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# Glucocorticoids boost stimulus-response memory formation in humans

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**Summary** Stress affects memory beyond hippocampus-dependent spatial or episodic memory processes. In particular, stress may influence also striatum-dependent stimulus-response (S-R) memory processes. Rodent studies point to an important role of glucocorticoids in the modulation of S-R memory. However, whether glucocorticoids influence S-R memory processes in humans is still unknown. Therefore, we examined in the current experiment the impact of glucocorticoids on the formation of S-R memories in humans. For this purpose, healthy men and women received either hydrocortisone or a placebo 45 min before completing an S-R association learning task and an S-R navigation task. In addition, participants performed also a virtual spatial navigation task and a spatial navigation task in a real environment. Memory of all four learning tasks was tested one week later. Our data showed that hydrocortisone before learning enhanced memory of the S-R association learning task. Moreover, hydrocortisone enhanced the memory of the virtual spatial navigation task, mainly in women. Memory performance in the other tasks remained unaffected by hydrocortisone. These findings provide first evidence that glucocorticoids may facilitate S-R memory formation processes in humans.


## 1. Introduction

Stress can influence hippocampus-dependent learning and memory processes (Roozendaal et al., 2009; Schwabe et al., 2012a). The direction of these stress effects depends critically on the timing of the stress exposure, i.e., on whether stress is experienced shortly before learning, before consolidation, or before memory retrieval (Schwabe et al., 2012a).

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Stress shortly after learning enhances memory consolidation (Cahill et al., 2003; Roozendaal et al., 2009), whereas stress before retention testing appears to impair memory retrieval (De Quervain et al., 1998; Kuhlmann et al., 2005; Buchanan et al., 2006). The effects of stress before learning, however, are more controversial because some studies reported enhancing (Smeets et al., 2007; Schwabe et al., 2008) and others impairing effects of pre-learning stress on hippocampus-dependent spatial or episodic memory (Kirschbaum et al., 1996; Diamond et al., 2006).

In addition to the timing of the stressor, participants' sex may be another factor that influences the nature of stress effects on memory. For example, it has been shown that stress after learning enhanced the consolidation of a hippocampus-dependent task in men, whereas the memory of women remained unaffected by stress (Andreano and Cahill, 2006). Similarly, the cortisol response to a stressor before learning was negatively correlated with subsequent memory performance in men but not in women (Wolf et al., 2001). These findings suggest that participants' sex should be taken into account when examining stress effects on memory processes.

Although most studies focused on stress effects on hippocampus-dependent memory (Lupien and Lepage, 2001), evidence is accumulating that stress can also affect striatum-dependent memory processes. Rodent studies indicated that stress hormones injected shortly after training enhance the consolidation of striatum-dependent inhibitory avoidance or stimulus-response (S-R) memories (Medina et al., 2007; Quirarte et al., 2009). S-R memory refers to learning of the association between responses and preceding stimuli. In our everyday life there are numerous examples of such S-R memories, for instance, stopping at red traffic lights or switching the light on when entering a dark room. In humans, stress has been shown to disrupt the retrieval of S-R memories when induced shortly before retention testing (Guenzel et al., 2013). These data suggest that stress may affect the consolidation and retrieval of striatum-dependent memory in a similar manner as hippocampus-dependent memory processes (Roozendaal, 2002; Cahill et al., 2003; Kuhlmann et al., 2005). Recently, it was reported that stress may also alter the formation of striatal S-R memories when induced shortly before learning. These effects, however, were sex-dependent, with stress impairing S-R memory formation in men but not in women (Guenzel et al., 2014).

For hippocampus-dependent memory, it is well established that stress effects are mainly mediated by catecholamines and glucocorticoids (corticosterone in rodents, cortisol in humans) that are released in response to stressful events. Pharmacological elevations of glucocorticoids have been shown to mimic the time-dependent effects of stress on memory in both rodents and humans (Kirschbaum et al., 1996; De Quervain et al., 1998, 2000; Buchanan and Lovallo, 2001; Roozendaal, 2002; Roozendaal et al., 2006b; De Quervain et al., 2007). First evidence from rodents shows that glucocorticoids may also affect striatum-dependent memory. In particular, post-learning injections of corticosterone into the dorsal striatum enhance the consolidation of striatum-dependent memories (Medina et al., 2007; Quirarte et al., 2009). However, whether glucocorticoids influence striatum-dependent memory processes also in humans is still unknown.

Therefore, the present experiment examined in humans the effect of glucocorticoids on the formation of S-R memories that are known to rely on the striatum (Iaria et al., 2003; Bohbot et al., 2007). Healthy men and women received either hydrocortisone or a placebo 45 min before completing two S-R learning tasks. In order to compare glucocorticoid effects on S-R memory with those on spatial memory processes, participants completed also two spatial tasks. Based on previous findings showing that stress before learning may affect both spatial and S-R learning (Guenzel et al., 2014), we expected that hydrocortisone would also affect both forms of memory. However, because previous studies on the influence of pre-learning stress on memory yielded inconsistent results (Kirschbaum et al., 1996; Diamond et al., 2006; Smeets et al., 2007; Schwabe et al., 2008), it was difficult to predict the direction of these effects. Moreover, because pre-learning stress had stronger effects on S-R memory in men compared to women (Guenzel et al., 2014), we expected that hydrocortisone effects on S-R memory would also be more pronounced in men than in women.

