Corticosteroids Operate as a Switch between Memory Systems

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Abstract

■ Stress and corticosteroid hormones are known to affect learning and memory processes. In this study, we examined whether stress and corticosteroids are capable of facilitating the switch between multiple memory systems in mice. For this purpose, we designed a task that allowed measurement of nucleus caudate-based stimulus–response and hippocampus-based spatial learning strategies. Naive mice used spatial strategies to locate an exit hole on a circular hole board at a fixed location flagged by a proximal stimulus. When the mice were either stressed or administered corticosterone before the task, 30–50% of the mice switched to a stimulus–response strategy. This switch between learning strategies was accompanied by a rescue of performance, whereas performance declined in the stressed mice that kept using the spatial strategy. Pretreatment with a mineralocorticoid receptor antagonist prevented the switch toward the stimulus–response strategy but led to deterioration of hippocampus-dependent performance. These findings (i) show that corticosteroids promote the transition from spatial to stimulus–response memory systems, (ii) provide evidence that the mineralocorticoid receptor underlies this corticosteroid-mediated switch, and (iii) suggest that a stress-induced switch from hippocampus-based to nucleus caudate-based memory systems can rescue performance. ■

INTRODUCTION

Stress affects cognitive functions, and its effects on the strength of learning and memory are well documented (Lupien & Lepage, 2001; de Kloet, Oitzl, & Joels, 1999; de Quervain, Roozendaal, & McGaugh, 1998). Memory, however, is not a single faculty but is supported by multiple systems that process distinct information and differ in both their mode of operation as well as in the underlying neural networks (White & McDonald, 2002; Kim & Baxter, 2001; Cohen & Squire, 1980). Two memory systems in particular have received a lot of attention in recent literature: (i) a "cognitive" memory system that is based on the hippocampus and the adjacent cortices, processes the relation between multiple stimuli, and allows the flexible use of learned information (White & McDonald, 2002; Eichenbaum, 1992); and (ii) a rather rigid "habit" memory that is based on the caudate nucleus and uses single stimuli (Packard & Knowlton, 2002; Eichenbaum & Cohen, 2001; Mishkin & Petri, 1984). Although most studies focus on the effect of stress within one memory system, little is known on how stress influences the capacity to switch between different memory systems.

Recently, we found in humans that prior exposure to stress shifts the preferred learning strategy toward nucleus caudate-based stimulus–response (S-R) learning at the expense of hippocampus-based spatial learning (Schwabe et al., 2007). Similar shifts in learning strategies occurred in chronically stressed humans and mice (Schwabe, Dalm, Schachinger, & Oitzl, 2008). So far, only one study in rats that used strong aversive stressors provided evidence for stress-induced switching from hippocampal to striatal memory-based learning strategies (Kim, Lee, Han, & Packard, 2001). In this study, we test the hypothesis that corticosteroid hormones secreted from the adrenal cortex after stress and acting in the brain can facilitate the stress-induced transition between memory systems.

Corticosteroid hormones (mainly cortisol in humans, corticosterone in rodents) act via low-affinity glucocorticoid (GR) and high-affinity mineralocorticoid receptors (MR) in the brain. Although GR is widely distributed throughout the brain, MR is predominantly expressed in the hippocampus, the amygdala, and the pFC and at low levels in the nucleus caudate (de Kloet, Joels, & Holsboer, 2005). Although GR is generally not active in the acquisition phase of learning, it promotes the effect of corticosteroids on memory consolidation (Ferguson & Sapolsky, 2007; Kim et al., 2001; Lupien & McEwen, 1997). Conversely, MR with its high affinity for corticosterone is constitutively activated and involved in the initial appraisal of novel situations, that is, the acquisition phase of hippocampal-dependent cognitive tasks (Oitzl & de Kloet, 1992). When mice with forebrain ablation of MR ($MR^{CaMKCre}$; Berger et al., 2006) were subjected

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to standardized behavioral paradigms, rather subtle behavioral changes were reported. However, under stressful testing conditions, pharmacological and endocrine manipulations of MR activity resulted in altered reactivity patterns of spontaneous behavior (Smythe, Murphy, Timothy, & Costall, 1997; Korte, de Boer, de Kloet, & Bohus, 1995; Oitzl, Fluttert, & de Kloet, 1994; Sandi & Rose, 1994). Changes in learning strategies were not addressed.

Accordingly, in the present study, we assessed whether MR is involved in the corticosteroid-dependent shift from spatial to S-R learning strategies that occurs during the acquisition phase of the learning task. For this purpose, we designed a task for mice that allows to distinguish between (i) a spatial learning strategy that uses maplike knowledge structures and is supported by the hippocampus-dependent memory system (White & McDonald, 2002; Squire, 1992; O'Keefe & Nadel, 1978) and (ii) an S-R strategy using a single cue that relies on the caudate nucleus-dependent "habit" system (Packard & Knowlton, 2002; White & McDonald, 2002; Eichenbaum & Cohen, 2001; Mishkin & Petri, 1984). Importantly, the central characteristics of the task are comparable to other tasks that have been used to study spatial and S-R learning separately and for which there is convincing evidence that performance relies on the hippocampus and caudate nucleus, respectively (Lee, Duman, & Pittenger, 2008; Kim et al., 2001; Packard & Teather, 1998; Kesner, Bolland, & Dakis, 1993; Packard, Hirsh, & White, 1989). The task used here allows a more elaborate analysis because of the higher number of choice alternatives.

