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ter Horst, J.P.; Kentrop, J.; de Kloet, E.R.; Oitzl, M.S.

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Stress and estrous cycle affect strategy but not performance of female C57BL/6J mice

J.P. ter Horst, J. Kentrop, E.R. de Kloet & M.S. Oitzl

Division of Medical Pharmacology, Leiden Amsterdam Center for Drug Research and Leiden University Medical Center, Leiden University, Leiden, The Netherlands

Corresponding author: J.P. ter Horst

Current Address Corresponding Author: University of Amsterdam, SILS-CNS, Science Park 904, 1098 XH Amsterdam

The Netherlands

Email: Judith_ter_Horst@hotmail.com

Highlights:

- There is a sex difference in the use of learning strategies

- Stress induces a switch from stimulus-response towards spatial strategy in female mice

- Stressed estrus females rescue their performance by switching to a spatial learning strategy

Abstract

Stress induces a switch in learning strategies of male C57BL/6J mice from predominantly spatial to more stimulus-response learning. To study generalization of these findings over **sex**, we investigated female C57BL/6J mice at three phases of the estrous cycle under non stress and acute (10 min) restraint stress conditions. On a circular hole board (CHB) task, about half of the naive female mice used spatial and stimulus-response strategies to solve the task. Under stress, female mice favored spatial over stimulus-response strategies, with 100 % of female mice in the estrus phase. Performance expressed as latency to solve the task is only improved in stressed female mice in the estrus phase. We conclude that the use of learning strategies is influenced by **sex** and this difference between **sexes** is aggravated by acute stress.

Keywords

Mice - female – stress - behavioral strategy – spatial learning – hippocampus – caudate nucleus

Intro

Multiple memory systems run in parallel when accessing the same information but differ in their mode of action and the underlying neuronal networks [1]. For example, to solve a learning task animals can either use a spatial or stimulus-response (S-R) strategy. These two strategies originate in different parts of the brain. Spatial memory involves the use of multiple stimuli and relies on the hippocampus [1, 2], whereas S-R memory depends on a single stimulus and is based on the caudate nucleus [3]. Male mice and rats seem to prefer the use of a spatial strategy [4-7], while females apply both strategies [6-8].

In response to stress, strategy preference of male mice and rats switches from spatial towards more S-R learning [Schwabe et al., 2010a; Schwabe et al., 2008; Kim & Baxter, 2001), thereby rescuing their performance [Schwabe et al., 2010a; Schwabe et al., 2008). The effect of stress on strategy use of female mice has not been investigated so far. The strategy of females during stress is of interest because of their higher basal and stress-induced corticosterone concentrations [9-14] than

observed in male rats. In addition, in multiple learning paradigms females behave differently during naive and in stressful situations compared to males [15]. In the present study, we assessed which strategy female mice use under normal and stress conditions and how stress does influence their performance. Here, we report that stressed females switch towards the use of a spatial strategy and improve their performance, but only when in the estrus phase.

Materials and Methods

The present study was designed to assess qualitative (strategy: spatial, stimulus response) and quantitative (performance expressed e.g., as latency) characteristics of the behavior of female C57BL/6J mice. Mice received acute restraint stress 30 minutes before training, and their phase of the estrous cycle was determined. Twenty-eight naive and 28 stressed female C57BL/6J mice (Janvier, France), approximately 5 months of age, were either subjected to the restraint stressor or not (n = 28 per condition) and observed on the circular hole board (CHB) [4, 16] 17]. Mice were housed individually in Macrolon cages one week before the start of the experiment (translucent plastic: 44 x 22 x 17 cm) with sawdust bedding, a tissue for nest building, water and food *ad libitum*, with controlled humidity on a 12h:12h light/dark cycle. Experiments were approved by the committee on Animal Health and Care from Leiden University, The Netherlands, in accordance with the EC Council Directive of November 1986 (86/609/EEC).

The CHB is a revolvable grey round plate (Plexiglas, 110 cm in diameter, situated 1 m about the floor) with 12 holes at equal distances from each other, 10 cm from the rim of the board. Holes are 5 cm in diameter and can be closed by a lid at a depth of 5 cm. If open, the hole is the exit to the animal's home cage via an S-shaped tunnel (15 cm long; 5 cm diameter). Numerous cues in the room allow spatial orientation. Procedure: First, mice were "pretrained" to climb through the tunnel. One week before the first training trial, mice were placed on the CHB for a 5 min free exploration trial (FET). All holes were covered with a lid. A transparent 0.5 l. plastic bottle filled with water stood next to the hole that was opened at the end of the exploration trial. The FET served to estimate possible differences in movement pattern of the mice, that might be influenced by the estrous cycle. No differences were

found. Each training trial started by placing the mouse in a grey cylinder, 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 gently guided there by the experimenter along a grid. 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 under the exit hole but was not visible for the mouse on the board. During six training trials, the position of the exit hole was fixed with respect to the distant extra-maze cues in the room. Also, the proximal intra-maze cue (the bottle) was placed next to this exit hole in all six training trials.