## 2. Methods

### 2.1. Participants and design

Sixty healthy, normal-weight university students (30 women; mean age: 24.75 years, SEM = 0.39 years; mean body mass index: 22.90 kg/m$^2$, SEM = 0.25 kg/m$^2$) participated in this experiment. Participants were prescreened in a standardized interview. Exclusion criteria comprised a history of psychiatric or neurological disorders, smoking, drug abuse, medication intake, and in women the use of oral contraceptives. Furthermore, women were not tested during their menses. One female participant was excluded from further statistical analysis because of technical failure.

We used a placebo-controlled, double-blind between-subject design in which participants were randomly assigned to the hydrocortisone (15 men, 15 women) or placebo group (15 men, 14 women). The study was conducted on two testing days with a time-interval of one week. All testing took place between 13:00 h and 19:00 h; starting times were counterbalanced across experimental groups. Participants were asked to refrain from physical exercise, food intake, and beverages except water, 1 h before testing. All participants provided written informed consent for their participation in the study, which was approved by the local ethics committee, and received a compensation of 30 € for their participation.

### 2.2. Day 1: training session

After their arrival on the first testing day, participants completed two training programs: (i) a training program in a 3D virtual environment and (ii) a computer-based S-R association learning program. The training programs resembled our learning tasks and served to familiarize participants with the navigation in a virtual room and the procedure of the S-R association task.

In the first training program, participants learned in two trials how to collect objects in a 3D virtual room by using the left-, right-, and forward-arrow keys of a keyboard. In the second training program, participants were presented three different symbols on a computer screen and instructed to assign one out of three buttons to each symbol. Feedback

about correct and incorrect responses was given by positive or negative smileys. All computer-based programs were created by means of a commercially available computer-game editor (Conitec, Gamestudio, Germany).

## 2.3. Day 1: drug administration

Participants received either 20 mg hydrocortisone (Jenapharm) or a placebo 45 min before the beginning of the learning tasks. Drugs were taken orally under supervision of the experimenter. Participants were allowed to read within the break of 45 min. Timing and dosage of the drug administration were chosen in accordance with previous studies (Schwabe et al., 2010a, 2012b).

In order to verify the action of the drug, participants collected saliva samples by means of Salivette collection devices (Sarstedt, Germany) before drug intake as well as 45 min, 75 min, and 105 min thereafter. On the second testing day, another saliva sample was collected to control for potential group differences in cortisol before memory testing. Saliva samples were stored at −20 °C until the end of the study. From saliva we analyzed the free fraction of cortisol by means of an immunoassay (IBL). Inter- and intra-assay coefficients of variance were below 10%.

## 2.4. Day 1: learning session

Forty-five minutes after pill intake, participants completed four learning tasks: (i) an S-R navigation learning task in a virtual environment, (ii) a spatial navigation learning task in a virtual environment, (iii) a computer-based S-R association learning task, and (iv) a spatial navigation learning task in a real environment. The virtual S-R and spatial navigation learning tasks were completed in random order, before participants performed the S-R association learning task and the spatial navigation task in the real environment.

### 2.4.1. S-R navigation learning task in a virtual environment

The S-R navigation task, which had been used in a previous study (Guenzel et al., 2013), was designed as a virtual eight-arm radial maze with a center platform and a single intra-maze cue (chair) for orientation (see Fig. 1A). Each maze arm was surrounded by high walls and contained a wooden hollow at the end. Three of these wooden hollows contained an object (book, cake, or bag) and participants were instructed to collect these objects in a given order (book — cake — bag) as quickly as possible by using the left-, right-, and forward-arrow keys. Order and location of the objects did not vary