In the first experiment, untreated, restraint-stressed, corticosterone-injected, and vehicle-injected mice were subjected to a circular hole board (CHB) task that can be solved by spatial and S-R strategies ("conflict task"). Like in the task in the human study (Schwabe et al., 2007), the applied strategy can be determined in a test trial. Previous studies indicate that the hippocampus dominates learning in early stages of training (Chang & Gold, 2003; Packard & McGaugh, 1996). For example, a study that used the release of acetylcholine as a neurochemical marker of system activation showed that the hippocampus is activated before the striatum during training in a task in which there is a sequential shift from spatial to S-R learning (Chang & Gold, 2003). Moreover, there is evidence suggesting that nonstressed rodents prefer hippocampus-based learning, at least early during learning (Schwabe, Dalm, et al., 2008; Kim et al., 2001). Therefore, we hypothesized that untreated mice would mainly use spatial strategies, whereas stressed and corticosterone-injected mice would also use the S-R strategy. In two additional experiments, a selective MR antagonist was used to reveal the molecular mechanism underlying stress and corticosterone action. The MR antagonist was given either alone or in combination with corticosterone or stress. We expected that blockade of MR would prevent the shift toward more S-R learning.

METHODS

Animals

Male C57BL/6J mice (n = 145; 12 weeks old; purchased from Janvier, France) were single housed with free access to food and water. A 12:12-hr light–dark cycle (lights on at 0730 hr; 120 lx) was maintained, with all testing carried out between 0830 and 1230 hr. The mean body weight of the mice at the time of testing was 28.2 g (*SEM* = 0.17 g). Mice were housed in the testing room (same light–dark cycle; temperature = 21 ± 1°C) 1 week before behavioral experiments started. Each second day before training started, mice were weighed. After weighing, they were "pretrained" to climb through the tunnel. This tunnel was part of the test equipment (see Learning Task). All experiments were approved by the Local Committee for Animal Health, Ethics and Research at the University of Leiden. Animal care was conducted in accordance with the EC Council Directive of November 1986 (86/609/EEC).

Learning Task

Apparatus

The CHB is a revolvable white round plate (Plexiglas, 110 cm in diameter, situated 1 m above the floor) with 12 holes at equal distances from each other, 10 cm from the rim of the board (Figure 1A). Holes are 5 cm in diameter and can be closed by a lid at a depth of 5 cm. Whether a hole is open or not can only be recognized if the mouse puts its head over the edge of the hole. If open, the hole is the exit to the animals' home cage via an S-shaped tunnel (diameter = 5 cm; 15 cm long). As in landmark studies in the field (Winocur, Moscovitch, Fogel, Rosenbaum, & Sekeres, 2005; de Quervain et al., 1998), numerous cues in the room allow spatial orientation. The design of this task is based on the CHB developed by Carol Barnes (Barnes, 1979). Others have demonstrated the central role of the hippocampus in the acquisition of this maze (Poucet, Hermann, & Buhot, 1991; see also Parron, Poucet, & Save, 2001).

General Procedure

Each trial started by placing the mouse in a gray cylinder (Plexiglas; 25 cm high; 10 cm diameter), which was located at the center of the board. After 5 sec, the cylinder was lifted and the animal could explore the board and exit through the tunnel. If a mouse did not enter the exit hole within 120 sec, it was guided there by the experimenter along a grid (20 × 6 cm). The board was cleaned after each trial with 1% acetic acid solution and turned clockwise until another hole was at the location of the exit to avoid an influence of odor cues. The home cage was placed hereunder so that it could not be seen by the mouse on the board.

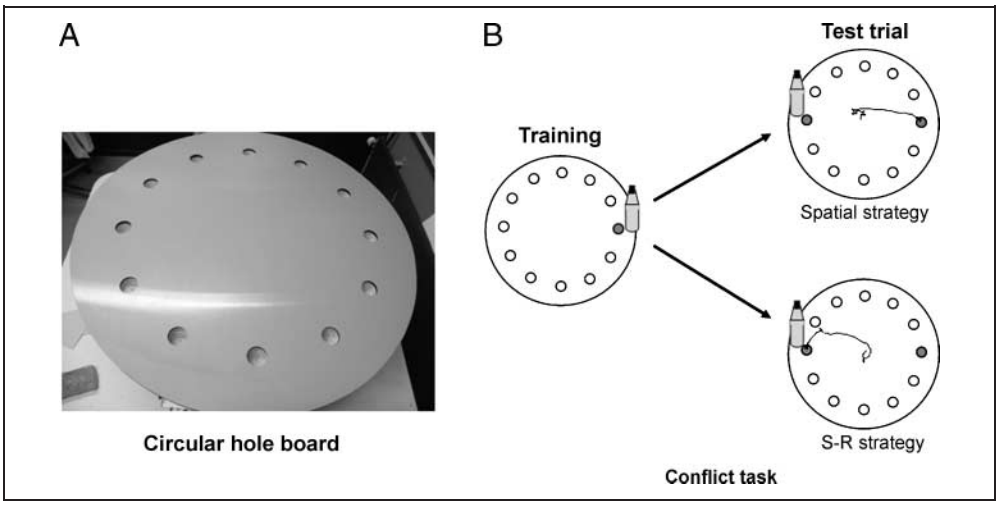

Figure 1. The CHB and the "conflict task." Mice were trained in six trials (intertrial interval = 15 min) to find the exit hole (marked gray in the drawings) that provided access to the home cage. (A) Picture of the CHB situated in a room providing several spatial cues. (B) The "conflict task" could be acquired by using the relation between multiple room cues (spatial strategy) or by using a single, proximal cue, the bottle (S-R strategy). Relocation of the bottle in the test trial revealed the used learning strategy. The lines in the schemes of the CHB are representative examples of walking patterns obtained in the present study.