The test-trial was used to detect the learning strategy. The hole of the training remained open, but the bottle was relocated to an additional exit hole opposite to the training position. The use of either S-R or spatial strategies is defined by the exit that is used: leaving the board through the exit of the training shows the use of a spatial strategy. Using the hole at the novel location, next to the bottle, reflects the use of a S-R strategy. To control for possible odor cues, we divided the bedding of the home cage of the mouse over two cages placed under both exit holes.

Thirty minutes before the first training trial mice were stressed by immobilization for 10 min in a narrow cylinder that still allowed breathing but no further movement. Immobilization was performed in a room adjacent to the experiment room. Mice returned to the experimental room and remained for 20 min in their home cage, before training started.

Vaginal smears were taken twice: after the FET and after the training. The mouse was placed on top of its cage, the tail was lifted slightly and a small smear loop (1 µl; Greiner Bio-one) was gently inserted above the major labia in the cloaca and carefully rubbed along the ventral / rostral side of the cloaca. Cells were transferred to a drop of water on a microscope glass slide. Slides were air-dried and stained with Giemsa (Sigma) to facilitate identification of the cycle stage. The four stages are proestrus, estrus, metestrus and diestrus (Figure 1). We did not encounter the metestrus phase so therefore it is not included in this study.

Behavior was digitally recorded and analyzed with Ethovision XT 6.1 (Noldus). Statistical analyses were performed with SPSS 17.0 including chi-square and GLM repeated measures.

Results

Performance: Female mice learned the CHB task. Latency of first visit to exit hole significantly decreased over the course of trials ($F(5,135)4.45$ $p=0.001$; Figure 2). Stressed mice had similar latencies as naive mice ($F(1,54)0.093$ $p = 0.762$). The phase of the estrous cycle did not affect the performance of stressed and naive mice (latency to exit hole: naive $F(2,25)0.187$ $p = 0.831$, stressed $F(2,25)2.508$ $p = 0.102$).

Strategy: Naive mice applied either the spatial (57%) or the S-R strategy (43%) to locate the exit hole, while stressed mice showed a significant switch towards more spatial learning strategies ($\chi^2(1)5.6$ $p = 0.018$; 86 % spatial, 14 % S-R). Sub-grouping the mice according to their estrous phase (which had been determined after the last training trial) revealed no effect of estrous phases in naive mice ($\chi^2(2)1.197$ $p = 0.55$; Figure 3). The stress-induced increase in spatial over S-R strategies tended to be higher in the three phases of the estrous cycle. Although all mice in estrus used the spatial strategy, this was not statistically different from the stressed mice in pro- en diestrus ($\chi^2(2)4.407$ $p = 0.110$). However, the stressed mice in estrus used significantly more often the spatial strategy than their naive counterparts ($\chi^2(1)9.579$ $p = 0.003$).

Test trial: Latency of first visit to exit hole in the test trial were comparable to trial 6 and did not differ between naive and stressed mice ($t(54)1.229$ $p = 0.224$). The latency of first visit of exit hole and the speed during the test trial were not influenced by either stress nor estrous cycle (estrous x stress: $F(2,50)$ 0.494 $p = 0.613$; $F(2,50)$ 0.013 $p = 0.987$, respectively).

Performance and strategy: The stress-induced switch towards a spatial strategy in estrus female mice was paralleled by shorter latencies for the first visit to the exit hole, specifically in the latter part of the training (trials 3 to 6: $F(1,24)6.403$ $p=0.018$; Figure 2B).

Discussion

Female C57BL/6J mice used either stimulus-response or spatial strategies to solve a task that allows the use of proximal and distal stimuli. However after acute stress, spatial strategies were favored over stimulus response in all estrus phases. During estrus, all mice used the spatial strategy, which was paralleled by shorter latencies to exit, which is indicative for an improved performance.