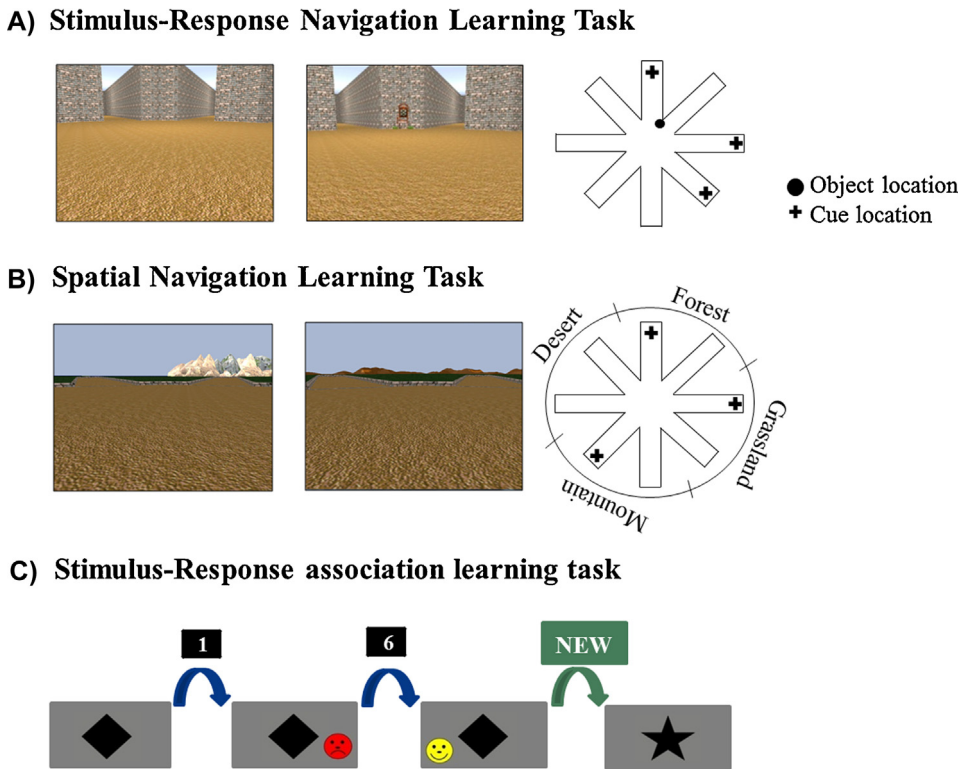


**Figure 1** Learning tasks. (A) S-R navigation learning task in a virtual environment. Left: The center platform, the single intra-maze cue (chair) and two radiating arms, right: Overview of the S-R navigation task. Crosses indicate the position of the objects, whereas the circle shows the location of the single intra-maze cue in relation to the position of the objects. (B) Spatial navigation learning task in a virtual environment. Left: Center platform with two radiating arms and three of the four different external landmarks. Right: Overview of the spatial navigation task. The crosses indicate the position of the objects in relation to the external landmarks. (C) S-R association learning task. Shown are two out of six different symbols together with the correct button as well as the positive or negative feedback in form of smilies.

Parts of Fig. 1A have been reproduced from Guenzel et al. (2013) whereas the parts of Fig. 1B have been reproduced from Guenzel et al. (2014), with permission from Elsevier.

across trials. Extra-maze cues were not provided. Participants could solve the task only by linking the position of an object to a sequence of movements relative to the single intra-maze cue, i.e. they had to learn the association between a single stimulus and a motor response, which defines S-R learning. Previous neuroimaging studies demonstrated that such 'response' learning relies on the striatum (Iaria et al., 2003; Bohbot et al., 2007).

The task was finished after solving two trials in a row error-free or after reaching the maximum number of nine learning trials. Each entry into an arm without an object or into an arm with an incorrect object was counted as an error and errors were taken, together with the time needed to solve a trial, as indicators of learning performance. There was a time limit of 3 min for each trial. The starting position of the participants was constant across trials, whereas the viewing direction varied between trials.

#### 2.4.2. Spatial navigation learning task in a virtual environment

The spatial navigation learning task resembled the S-R navigation learning task. This task was also created as a virtual maze with eight radiating arms, a center platform and wooden hollows at the end of the maze arms (Guenzel et al., 2014). In the spatial task, however, the maze was surrounded by several extra-maze cues (mountain, desert, forest, grassland) whereas there were no intra-maze cues (see Fig. 1B). Again participants were instructed to collect three objects (book, cake, and bag) as quickly as possible; the order and location of the objects was constant across the learning trials. Because the viewing direction of the participants varied between trials and due to the absence of any intra-maze cues participants could solve the task solely by using the relationship between the extra-maze cues. Neuroimaging studies indicated that such spatial learning depends on the hippocampus (Iaria et al., 2003; Bohbot et al., 2007). Notably, the location of a certain object was not associated with a single extra-maze cue, thus ruling out the use of a response strategy.

Same as in the S-R navigation learning task, the spatial navigation task was finished if a participant solved two trials in a row error-free or if the participants reached the maximum number of nine trials. Again, each trial was time limited (at maximum 3 min per trial) and each entry into an incorrect arm (e.g. an arm without an object or with an incorrect object) was counted as an error and the number of errors was taken, together with the time needed to solve a trial, as an indicator of learning performance.

#### 2.4.3. S-R association learning task

On each trial of the S-R association learning task, one of six symbols (moon, star, triangle, rectangle, rhombus, and circle) was presented on a computer screen. Participants were instructed to press as quickly as possible the button of a keyboard that corresponded to that symbol. At the beginning of the task, participants did not know which button corresponded to which symbol. However, once participants pressed a button, they received immediate feedback about whether this was the correct button or not (positive vs. negative smiley). The symbol remained on the screen until the correct button was pressed. Thus, participants learned to associate single stimuli with distinct responses. All symbols were presented eight times, in randomized order (see Fig. 1C). The time needed and the errors made per trial were taken as indicators of learning performance. Previous evidence indicates that this kind of association learning relies on the striatum (Pasupathy and Miller, 2005; Graybiel, 2008).

#### 2.4.4. Spatial navigation learning task in a real environment

The final spatial navigation task took place in the psychology building of the Ruhr-University Bochum (Guenzel et al., 2014). Participants were guided along a predefined route, which was about 70 m long and comprised 15 forks. Participants were not instructed to memorize the route, nor were they informed that they had to retrieve the route on the second experimental day. Importantly, psychology students were excluded from study participation, thus participants were unfamiliar with the building.

### 2.5. Day 2: memory testing

Participants' memory of all four learning tasks was assessed one week after the first testing day. All tasks were completed in the same order as on day 1. This time, however, participants completed only one test trial for each of the virtual navigation tasks. Retention performance was expressed as the time needed to complete a task and the number of errors made.

### 2.6. Statistical analysis

Changes in salivary cortisol were analyzed by means of a mixed-design ANOVA, followed by simple effects analyses. The learning performance in the virtual navigation tasks was compared between the experimental groups by using the last three learning trials of each participant for further statistical analysis. In order to assess the performance in the S-R association learning task, the 48 trials were subdivided into 6 blocks of 8 trials each. Group differences in learning and retention performance were analyzed by means of mixed-design ANOVAs and t-tests. Pearson correlation coefficients were analyzed to assess whether cortisol increases relative to baseline or absolute cortisol levels are related to learning or memory performance. The statistical analysis was conducted with SPSS (version 20, IBM). All reported $p$-values are two-tailed.