Free Exploration Trial

One week before the first training trial, mice were placed on the CHB for 5 min. All holes were covered with a lid. There was a customary transparent 0.5-L bottle (22 cm high, 5 cm diameter) filled with water next to the hole that was opened at the end of this exploration trial. Then, the mouse was gently guided by the experimenter toward the exit hole using a grid (20 × 6 cm). This initial exploration trial served to estimate possible differences in mouse exploratory behavior before treatment. In retrospect, we did not find behavioral differences between the groups with respect to velocity, distance moved, and number of holes visited (all $F < 0.80$, all ps $> .50$).

Training in the "Conflict Task"

During six training trials, given in sequence on 1 day with an intertrial interval of 15 min, the position of the exit hole was fixed with respect to the distal extramaze cues in the room. Also, the proximal cue (the bottle) was placed next to this exit hole in all six training trials. Thus, the location of the exit hole could be acquired by using the relation between spatial cues and by association with a single cue, the bottle. Trial 7, which started 15 min after the last training trial, was used to detect the learning strategy (test trial). The hole in the training position remained open, but the bottle was relocated to another exit hole on the opposite part of the board. Leaving the board through the exit hole in the training position shows the use of a spatial strategy. Using the hole at the novel location, next to the bottle, reflects the use of an S-R strategy (Figure 1B). To control for possible odor cues in Trial 7, we divided the bedding of the home cage of the mouse over two cages, placed under both exit holes.

Blood Sampling and Hormone Assays

To determine corticosterone and ACTH concentrations at the time when behavioral testing starts, we decapitated 30 mice (untreated, vehicle injected, restraint stressed, corticosterone injected, and MR antagonist injected, respectively; 6 mice per group) 30 min after treatment. Until decapitation, these mice were treated like the other animals, that is, they were weighed every second day and received an exploration trial 1 week before decapitation. Blood obtained via decapitation was collected individually in capillaries (coated with potassium–EDTA, Sarstedt, Germany) and stored frozen at −20°C. Plasma corticosterone and ACTH concentrations were determined (in 10 and 100 μl plasma, respectively) using commercially available radioimmunoassay kits with ^{125}I-corticosterone and ^{125}I-ACTH (MP Biomedicals Inc. Europe, Illkirch, France; sensitivity 3 ng/ml and 10 pg/ml, respectively).

Analysis of Behavior

Behavior was recorded on videotape and analyzed by EthoVision 1.95 (Noldus Information and Technology BV, Wageningen, The Netherlands). This image analysis system sampled the position of the mouse 12.5 times per second. To calculate the distance walked, we chose a minimal distance between samples of 3 cm. For the purpose of a more detailed analysis, we subdivided the CHB into several subareas (center, rim, and four quadrants). In particular, we were interested in mouse preference for the quadrants that contained either the bottle and exit in the novel position ("S-R quadrant") or the exit during training ("spatial quadrant"; the remaining two quadrants were combined and referred to as "control quadrants"). The following parameters were measured for all trials: latency to exit hole (sec), velocity (cm/sec), distance moved (cm), and time in the different subareas (in seconds and as percentage

of total time on the CHB) as well as the holes visited (a hole visit was counted, when the animal at least put its nose in the hole). Latency measures were cross-checked with manual protocols.

Statistical Analysis

Statistical analyses included chi-square and *t* tests, one-way or mixed-design ANOVAs, followed by post hoc Tukey tests to correct for Type I error accumulation. Reported *p* values are two-tailed. $p < .05$ was accepted as statistically significant. Statistical calculations were done with SPSS software (version 15.0; SPSS Inc., Chicago, IL).

Experiment 1: Switch of Learning Strategies

Mice were randomly assigned to one of four treatment groups: untreated (taken from the home cage at the beginning of the task), vehicle injection stressed, restraint stressed, and corticosterone. Stress or corticosterone were administered 30 min before the first training trial in the conflict task ($n = 12$ mice/group).

Drugs

Corticosterone (250 μg/kg body weight in 200 μl/25 g body weight HBC complex dissolved in physiological saline) and vehicle were administered subcutaneously. Solutions were freshly prepared the day before injection. After the injection, animals were placed back in their home cage.

Restraint

Mice were immobilized for 10 min in a cylinder (transparent Plexiglas; diameter = 2.5 cm, 8 cm long) in a room adjacent to the testing room. After immobilization, mice returned to their home cage in the testing room for 20 min.

Experiment 2: Molecular Mechanism Underlying the Stress-induced Switch of Strategy

To unravel the mechanism underlying the effect of stress and corticosterone on spatial and S-R learning and memory, we injected mice with an MR antagonist before training in the conflict task. Mice were randomly assigned to one of three treatment groups: untreated ($n = 6$), vehicle injection stressed ($n = 6$, 200 μl saline/25 g body weight), and MR antagonist ($n = 8$; RU 28318; Biotrend, Cologne, Germany; 50 mg/kg of body weight dissolved in physiological saline). The dose of RU28318 for peripheral treatment was chosen according to Spencer, Kim, Kalman, and Cole (1998). Injections were given subcutaneously. Solutions were prepared on the day before the injection, stored at −80°C, and defrosted 45 min before injection. After injection of the compound, mice returned to their home cage and training started 45 min later. The procedure of the conflict task, the behavioral analysis, the blood sampling, and the hormone assays were the same as described above.

In the next experiment, mice received the MR antagonist and vehicle via oats to prevent the stress of the injection procedure (Dalm, Brinks, van der Mark, de Kloet, & Oitzl, 2008). Due to the high affinity of the MR for corticosterone, MR is almost continuously occupied. To allow blockade of the MR by the competitive MR antagonist, we administered the compound 30 min before restraint or corticosterone injection. Training started 30 min later. Mice were randomly assigned to four groups and tested in the conflict task: vehicle ($n = 8$), MR antagonist ($n = 13$), MR antagonist + restraint stressed ($n = 13$), and MR antagonist + corticosterone injection ($n = 13$).