Strategy: The design of our circular holeboard task allowed to identify the use of spatial and S-R strategies in mice. Half of the naive female mice used spatial as well as S-R strategies. This is in contrast to male C57BL/6J mice that all used spatial strategies [4, 17]. This clear-cut sex difference was further extended to learning strategies under acute stress. Stressed females increasingly used a spatial strategy on the CHB task, whereas stressed male mice switched towards a S-R strategy [4, 5, 16]. Our results are supported by previous findings that naive females have no preference for either of the two strategies. On a plus maze female rats were reported to use either a place strategy (spatial navigation) or a response strategy (S-R navigation) [6]. In addition, on a ladder-rewarded plus maze female mice did use intra-maze and extra-maze cues while male mice mainly employed extra-maze cues, implying a spatial strategy [7]. Strategy use, therefore, seems to be sex specific.

Does the estrous cycle have an effect on strategy use on the CHB? Here, we observed no significant interference of the estrous cycle on strategy use, neither in naive nor stressed females. However, stress did induce a switch towards the spatial strategy in estrus female mice; all stressed mice in estrus used the spatial strategy. It should be taken into account however, that behavior of female mice and rats depends on several factors, such as the type of learning task (complex or simple, emotional or cognitive), phase of the task (acquisition or memory retrieval), the type of stressor and the estrous cycle phase [15]. The adversity of a task influences the task-inherent activation of the stress system and concurrently also emotionality. For example, estradiol did not influence strategy choice in a water maze task in ovariectomized rats [18]. However, after exposure to a water-based T-maze task a preference for spatial strategies has been reported in proestrus rats during the memory test [6] and also after extensive training on a dry T-maze task [19].

Rats deprived of estrogen by ovariectomy perform better in S-R tasks than in spatial tasks [20]. Moreover, injection of estradiol into the hippocampus enhanced spatial learning whereas injection of estradiol into the striatum impaired response learning [21]. Acute stress was reported to increase estrogen levels in female rats [22]. Therefore one explanation of these findings could be that a stress-induced increase in estrogen levels might have facilitated the estrus mice switch towards a spatial strategy. We found one human study that specifically tested the effect of stress on learning strategy in women. In support of our findings, high cortisol levels increased the number of women that switched from S-R to a spatial strategy [23]. Other studies with men and women did not report sex differences, neither for basal nor for the stress-induced switch of learning strategies [5, 24]. The lack of a sex-dependent effect might be related to the number of participants and also to the use of oral contraceptives. Clearly, the interaction of stress and sex hormones plays a role in the switching of the choice of learning strategy.

The phase of the estrous cycle influences learning in a task-dependent fashion. Tasks that require a functional hippocampus such as trace eyeblink conditioning showed improved learning during the proestrus [25-27], while studies using spatial learning tasks, reported impairments in learning during the proestrus phase on the radial arm maze [28] or during the estrus phase in the Morris water maze [29, 30]. We detected no effect of the estrous cycle phase on the learning of the CHB in mice. It is conceivable therefore that the following two factors could play a role: (i) the training schedule, i.e., several training trials on one day in the present study compared with extensive training trials and training over days in the studies mentioned above and (ii) the involvement of other brain regions than the hippocampus.

As we have demonstrated previously, mice can acquire the CHB by employing either the hippocampus-based spatial strategy or by the caudate nucleus-based S-R strategy [4, 31, 32]. Other brain areas that might become involved are the amygdala, which is an area relevant for the emotional modulation of strategies [33] and the prefrontal cortex, which is associated to the switching between strategies [34]. During learning and memory remodeling of hippocampal regions occurs with alterations in dendrite complexity, spine density and neurogenesis [35-37]. Sex hormones modulate

these morphological substrates of learning and memory, which may explain why the estrous cycle of female rats is paralleled by fluctuations in spine density and number of new born cells in the hippocampus. Spine density and number of synapses is the highest during proestrus, followed by a rapid decrease in the estrus phase and intermediate spine density during diestrus [35-37]. Thus, enhancing effect of estrogen on dendritic growth and plasticity was found. In addition to sex hormones, corticosterone is also known to modulate neuronal plasticity resulting in decreased dendritic morphology in the dorsomedial striatum and CA3 region of the hippocampus [38-40]. Chronic stress and estradiol were found to affect spatial memory processes, which correlated with CA1 apical spine expression rather than CA3 dendritic organization [41]. It is likely that this interplay of sex and stress hormones on hippocampal plasticity modulates the changes in performance and strategy we have observed in the current study.