## 3. Results

### 3.1. Manipulation check: salivary cortisol concentrations across the experiment

As expected, the intake of hydrocortisone resulted in a significant increase in salivary cortisol (time point of measurement $\times$ group interaction effect: $F_{(1.21,\ 66.42)} = 25.50$; $p < .001$; $\eta^2 = .32$; see Fig. 2). Follow-up tests revealed significantly higher cortisol concentrations in the hydrocortisone group than in the placebo group 45 min, 75 min and 125 min after pill intake (all $p < .001$), whereas groups did not differ in their cortisol concentrations before pill intake ($t_{(57)} = -0.84$; $p = .40$). Cortisol concentrations were similar in men and women (main effect sex and all interaction

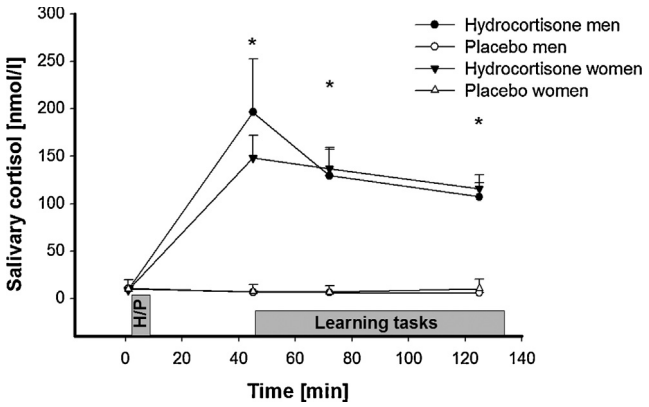


**Figure 2** Mean cortisol concentrations (nmol/l; ± SEM) in men and women of the experimental groups. The gray bars indicate the time of hydrocortison/placebo (H/P) administration and the duration of the learning tasks. Data represent mean ± SEM. $^{*}p < .001$ indicates the results of the *t*-tests for men and women.

effects with the factor sex: all $F < 0.98$; all $p > .34$; all $\eta^2 < .03$).

Before memory testing on day 2, groups did not differ in their cortisol levels (placebo group: M = 9.81 nmol/l, SEM = 1.18; hydrocortisone group: M = 8.56 nmol/l, SEM = 1.14; $F_{(1,\ 55)} = 0.54$; $p = .47$; $\eta^2 = .01$), nor was there a difference between cortisol concentrations in men and women (main effect sex: $F_{(1,\ 55)} = 1.82$; $p = .18$; $\eta^2 = .03$).

## 3.2. Learning performance on day 1

### 3.2.1. S-R navigation learning task in the virtual environment

Overall, participants needed on average 5.88 (SEM = 0.30) trials to reach the learning criterion in the S-R learning task, without differences between the experimental groups ($p = .40$) or between men and women ($p = .16$).

A sex × group × trial ANOVA indicated that performance improved across trials (main effect trial for the time needed: $F_{(1.28,\ 70.40)} = 66.46$; $p < .001$; $\eta^2 = .55$; main effect trial for the number of errors made: $F_{(1.20,\ 65.95)} = 61.72$; $p < .001$; $\eta^2 = .53$) without any differences between the experimental groups (main effect group and interaction effect trial × group for both the time needed and the errors made: all $F < 1.40$; all $p > .24$; all $\eta^2 < .03$; see Fig. 3A). Although men completed the S-R navigation task generally faster than women (main effect sex: $F_{(1,\ 55)} = 7.35$; $p = .01$; $\eta^2 = .12$), hydrocortisone did not affect the learning performance of men and women differently (interaction effects sex × group for the time needed and the errors made: both $F < 0.70$; both $p > .40$; both $\eta^2 < .02$).

### 3.2.2. Spatial navigation learning task in the virtual environment

Participants needed on average 6.39 (SEM = 0.32) trials to reach the learning criterion in the virtual spatial navigation learning task. The number of trials needed to complete the task was comparable between the experimental groups ($p = .39$) and between men and women ($p = .27$).

A sex × group × trial ANOVA showed that performance improved across trials (main effect trial for the time needed: $F_{(1.54,\ 84.63)} = 34.33$; $p < .001$; $\eta^2 = .38$; main effect trial for the number of errors made: $F_{(1.58,\ 87.03)} = 38.96$; $p < .001$; $\eta^2 = .42$) and to a similar extent in the experimental groups (main effect group and interaction effect trial × group for both the time needed and the errors made: all $F < 2.10$; all $p > .13$; all $\eta^2 < .05$; see Fig. 3B). Moreover, hydrocortisone had no differential effect on learning performance in men and women (interaction effects sex × group for the time needed and the errors made: both $F < 0.35$; both $p > .55$; both $\eta^2 < .02$). Overall, however, men completed trials faster than women (main effect sex: $F_{(1,\ 55)} = 5.35$; $p = .03$; $\eta^2 = .09$).