Drugs

The MR antagonist RU 28318 (Biotrend) was dissolved in physiological saline and pipetted on two flakes of oats that were placed in a well. Consumption of the oats corresponds to an RU28318 dose of 50 mg/kg of body weight (for a detailed description of the method, see Dalm et al., 2008). When offered to the mouse, the oats are consumed within 5 min. Another group of mice received the vehicle-treated oats as control. Dose, preparation, and injection of corticosterone and method of restraint were done as described in Experiment 1.

RESULTS

Experiment 1: Stress, Corticosteroids, and the Switch from Spatial to S-R Learning

Mice were either untreated, restraint stressed for 10 min, or injected with vehicle or corticosterone 30 min before training in the CHB task. It is important to note that vehicle injection is a stressor itself leading to increased corticosterone secretion (Dalm et al., 2008; see also Table 1).

Groups differed significantly regarding the used strategy in the test trial (Figure 2A). All untreated mice used the spatial strategy, whereas 58% of the vehicle injection-stressed group and 67% of both the restraint-stressed and the corticosterone group used the spatial strategy (vs. untreated), vehicle injection stressed, $\chi^2(1) = 6.32$, $p = .01$, restraint stressed and corticosterone, both $\chi^2(1) = 3.77$, $p < .05$. The percentage of mice switching toward the S-R strategy is comparable to reports in other rodent studies after severe or chronic stress (Schwabe, Dalm, et al., 2008; Kim et al., 2001). Importantly, mice classified as spatial and S-R learners differed significantly in their preference for the four quadrants of the CHB in the test trial, Quadrants × Strategy interaction, $F(3,126) = 15.80$, $p < .001$. Although S-R learners preferred the quadrant containing the bottle and exit in the novel position, spatial learners showed a preference for the quadrant and

Table 1. ACTH and Corticosterone (CORT) Concentrations at the Time When Behavioral Testing Started (n = 6 per Group)

	Untreated	*Vehicle Injection*	*Corticosterone*	*Restraint Stress*	*aMR*
ACTH (pg/ml)	48.2 ± 11.3	25.0 ± 6.7	71.4 ± 23.3	46.0 ± 6.8	127.7 ± 26.1*
CORT (ng/ml)	4.5 ± 0.7**	66.4 ± 3.62***	197.4 ± 16.7**	95.5 ± 19.72***	128.6 ± 24.8***

aMR = mineralocorticoid receptor antagonist. Data represent means ± *SEM*.

*$p < .05$ versus untreated, vehicle injection-stressed, and restraint-stressed mice.

**$p < .05$ versus all other groups.

***$p < .05$ versus untreated and corticosterone-injected mice.

exit of the training trials (Figure 3). This clearly indicates that mice were not leaving the CHB through a randomly chosen hole but used different strategies, either based on distal or spatial cues or on a single proximal cue.

Besides strategies, also quantitative performance parameters were affected by stress and corticosterone. A mixed-design ANOVA over the latencies to the exit hole of the six training trials revealed significant trial and group effects, Trial, $F(5,220) = 15.02$, $p < .001$; Group, $F(3,44) = 7.15$, $p < .001$. Although all groups improved with training, corticosterone-treated and restraint-stressed mice showed longer latencies than untreated (vs. corticosterone: $p = .01$; vs. restraint: $p < .001$) and vehicle injection-stressed mice (vs. corticosterone: $p = .11$; vs. restraint: $p = .02$). Performance differed most strongly in Trial 1, $F(2,33) = 4.63$, $p = .007$, and the test trial, $F(3,44) = 4.20$, $p = .01$ (Figure 2B). Group differences in latencies were paralleled by distance moved, $F(3,44) = 3.73$, $p = .02$, and number of holes visited, $F(3,44) = 5.48$, $p < .01$. Furthermore, corticosterone-injected mice moved slower than untreated and vehicle injection-stressed mice, $F(3,44) = 3.03$, $p = .04$ (untreated/vehicle injection stressed vs. corticosterone: both ps $< .05$). This difference was most pronounced in the last three training trials suggesting effects of learning, $F(3,44) = 4.88$, $p < .01$ (untreated/vehicle injection stressed vs. corticosterone: both $p < .01$). Within the group of spatial learners, corticosterone-injected mice had overall longer latencies than untreated and vehicle injection-stressed mice, $F(3,29) = 4.76$, $p < .01$ (corticosterone vs. untreated: $p < .006$; corticosterone vs. vehicle injection stressed: $p = .05$).

Experiment 2: The MR Is Involved in the Shift toward S-R Learning Strategies

In Experiment 1, the shift toward S-R strategies was most pronounced in vehicle injection-stressed mice. To identify MR-mediated corticosterone action as the mechanism operating this switch between memory systems, we used an MR antagonist in the injection-stressed procedure. Replicating our results of Experiment 1, all untreated mice used the spatial strategy, whereas 50% of the vehicle injection-stressed mice used the S-R strategy, $\chi^2(1) = 4.00$, $p < .05$ (n = 6 per group). The learning strategy of mice injected with the MR antagonist did not differ significantly from untreated mice (spatial = 88%; n = 8), $\chi^2(1) = 0.92$, $p = .34$ (Figure 4A).