Performance: In the present study, the performance related to the spatial strategy was not altered by either stress or estrous cycle. However, when in estrus and stressed, we found an improved performance in mice using the spatial strategy. In contrast, performance of stressed male mice that remained in the spatial learning mode deteriorated, but was rescued in those who switched to S-R learning [4]. Apparently it is the switch of the learning strategy in a certain group of individuals rather than the perseverance of learned behavior *per se* that is relevant to keep an optimal level of performance.

Conclusion

Naïve female C57BL/6J mice use either the spatial or the S-R strategy to solve the CHB task. After acute stress female mice switch to spatial strategies. This switch occurs especially in the estrus phase with a concomitant improvement in performance. Previously we have reported that naive male C57BL/6J mice favor the spatial strategy and switch to S-R when stressed. While stress can induce a sex-dependent switch of learning strategies, at the same time this switch in strategy rescues performance.

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Figure captions:

Figure 1: Phases of the estrous cycle. Proestrus is characterized by single nuclei cells; estrus contains epithelial cells and in diestrus macrophages and single nuclei cells are present.

Figure 2: Latency of the first visit to the exit hole (in seconds) of naive and stressed female mice during training in proestrus, estrus and diestrus. A) Naive mice; B) stressed mice. Insert: scheme of the circular hole board; gray circle: location of the exit hole; bottle: location of the proximal stimulus. Data expressed as mean ± SEM. * $p<0.05$ compared to naive estrus over trials 3 to 6.

Figure 3: Percentage of female mice during proestrus, estrus and diestrus under naive and stressed conditions, showing S-R: Stimulus-Response or spatial strategy. Numbers in bars represent the total number of mice using either strategy. Insert: scheme of the circular hole board; gray circle: location of the exit hole; bottle: location of the proximal stimulus.* $p<0.05$ compared to naive females in the same phase of the estrous cycle.

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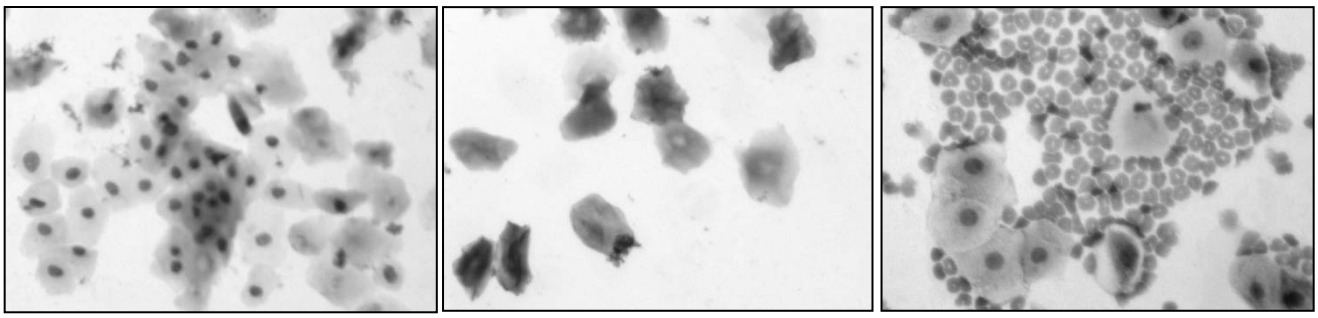
Figure 1