### 3.2.3. S-R association learning task

A sex × group × learning block ANOVA indicated that the time needed to complete a trial (main effect block: $F_{(3.17,\ 174.51)} = 49.72$; $p < .001$; $\eta^2 = .48$) and the errors made per trial (main effect block: $F_{(2.86,\ 157.00)} = 127.38$; $p < .001$; $\eta^2 = .70$) decreased across trials without differences between the experimental groups (main effect group for both the time needed and the errors made: both $F < 0.75$; both $p > .38$, both $\eta^2 < .02$). Overall, men tended to solve the learning task faster (main effect sex for the time needed: $F_{(1,\ 55)} = 2.28$; $p = .14$; $\eta^2 = .04$) and to make fewer errors (main effect sex for the errors made: $F_{(1,\ 55)} = 3.81$; $p = .06$; $\eta^2 = .07$) than women. However, there was no differential effect of hydrocortisone on learning performance in men and women (main effects sex × group and interaction effects block × sex × group for both the time needed and the errors made: all $F < 0.67$; all $p > .41$; all $\eta^2 < .02$; see Fig. 3C).

## 3.3. Memory performance on day 2

### 3.3.1. S-R navigation learning task in the virtual environment

A sex × group ANOVA indicated that hydrocortisone prior to learning did not influence the memory of the S-R navigation task (main effect group for both the time needed and the errors made: both $F < 0.40$; both $p > .52$; both $\eta^2 < .02$; see Fig. 4A), nor did hydrocortisone influence the retention performance of men and women differently (interaction effect sex × group for both the time needed and the errors made: both $F < 1.65$; both $p > .20$; both $\eta^2 < .04$). Irrespective of the experimental groups, however, men tended to complete the retention test trial faster than women (main effect sex: $F_{(1,\ 55)} = 3.04$; $p = .09$; $\eta^2 = .05$).

### 3.3.2. Spatial navigation learning task in the virtual environment

A sex × group ANOVA showed that participants who were administered hydrocortisone before learning had better memory for the virtual spatial navigation task than participants that had received a placebo (main effects group for both the time needed and the errors made: both $F > 4.35$; both $p < .05$, both $\eta^2 > .06$). A trend for a sex × group interaction (interaction effect sex × group for the time needed: $F_{(1,\ 55)} = 3.48$; $p = .07$; $\eta^2 = .06$; interaction effect

sex × group for the errors made: $F_{(1,\ 55)} = 2.53$; $p = .12$; $\eta^2 = .04$; see Fig. 4B), suggested that this effect was mainly due to the influence of hydrocortisone in women. As shown in Fig. 4B, women of the hydrocortisone group completed the task faster ($t_{(19.07)} = -2.33$; $p = .03$) and tended to make fewer errors ($t_{(17.99)} = -2.06$; $p = .06$) than women of the placebo group; whereas there was no effect of hydrocortisone in men (for the time needed: $t_{(28)} = -0.40$; $p = .69$; for the errors made: $t_{(28)} = -0.50$; $p = .62$). Irrespective of the experimental group, men completed the test trial faster (main effect sex: $F_{(1,\ 55)} = 12.18$; $p = .001$; $\eta^2 = .18$) and made fewer errors than women (main effect sex: $F_{(1,\ 55)} = 6.73$; $p = .01$; $\eta^2 = .11$).

### 3.3.3. S-R association learning task

A sex × group × testing block ANOVA showed, in addition to a general improvement across retention testing blocks (main effect block for the time needed to complete a trial: $F_{(3.28,\ 180.23)} = 41.38$; $p < .001$; $\eta^2 = .43$; main effect block for the errors made: $F_{(2.16,\ 118.95)} = 54.25$; $p < .001$; $\eta^2 = .50$; see Fig. 4C), that participants that had received hydrocortisone before learning made fewer errors than those participants that had received a placebo (main effect group for the errors made: $F_{(1,\ 55)} = 6.01$; $p = .02$; $\eta^2 = .10$; interaction effect block × group: $F_{(2.16,\ 118.95)} = 2.26$; $p = .10$; $\eta^2 = .04$). For the time needed, we obtained a time-dependent effect of hydrocortisone (effect block × group for the time needed:

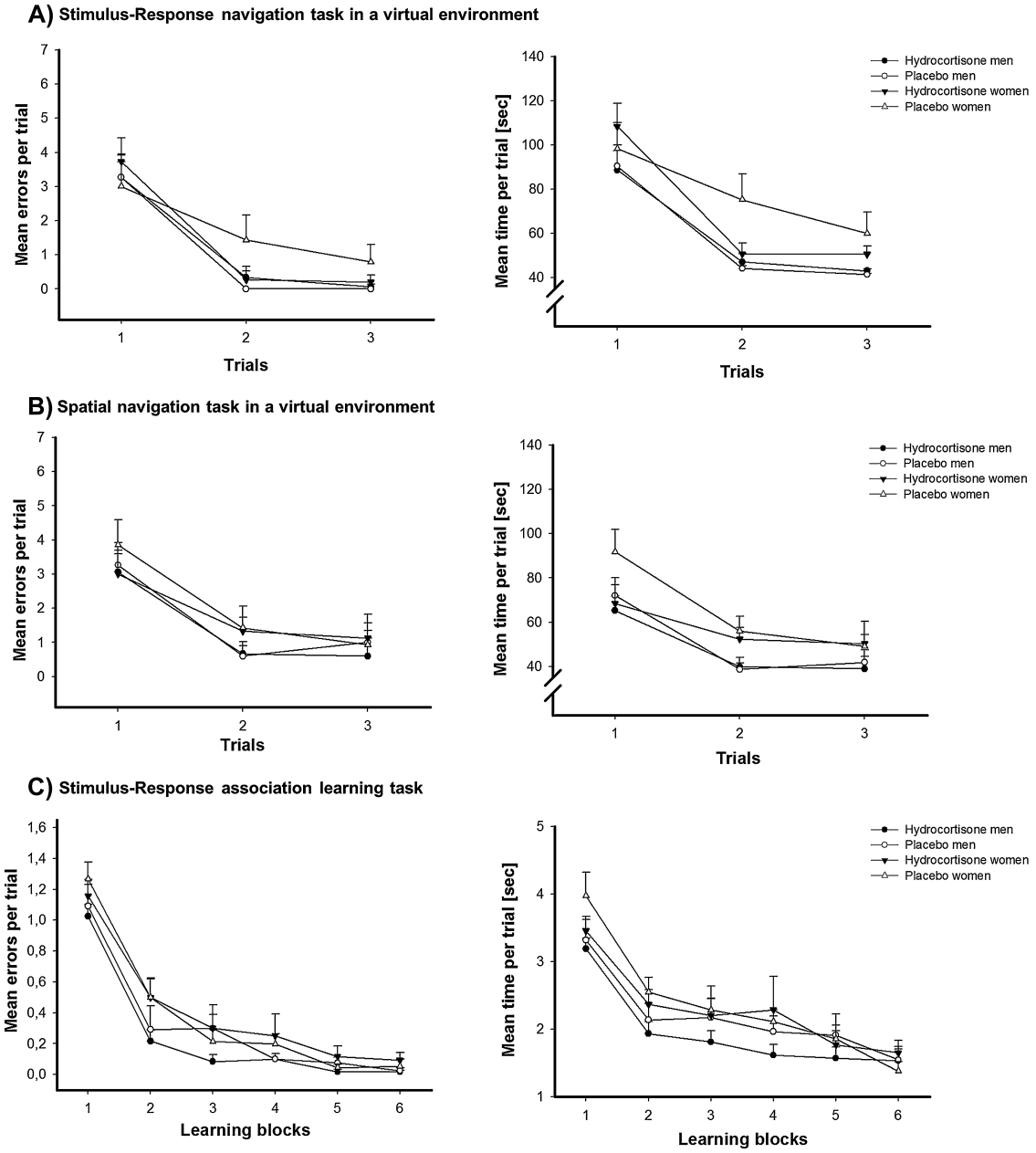


**Figure 3** Mean time and number of errors in the learning session on day 1. Learning performance expressed as the time needed to complete a trial and the errors made in men and women of the experimental groups for (A) the S-R navigation learning task, (B) the computer-based spatial navigation learning task, and (C) the S-R association learning task. Data represent mean ± SEM.