All but the MR antagonist-treated mice showed decreasing latencies over trials, Trial, $F(5,85) = 4.74$, $p = .01$,

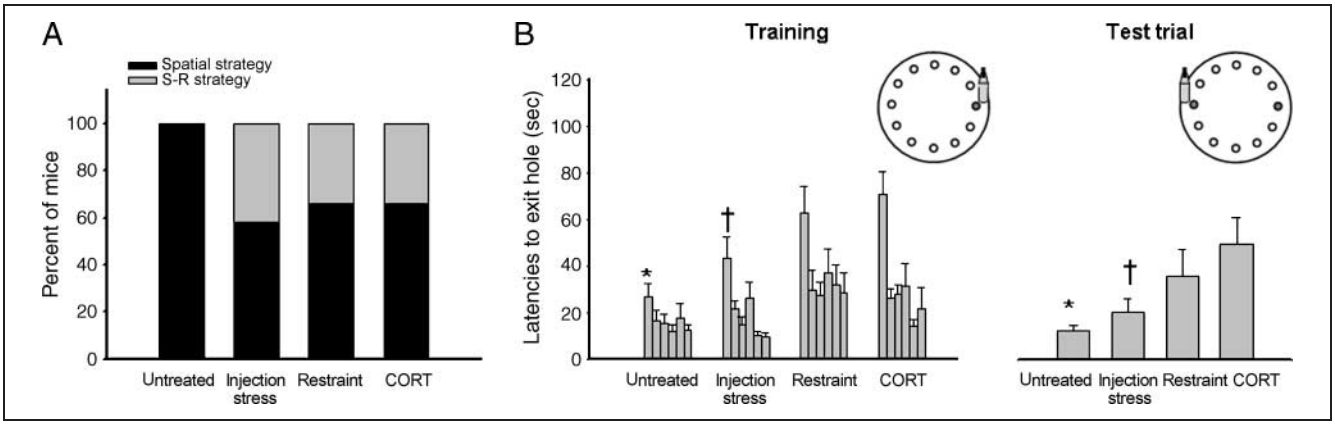

Figure 2. (A) Treatment-dependent switch from spatial to S-R learning strategies. The injection of vehicle (injection stress) and corticosterone (CORT) as well as restraint stress before training in the CHB task changes learning strategies toward more S-R learning. (B) In addition to the switch in learning strategies, injection-stress, restraint, and CORT-impaired quantitative learning performance are expressed as latencies to the exit hole. Insert: scheme of the CHB; gray circle: location of the exit hole; bottle: location of the proximal stimulus. *$p < .05$ versus restraint-stressed and corticosterone-injected mice; †$p < .05$ versus corticosterone-injected mice. Bars represent mean ± *SEM*.

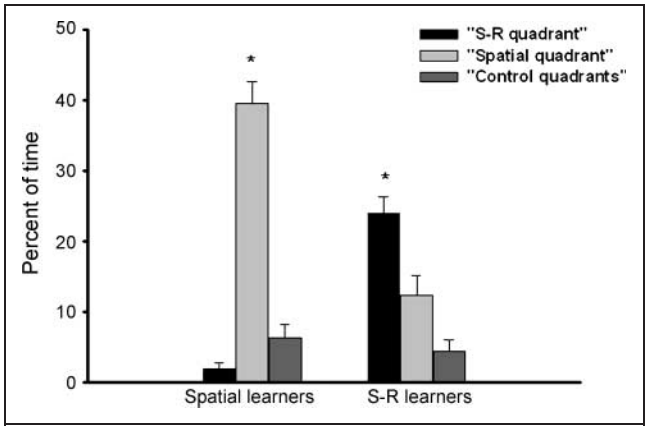

Figure 3. Percent of time mice classified as spatial or S-R learners spent in the quadrant containing the exit and the bottle in the novel position ("S-R quadrant"), the exit hole that was open during training ("spatial quadrant"), or in the two other quadrants (combined to "control quadrants") in the test trial. Please note that the sum of the percentages is below 100% because the time in the center of the CHB (about 50% in both groups) is not included. *$p < .05$ versus the other quadrants. Bars represent mean ± *SEM*

Treatment, $F(2,17) = 3.47$, $p = .05$ (Figure 4B), and decreasing numbers of hole visits, Trial, $F(5,85) = 2.48$, $p = .04$, Treatment, $F(2,17) = 2.89$, $p = .07$. Untreated mice had the shortest latencies and visited fewer holes than MR antagonist-treated mice in the test trial (both $p = .03$). Velocity and distance moved were comparable between groups.

Stress-free Oral Application of the MR Antagonist to Block the Effect of Stress and Corticosterone on the Switch of Learning Strategies

To dissect the action of the MR antagonist from the injection-associated stress, we used a nonstressful method (Dalm et al., 2008) and fed the mice oats containing the MR antagonist (or the vehicle) before restraint or corticosterone injection, followed by training in the CHB task: vehicle controls ($n = 8$), MR antagonist ($n = 13$), MR antagonist + restraint stressed ($n = 13$), and MR antagonist + corticosterone injection ($n = 13$). Again, control mice used the spatial strategy, just like the mice that received vehicle via oats (Figure 5A). Not a single mouse of the MR antagonist-treated groups switched strategy: the restraint and the corticosterone-induced switch between learning strategies was eliminated. Additionally, the MR antagonist had a significant impairing impact on performance, expressed by significantly longer latencies, $F(3,43) = 5.84$, $p = .002$ (Figure 5B), longer distances, $F(3,43) = 2.86$, $p < .05$, and the tendency to visit more holes, $F(3,43) = 2.34$, $p = .08$, during training trials than controls. Also in the test trial, MR antagonist-treated mice had a significantly lower performance compared with controls: latency, $F(3,43) = 3.07$, $p < .04$; holes visited, $F(3,43) = 3.05$, $p < .04$; distance moved, $F(3,43) = 1.49$, *ns*. Vehicle-treated mice moved significantly slower than mice of the three MR antagonist-treated groups, $F(3,24) = 10.41$, $p < .001$.