Proestrus **Estrus** **Diestrus**

Figure 2

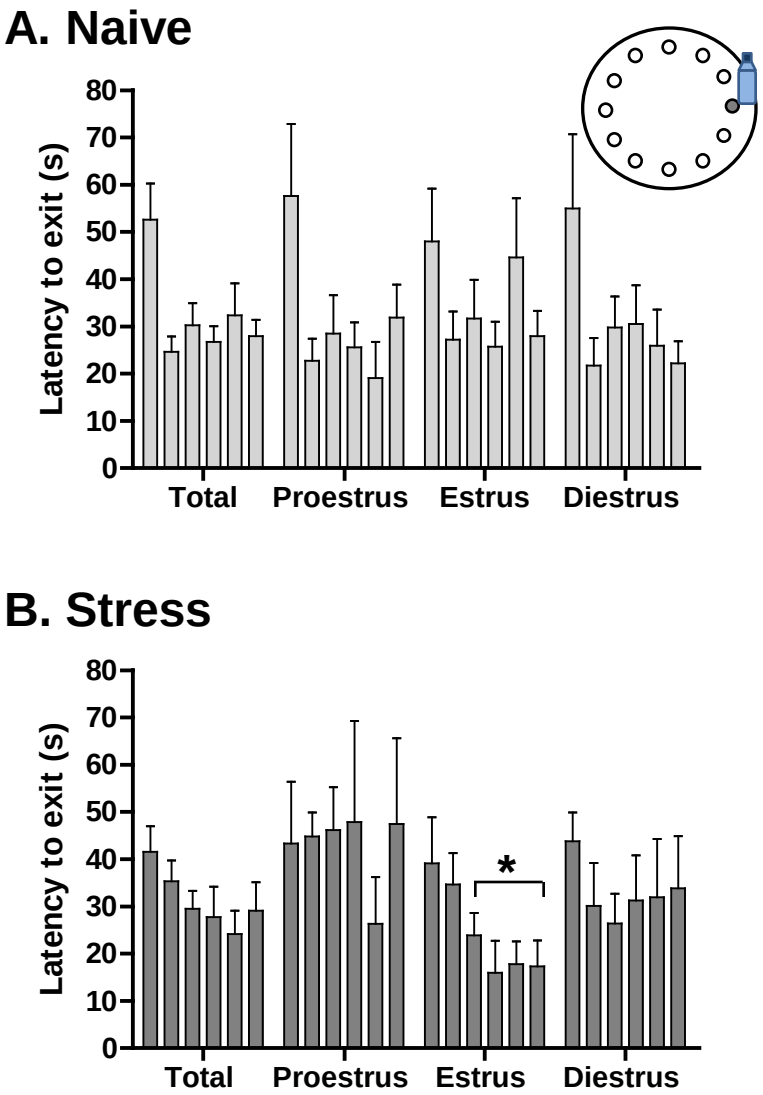
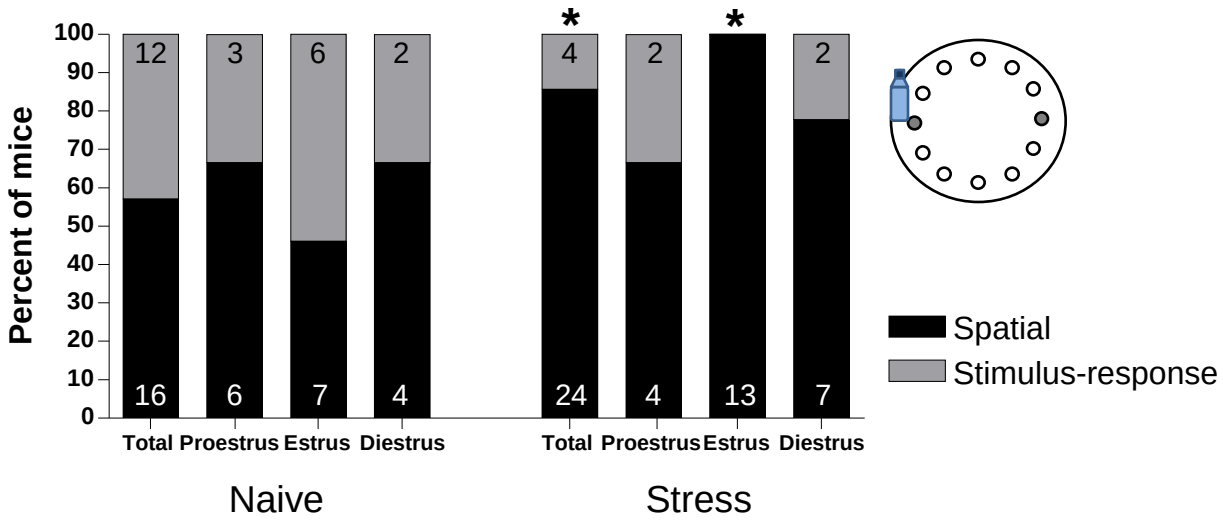

A. Naive
Latency to exit (s)
80
70
60
50
40
30
20
10
0
Total
Proestrus
Estrus
Diestrus
B. Stress
Latency to exit (s)
80
70
60
50
40
30
20
10
0
*
Total
Proestrus
Estrus
Diestrus

Figure 3


Percent of mice
100
90
80
70
60
50
40
30
20
10
0
12
3
6
2
16
6
7
4
*
*
4
2
2
24
4
13
7
Total
Proestrus
Estrus
Diestrus
Total
Proestrus
Estrus
Diestrus
Naive
Stress
Spatial
Stimulus-response