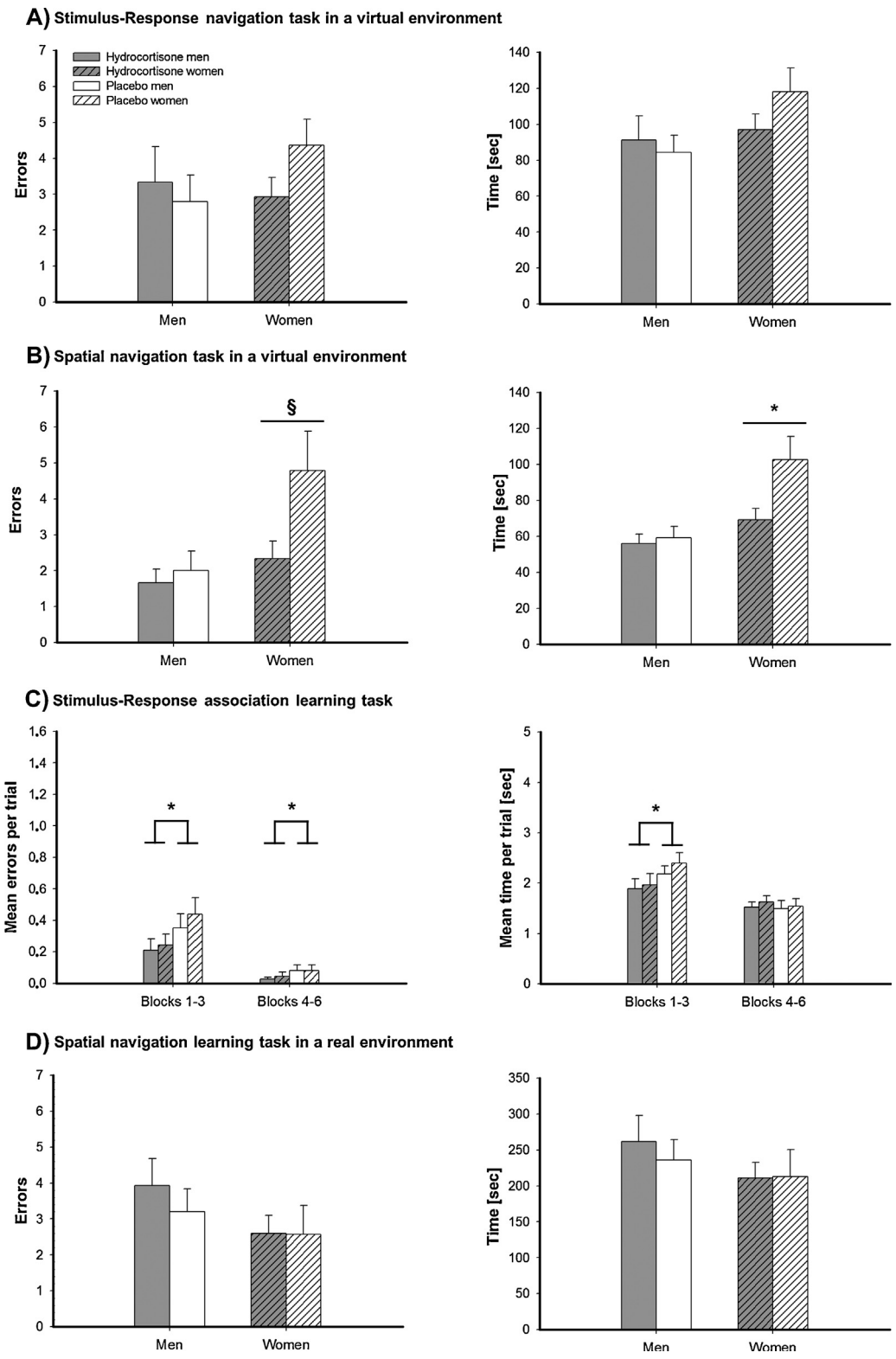


**Figure 4** Mean time and number of errors in the testing session on day 2. Retention performance expressed as the time needed and the errors made in men and women of the experimental groups for (A) the S-R navigation learning task, (B) the computer-based spatial navigation learning task, (C) the S-R association learning task, and (D) the spatial navigation learning task in a virtual environment. Data represent mean $\pm$ SEM. $^{*}p < .05$; $^{\S}p = .06$.

$F_{(3.28,\ 180.23)} = 2.93$; $p = .03$; $\eta^2 = .05$; main effect group for the time needed: $F_{(1,\ 55)} = 1.81$; $p = .18$; $\eta^2 = .03$;): compared to the placebo group, participants that had received hydrocortisone before learning were faster in the first half of the retention test ($t_{(57)} = -2.19$; $p = .03$), when the effect of S-R memory was more prominent, whereas groups did not differ in the second half of the test session ($t_{(57)} = 0.52$; $p = .60$). Moreover, men and women did not differ in test performance (main effect sex and all interaction effects including the factor sex: all $p > .30$).

#### 3.3.4. Spatial navigation learning task in the real environment

As shown in Fig. 4D, hydrocortisone did not affect the retention performance in the spatial task in the real environment (main effect group for the time needed and the errors made: both $F < 0.35$; both $p > .55$; both $\eta^2 < .02$), nor did hydrocortisone influence the retention performance of men and women differently (interaction effects sex $\times$ group for the time needed and the errors made: both $F < 0.30$; both $p > .60$; both $\eta^2 < .02$). Moreover, there were no overall differences between men and women in the retention performance in this task (main effects sex for the time needed and the errors made: all $F < 2.10$; all $p > .15$; both $\eta^2 < .05$).

### 3.4. Correlations between cortisol and performance

In order to examine whether individual cortisol concentrations after hydrocortisone intake were directly related to learning and memory performance, we performed correlational analyses assessing the relation between either peak cortisol concentrations or cortisol increases from baseline to peak and learning or memory performance. These analyses, however, revealed no significant correlations, neither between peak cortisol concentrations and performance on day 1 or 2, nor between cortisol increases and learning or retention performance (all $r$ between $-.29$ and .22, all $p > .11$).

## 4. Discussion

This study examined whether glucocorticoids affect S-R memory formation in humans and, if so, whether these glucocorticoid effects differ between men and women. Therefore, our participants received either hydrocortisone or placebo 45 min before completing two S-R and two spatial learning tasks that are known to rely on the activation of the striatum and hippocampus, respectively (Iaria et al., 2003; Bohbot et al., 2007). Our data show that hydrocortisone before learning (i) enhanced subsequent memory of the S-R association learning task, and (ii) enhanced the retention performance of the virtual spatial navigation learning task, mainly in women.

By now, accumulating evidence shows that stress and glucocorticoids affect not only hippocampus-dependent (De Quervain et al., 1998, 2000; Buchanan and Lovallo, 2001; Roozendaal et al., 2006b) but also striatum-dependent memory processes (Medina et al., 2007; Quirarte et al., 2009). Human studies demonstrated that stress prior to learning may alter S-R memory formation, particularly in men (Guenzel et al., 2014), and that stress before retrieval impairs the retention of S-R memories (Guenzel et al., 2013). The present data extend these findings by showing for the first time that glucocorticoids affect the formation of S-R memories in humans. In particular, our data indicate that hydrocortisone administration before learning enhances subsequent S-R memories. These data are in line with recent evidence suggesting that stress or glucocorticoids, in combination with noradrenergic activation, facilitate striatum-dependent habit learning, at the expense of prefrontal cortex-dependent goal-directed learning (Schwabe and Wolf, 2009; Schwabe et al., 2010a; Gourley et al., 2012; Schwabe and Wolf, 2012; Braun and Hauber, 2013). However, whereas these previous studies on habit learning used mainly dual-solution tasks that can be solved by different memory systems, the present tasks were explicitly designed as single solution tasks, allowing solely spatial or S-R learning. Our findings suggest that glucocorticoids may enhance both spatial and S-R memory.

Moreover, the present findings corroborate previous rodent studies showing that post-learning injection of corticosterone into the dorsal striatum enhanced the consolidation of striatum-dependent memories (Medina et al., 2007; Quirarte et al., 2009). However, in the present study hydrocortisone was administrated before learning and cortisol levels were elevated both during and after learning. Thus, we cannot separate glucocorticoid effects on S-R memory consolidation for those on S-R memory encoding. The finding that the hydrocortisone and placebo groups did not differ in learning performance might be taken as evidence against hydrocortisone effects on encoding. However, because this study did not include measurements of baseline performance, which is hardly feasible in studies that aim to assess the effect of glucocorticoid elevations before learning, it cannot be concluded that hydrocortisone did not affect learning performance.