Switching to S-R Strategy Prevents Deterioration of Performance in Vehicle Injection-stressed Mice

Comparing the performance of all vehicle injection-stressed mice that were classified as using S-R ($n = 8$) and spatial strategies ($n = 10$) revealed that mice that used the S-R strategy performed significantly better: holes visited, mean ± *SEM*, 2.1 ± 0.4 versus 3.1 ± 0.3, $F(1,16) = 4.49$, $p < .05$; latencies, mean ± *SEM*, 17.7 ± 3.2 versus 29.8 ± 3.0 sec, $F(1,16) = 7.40$, $p < .02$ (Figure 6). The performance of vehicle injection-stressed mice using the S-R strategy was comparable to untreated mice using the spatial strategy. Vehicle injection-stressed mice that did not switch their strategy had longer latencies, $F(1,26) = 4.94$, $p < .04$,

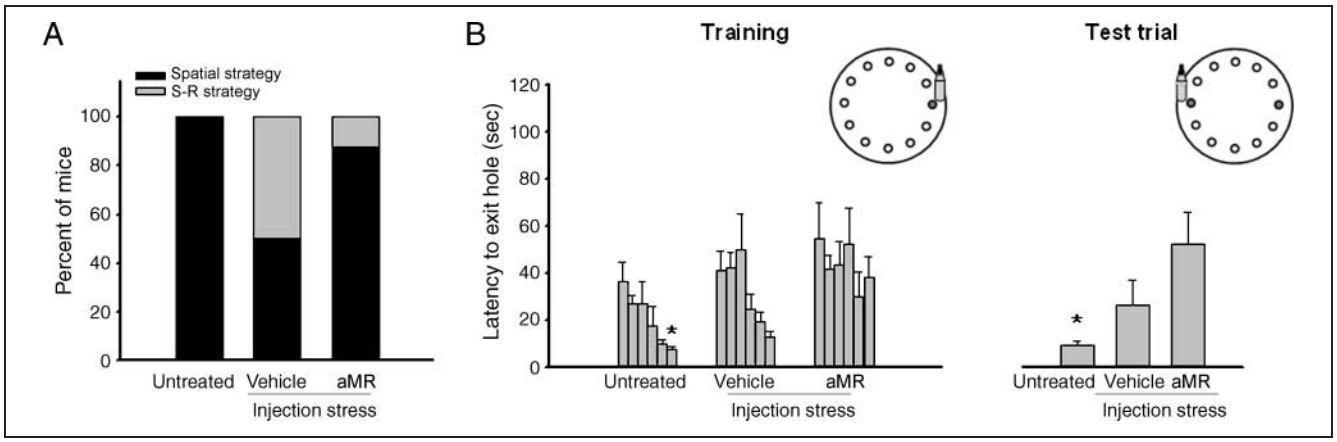

Figure 4. (A) The injection stress-induced facilitation of S-R learning strategies is blocked by the MR antagonist RU28318. (B) Latency to exit hole of (A) untreated ($n = 6$), injection-stressed ($n = 6$), and MR antagonist-injected (aMR, $n = 8$) mice. Decreasing latencies were observed in all but the mice that received aMR. Insert: scheme of the CHB; gray circle: location of the exit hole; bottle: location of the proximal stimulus. *$p < .05$ vs. aMR injected mice. Bars represent mean ± *SEM*.

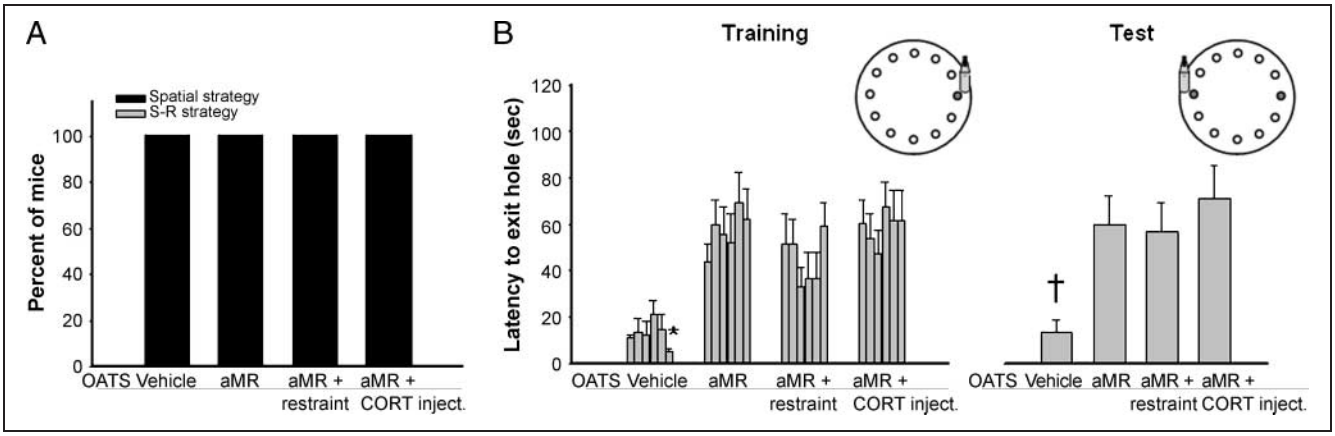

Figure 5. Stress-free application of the mineralocorticoid receptor antagonist (aMR) via oats to prevent injection stress. (A) The MR antagonist eliminates the restraint and the corticosterone injection-induced change of learning strategies. (B) Mice receiving vehicle on oats performed significantly better than all groups treated with the MR antagonist: aMR alone or aMR in combination with restraint or corticosterone injection (CORT). Insert: scheme of the CHB; gray circle: location of the exit hole; bottle: location of the proximal stimulus. $^{*}p < .05$ versus all other groups; $^{\dagger}p < .05$ versus aMR and aMR + CORT mice. Bars represent mean ± *SEM*.

and visited more holes, $F(1,26) = 4.78$, $p < .04$, than untreated mice that all used spatial strategies.

