were analyzed by treatment (control vs. stress) × drug (placebo vs. spironolactone) × time point of measurement analyses of variance (ANOVAs), classification performance by a treatment × drug × block ANOVA, explicit task knowledge by a treatment × drug ANOVA, and learning strategies by  $\chi^2$ -tests. Significant main effects were followed by least significant difference post-hoc tests if indicated. All analyses were performed with SPSS 20 (IBM); all reported *P*-values are two-tailed.

Preprocessing and analysis of the event-related fMRI data was performed using SPM8 (Wellcome Trust Center for Neuroimaging, University College London). Functional data were corrected for slice-timing and head motion. Structural images were segmented into gray matter, white matter, and cerebrospinal fluid. Gray matter images were normalized to the Montreal Neurological Institute template image. Normalized gray matter images were used for normalization of the structural and functional images. Finally, data were spatially smoothed using an 8 mm full-width half-maximum Gaussian kernel.

Correct and incorrect PCL trials as well as control trials were modeled using the canonical hemodynamic response function. Furthermore we included the button presses as well as the six movement regressors counting information about motion correction into our model. The data were filtered in the temporal domain using a nonlinear high-pass filter with a 128 s cut-off. Contrast images were generated for PCL trials minus control trials and for correct minus incorrect PCL trials. These difference contrasts were then entered into a second-level (group) analysis, treating subject as a random effect and using a full factorial model with the factors treatment (control vs. stress) and drug (placebo vs. spironolactone). In addition, on the second level, we also conducted whole brain correlation analyses (simple regression) for each group, in which we correlated the difference in brain activity between correct and incorrect PCL trials with classification performance (expressed as percent correct responses).

Finally, we performed a psychophysiological interaction (PPI) analysis in order to assess whether the coupling of the amygdala with the hippocampus and dorsal striatum was altered by stress and/or mineralocorticoid receptor blockade. Therefore, we extracted the deconvolved time series from a seed region in the amygdala (centered at 26, -8, -12; with a 6-mm radius) in the contrast PCL correct – PCL incorrect. This cluster was chosen because it was the region of the amygdala with the strongest activation during PCL (see Table S1) and, in addition, because this cluster has been identified as a locus of stress (hormone) effects before (9). The PPI was then computed as the element-by-element product of the blood oxygen level-dependent signal time course from this sphere and a vector coding for successful classification learning (i.e., the contrast PCL correct – PCL incorrect). For each subject, we created a new statistical model containing the PPI as regressor together with the physiological and the psychological vectors. Subjects' specific contrast images were then entered into random effects group analyses.

We used explorative whole brain analyses as well as region of interest (ROI) analyses. A priori ROIs were the hippocampus, the caudate nucleus, the putamen, and the orbitofrontal cortex as these structures were implicated in PCL in earlier studies (4, 7, 10). The referring masks were taken from the Harvard-Oxford subcortical and cortical atlases. For the explorative whole brain analyses, the significance threshold was set to  $P < 0.05$  on voxel-level, corrected for multiple testing (family-wise error (FWE) correction), and a minimum cluster size of 5 voxels. ROI analyses were performed using the small volume correction of SPM8 ( $P < 0.05$ , FWE corrected).



**Figure S1.** Probabilistic classification learning (PCL) task and control task. In the PCL task, participants were presented one to three cards per trial and asked to predict the weather ('rain' or 'sunshine') based on these cards. Feedback about the correct outcome was given after each trial. The control task had similar motor and perceptual characteristics as the PCL task but no learning demands; here, participants were asked to indicate if two or less than two cards were presented. Reproduced, with permission, from (7).

**Table S1. Subjective and blood pressure response to the stress and control manipulation.**

	Con-plac	Con-aMR	Stress-plac	Stress-aMR
Subjective ratings				
Stressfulness	1.1 ± 1.1	3.2 ± 2.2	63.7 ± 5.7**	50.6 ± 7.9**
Painfulness	0.5 ± 0.5	2.2 ± 2.2	69.5 ± 5.3**	71.7 ± 4.2**
Unpleasantness	1.6 ± 1.2	3.3 ± 2.4	72.1 ± 5.7**	58.9 ± 5.2**
Systolic blood pressure (mmHg)				
Baseline	122.7 ± 2.4	127.5 ± 3.3	118.8 ± 3.8	127.5 ± 3.7
Before SECPT/control manipulation	118.1 ± 2.7	118.2 ± 3.0	114.1 ± 4.1	121.8 ± 3.9
During SECPT/control manipulation	117.8 ± 2.6	118.0 ± 3.1	134.4 ± 4.4**	144.5 ± 3.6**
After SECPT/control manipulation	116.6 ± 2.6	115.5 ± 2.8	115.3 ± 3.8	122.6 ± 3.6
Diastolic blood pressure (mmHg)				
Baseline	69.0 ± 1.7	68.9 ± 1.4	68.0 ± 1.9	69.6 ± 2.4
Before SECPT/control manipulation	67.8 ± 1.8	64.7 ± 1.3	64.9 ± 2.0	69.2 ± 2.0
During SECPT/control manipulation	70.4 ± 1.5	65.2 ± 1.4	83.1 ± 3.2**	87.3 ± 2.6**
After SECPT/control manipulation	69.1 ± 1.8	65.6 ± 1.3	66.3 ± 2.0	71.0 ± 2.1

aMR, mineralocorticoid receptor antagonist; Con, control; plac, placebo; SECPT, socially evaluated cold pressor test.

Subjective ratings were given on a scale from 0 (“not at all”) to 100 (“very”). Data represent means ± SEM.

\*\*  $P < 0.005$ .

**Table S2. Significant brain activation during the PCL task compared to the control task.**

	Cluster	MNI coordinates (mm)			$F_{\max}$	$P_{\text{corr}}$
		x	y	z		
Superior frontal gyrus	1912	6	28	40	343	<0.001
R orbitofrontal cortex	3739	32	22	-2	276	<0.001
R middle frontal gyrus	944	44	22	26	129	<0.001
R caudate nucleus	373	10	6	0	127	<0.001
L caudate nucleus	575	-10	10	0	137	<0.001
L orbitofrontal cortex	663	-36	20	-4	241	<0.001
Frontal medial cortex	5061	8	52	-8	143	<0.001
R superior parietal lobe	4709	36	-54	44	220	<0.001
L superior parietal lobe	706	-36	-58	46	154	<0.001
R amygdala	148	26	-8	-12	70	<0.001
R hippocampus	72	28	-22	-18	54	<0.001
L hippocampus	11	-22	-14	-16	74	<0.001*
L putamen	151	-30	-8	-2	70	<0.001*
R putamen	50	16	8	-4	67	<0.001*

Corr, corrected; FWE, family-wise error; L, left; MNI, Montreal Neurological Institute; PCL, probabilistic classification learning; R, right; ROI, region of interest.

The significance threshold was set to  $P < 0.05$  (FWE corrected).

\*small volume corrected (ROI); all other activations were significant at the whole brain-level.

## Supplemental References

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