The enhancing effect of hydrocortisone on S-R memory formation was found in both men and women. For spatial memory, however, we obtained a trend for a sex difference, showing that hydrocortisone before learning enhanced subsequent memory particularly in women. It is, however, important to note that the spatial memory performance of men and women differed mainly under placebo and that hydrocortisone in a way equalized performance of men and women. The observed spatial memory enhancement (in women) is in line with some previous studies on the influence of stress or glucocorticoids before learning on hippocampus-dependent memory (Buchanan and Lovallo, 2001; Smeets et al., 2007; Schwabe et al., 2008; but see also Kirschbaum et al., 1996; Diamond et al., 2006 for opposite effects). For example, stress shortly before episodic memory encoding has been shown to facilitate later recall (Smeets et al., 2007; Schwabe et al., 2008). Pharmacological studies support these findings and suggest that hydrocortisone before learning may boost subsequent episodic memory (Buchanan and Lovallo, 2001). Sex differences in stress effects on hippocampus-dependent memory have also been reported before. In particular, it has been shown that stress after learning may enhance the consolidation of hippocampus-dependent memories in men, but not in women (Andreano and Cahill, 2006). The opposite direction of the sex differences in that study and the present one may be related to the different tasks used, the different timing of the stress exposure (before vs. after learning), or to differences in the hormonal status of the female participants.

Sex hormones are known to play a critical role in sex differences in the effects of stress and glucocorticoids on

memory processes. Rodent studies, for instance, reported that estrogen may enhance the performance of stressed female rats (Bowman et al., 2002; Conrad et al., 2004). Moreover, estrogen also influences hippocampal plasticity (Maren et al., 1994; Gupta et al., 2001) and testosterone affects the size of the dentate gyrus and CA3 region in the rat brain (Roof and Havens, 1992). In order to assess the role of sex hormones in the different effects of hydrocortisone on memory in men and women, future studies are required to measure or directly manipulate the sex hormone concentrations of the participants. In addition, future studies should control for the luteal vs. follicular phase of the menstrual cycle because these phases differ significantly regarding sex hormone concentrations and there is evidence that stress may have different effects on memory processes depending on the cycle phase (Andreano et al., 2008; Espin et al., 2013). The fact that we did not control whether our female participants were in the luteal or follicular phase can be considered as a limitation of the present study.

Our previous study on the influence of stress on S-R and spatial memory formation showed that S-R memory formation and retention testing was impaired in stressed men, but not in women, whereas the retention of spatial memories was impaired in stressed women, but not in men (Guenzel et al., 2014). In the present study, however, we found that glucocorticoids enhance the retention performance of S-R memories without a difference between men and women, whereas the retention performance of spatial memories was enhanced only in women of the hydrocortisone group. These discrepancies are most likely due to the critical differences that exist between an experimental stress-induction and a pharmacological manipulation of glucocorticoid concentrations, although these manipulations are often equated. For instance, depending on drug dosage, glucocorticoid concentrations are often higher after pharmacological manipulations than after a stress exposure (De Quervain et al., 1998; Abercrombie et al., 2003; Roozendaal et al., 2006b; Schwabe et al., 2012b). Furthermore, stress triggers not only the release of cortisol but also of many other hormones, neuropeptides and neurotransmitters, many of which may also have an effect on memory processes (Contarino et al., 1999; Radulovic et al., 1999). Pharmacological glucocorticoid manipulations, however, may even inhibit at least some of these stress mediators via negative feedback processes. Moreover, at least some effects of glucocorticoids require concurrent noradrenergic activity (Roozendaal et al., 2006a, 2006b, 2009) which occurs after stress but not after glucocorticoid administration.

Finally, it is important to note that the observed effects of hydrocortisone occurred in some of the used tasks but not in others. More specifically, hydrocortisone before learning increased memory for the S-R association learning task but not for the virtual S-R navigation task and, in women, for the virtual spatial navigation task but not for the navigation task in the real environment. We included different spatial and S-R learning tasks that shared some central characteristics but differed in other characteristics, in order to assess to what extent potential glucocorticoid effects could be generalized across tasks. The finding that glucocorticoids enhance memory formation in some S-R (and spatial) tasks but not in others is important as it shows that, although glucocorticoids may enhance S-R memory, these effects are dependent on certain task characteristics. One critical task characteristic might be the number of learning trials which was higher in the S-R association learning task than in the S-R navigation task and in the virtual spatial task than in the spatial task in the real environment. Another important difference between the S-R association task and the S-R navigation task was that participants received more direct feedback in the S-R association task. Moreover, the spatial navigation tasks in the virtual and real environment differed, for instance, also in the cognitive demands, the perceptual input, and the task-related locomotor activity. However, which of these factors accounts for the task-dependent effects of hydrocortisone remains at this point somewhat speculative and should be addressed in future studies.

Taken together, we investigated the impact of glucocorticoids on striatum-dependent S-R memory formation. Our findings show that hydrocortisone before learning enhanced the retention of S-R (association) memories both in men and women. Understanding how exactly stress and stress hormones shape memory processes beyond the hippocampus, may have important implications for mental disorders that are characterized by altered stress responses on the one hand and abnormal S-R memory processes on the other hand, such as posttraumatic stress disorder (PTSD) or drug addiction (Schwabe et al., 2010b; Goodman et al., 2012).

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## Conflict of interest statement

The authors report no conflict of interest.

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