Treatment-dependent Modulation of ACTH and Corticosterone Concentrations at the Start of Behavioral Testing

The endocrine markers were measured in blood plasma at the same time that the training in the CHB task had started in the other experiments (separate groups of mice: untreated, vehicle injection stressed, restraint stressed, corticosterone, and MR antagonist injection; $n = 6$ per group). They indicate the activation of the hypothalamus–pituitary–adrenal axis and corticosterone affinity-dependent activation of MR and/or GR influencing the consecutive performance of the mice. The MR antagonist-treated mice showed significantly elevated ACTH concentrations at the start of testing (Table 1), proving that the MR antagonist targets the brain MR (van Haarst, Oitzl, & de Kloet, 1997). Corticosterone was lowest in untreated controls and highest in corticosterone-injected mice: untreated < vehicle injection stressed = restraint stressed = MR antagonist < corticosterone (Table 1).

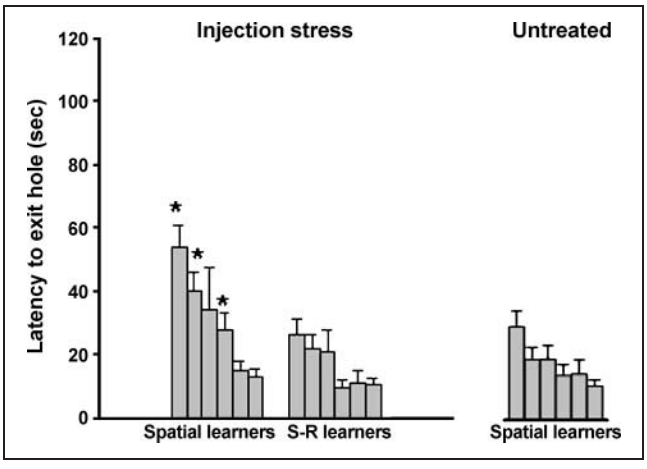

Figure 6. Switching to the S-R strategy rescued performance in injection-stressed mice. Their latencies to the exit hole were comparable to untreated mice. Performance was impaired in mice that did not switch strategies. Bars represent mean ± *SEM*. $^{*}p < .05$ versus untreated mice and injection-stressed mice that used the S-R strategy.

DISCUSSION

Our findings demonstrate that stressors and corticosterone can operate as a switch between memory systems in mice. All untreated mice used the spatial learning strategy, whereas 30–50% of the mice with elevated corticosterone levels either due to stress (vehicle-injection or restraint) or corticosterone injection displayed a shift toward S-R learning. This switch between learning strategies was accompanied by a rescue of performance. The role of corticosterone in mediating this stress effect on the use of learning strategies was revealed by pharmacological blockade of MR. When MR antagonist treatment was combined with injection stress, corticosterone, or restraint, mice no longer displayed a switch between strategies. In parallel, performance was impaired. Collectively, these experiments show that a stressor is necessary to operate the switch from one memory system to another, which is in line with our findings in humans (Schwabe et al., 2007). Furthermore, we have demonstrated that this stressor effect is mediated by the action of corticosterone via MR.

A theoretical framework for the present results can be found in the cue-utilization hypothesis proposed by Easterbrook (1959) in the middle of the past century. In this hypothesis, emotional arousal is postulated to consistently

act to reduce the range of cues an organism uses. This reduction in the range of cues "influences action in ways that are either organizing or disorganizing, depending on the behavior concerned" (Easterbrook, 1959). Here, we show that stress leads to a shift in learning strategies, from one that relies on the use of multiple cues to one that is based on a single stimulus. Interestingly, this shift in strategies or used memory systems may be paralleled by a decrease in the flexibility of thought. Indeed, it has been suggested that the flexibility in the use of knowledge is the key difference between hippocampus-dependent (e.g., spatial) and hippocampus-independent (e.g., S-R) learning and memory (Eichenbaum & Cohen, 2001; Eichenbaum, 1992). In line with this, very recent evidence indicates that the individuals' flexibility in thinking is reduced under stress (Liston, McEwen, & Casey, 2009). Our findings might imply that corticosteroids affect cognitive flexibility and the range of cues that are registered via a mechanism governed by the brain MR.

Behavioral strategies are one way of coping with stress, of relevance is also the actual performance. The majority of the human literature reports impairments of performance in memory tasks in response to prior stressors and corticosteroids (Schwabe, Bohringer, Chatterjee, & Schachinger, 2008; Payne et al., 2006, 2007; Buss, Wolf, Witt, & Hellhammer, 2004; Newcomer, Craft, Hershey, Askins, & Bardgett, 1994). These studies mostly describe a decline in performance within the hippocampus-dependent memory system, such as a reduction in the number of words or slides that are remembered in a stress or corticosteroid-treated group relative to a control group. The longer latencies and distances, the more errors in stressed or corticosterone-injected mice in the CHB task demonstrate a comparable decline in performance. The impairment of stressed and corticosterone-treated mice was most pronounced in the first training trial. Given that during training the exit hole was in the same position as in the exploration trial presented 1 week before training, this initial impairment in stressed, and corticosterone-treated mice might reflect impaired spatial memory retrieval (de Quervain et al., 1998) or an unsuccessful attempt at spatial learning. The latter alternative is related to the interesting question whether the switch from spatial to S-R learning happens automatically in the presence of stress or whether this switch is expressed more slowly via learning. Unfortunately, this question cannot be directly answered with the present task setup because this does not allow disentangling of the strategies during training (note that during training both strategies are directed at the only exit hole, located in the fixed spatial position and flagged by the bottle). Future studies could use neurochemical markers such as the release of acetylcholine (Chang & Gold, 2003) to assess the contribution of multiple memory systems across learning.

What we could show here is the intriguing relationship between strategy and performance in the group of injection-stressed mice. Switching to the S-R strategy allowed rescue of performance (Figure 6), whereas being stressed without switching the learning strategy resulted in deterioration of performance. Although the switch between memory systems is adaptive with respect to current performance, it is uncertain whether the long-term consequences of this strategy shift are favorable. As S-R "habit" learning is independent of conscious reflection and thus enables fast responses, it is also characterized by rigidity that might hamper behavioral adaptation to changing environmental demands.

Although most learning tasks allow the use of multiple strategies, it is difficult to discriminate between them, and generally the focus is only on a single strategy. Uniquely, here we could assess the use of spatial and S-R learning strategies in the same task and found that use of an S-R learning strategy only was not influenced by prior exposure to stress and/or corticosterone. In the conflict task, spatial strategy and optimal performance coincided in untreated mice. Stressed, corticosterone, and MR antagonist-treated mice that did not switch to S-R strategies remained to use the spatial strategy; however, their performance was impaired. These mice were neither efficient in processing the distal nor the proximal cues, which might be indicative of a decline of function within the hippocampus as well as of coordinated brain functions in general.

Considering the surprisingly poor performance of mice treated with the MR antagonist, one might question the view that these mice were using a spatial strategy. The active movement pattern of the MR antagonist-treated mice included more hole visits; velocity was either comparable or higher than in control mice, thereby excluding a deficit in motor abilities or sedative effects of the drug. Indeed, a more extensive exploration pattern (Smythe et al., 1997; Oitzl et al., 1994; Sandi & Rose, 1994) might interfere with the proper execution of a learning strategy. It could also be argued that in the presence of such a profound performance deficit, animals did not use any particular strategy and behavior was more or less random. According to this interpretation, a roughly 50:50% performance would be expected with half of the mice using the exit next to the novel position of the bottle and the other half the exit in the old position. This, however, was clearly not the case. Out of 47 mice that received the MR antagonist, there was only one mouse leaving the CHB via the hole next to the novel position of the bottle; all other mice escaped via the exit that had been open during training, which we interpreted as the use of a spatial strategy, although performance is deteriorated. This clearly argues against the possibility that MR antagonist-treated mice chose the exit hole at random (note that the bedding of the home cage was distributed over the two cages and placed under the two exits, thus ruling out an influence of odor cues). In contrast, it supports the view that these mice used a spatial strategy. In line with previous findings, our data show that the use of a spatial strategy is impaired in the face of high corticosteroid concentrations that activate GR (Diamond, Park, Heman, &

Rose, 1999; McEwen, 1999). A shift toward an S-R strategy might have rescued performance; this, however, was prevented by MR blockade.

Besides the hippocampus and the caudate nucleus, the amygdala has been assigned a critical role in acute stress effects on memory functions (Roozendaal, Okuda, Van der Zee, & McGaugh, 2006; Kim et al., 2001) and in the "emotional" modulation of spatial and S-R learning (Packard & Wingard, 2004). Intra-amygdala infusions of anxiogenic drugs were sufficient to shift learning strategies from predominantly spatial to more S-R learning in rats. Indeed, we cannot preclude a role of the amygdala and also of other brain areas like pFC in the modulation of spatial and S-R strategies and performance.

MR is known as a nuclear receptor acting as a transcription factor (de Kloet et al., 1999, 2005). However, recently, evidence has been obtained for nongenomic actions mediated by MR in response to high corticosterone (Joels, Karst, DeRijk, & de Kloet, 2008; Karst et al., 2005). Hence, it cannot be excluded that these nongenomic MR-mediated actions are involved in the fast MR-mediated effects in the acquisition phase. It would be highly interesting to develop a method that allows genomic and nongenomic MR-mediated effects to be dissected at the behavioral level.

Another challenging question derives from the fact that a certain percentage of the tested population of mice (and man; Schwabe et al., 2007) is resistant or vulnerable to the effects of stress and corticosterone. In humans, MR polymorphism is known to alter the endocrine stress response (DeRijk et al., 2006), whereas behavioral consequences are not known. In rodents, we might also test the contribution of an epigenetic predisposition due to experiencing discrete early life events like maternal care (Champagne et al., 2008; Meaney, Szyf, & Seckl, 2007). Assessing the degree of emotionality, which is known to modulate cognitive performance (Pessoa, 2008), could also contribute to the understanding of a resistant or vulnerable phenotype.

Although we view the observed changes in test trial behavior as a result of a stress (hormone)-induced transition from hippocampus-based spatial to more caudate-based S-R learning, there are of course other mechanisms that might have contributed to our findings. For example, stress and corticosteroids might have facilitated the overshadowing of the distal (room) cues by the salient proximal cue (the bottle). Future studies should explicitly address these alternative explanations.

In summary, we have obtained evidence that corticosterone acting via MR is responsible for the stress-induced switch from the hippocampal spatial to the nucleus caudate S-R memory system. If this switch does not take place, performance deteriorates. That corticosterone can facilitate the switch from hippocampal to caudate memory systems is an integral part of the amazing plasticity and resilience in cognitive operations aimed to facilitate behavioral adaptation to stress.

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