

Intramaze cue utilization in the water maze: Effects of sex and estrous cycle in rats

Simona Sava, Etan J. Markus*

Department of Psychology, Behavioral Neuroscience Division, University of Connecticut, 406 Babbidge Road Box U-20, Storrs, CT 06269, USA

Received 7 July 2004; revised 28 December 2004; accepted 5 January 2005

Available online 19 April 2005

Abstract

Rats can use a wide spectrum of intra- and extramaze information while navigating through the environment. The current study examined the relative contribution of an intramaze cue with regard to its proximity to the goal. Three experiments were conducted and the impact of intramaze cue removal or rotation on water maze search was examined. In males, the effect of the intramaze cue declined monotonically in relation to the proximity of the cue to the goal. A more complex relationship between cue location and utilization was found in estrous and proestrus females. Estrous females showed a strong effect of the cue only when it was near the goal, ignoring it when it was situated further away. Conversely proestrus females were affected by the cue under all conditions. It is concluded that previous reports of behavioral differences may stem from the fact that proestrus females are affected by and attend to a wider range of stimuli, while estrous females are more affected by salient stimuli.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Estrogen; Landmark; Learning; Navigation; Spatial; Hippocampus

Introduction

When searching for food or finding their way back home, animals can use a wide spectrum of strategies that allow them to successfully navigate through their environment. For example, food storing birds use visual landmarks and the sun compass (Sherry and Duff, 1996), bats use echolocation (Gallistel, 1990), honeybees use visual landmarks and path integration (Collett and Collett, 2002). Even when one limits the task to spatial navigation, and the species to rats, there are a multitude of cues the animal can attend to. Some studies show that rats ignore landmark information and preferentially encode the geometric layout of the environment defined by large areas (e.g., walls) and corners (Cheng, 1986; Gallistel, 1990; Margules and Gallistel, 1988). Other studies show that landmarks are important for navigation (Greene and Cook, 1997) and that rats are able to encode the

spatial information derived from the geometric relations among landmarks (Benhamou and Poucet, 1998; Greene and Cook, 1997). One possible reason for the differences in these studies is that the landmarks differed in their salience, location to the horizon, and proximity to the goal, all of which could affect how much the rats will rely on these stimuli.

In the above studies, the subjects were male rats. When females were tested on these types of tasks, a picture of sexually dimorphic spatial navigation emerges. Using a plus maze, Tropp and Markus (2001) found that during early exposure to an environment, female rats attend to many different types of cues and with additional training they rely more on distal visual information, while male rats predominantly used the distal visual information at all times. Williams et al. (1990) used a 12-arm radial maze with only 8 arms reinforced and showed that male rats selectively attend to the geometry of the room and ignore the distal landmarks, while females attend to both room geometry and distal landmarks. Kanit et al. (1998) showed that female rats relied more on a local visual cue than males

* Corresponding author. Fax: +1 860 486 2760.

E-mail address: markus@psych.psych.uconn.edu (E.J. Markus).

in a water maze task. These findings indicate sex differences in navigation.

It has been known for some time that the hippocampus is an important structure for spatial navigation (O'Keefe and Nadel, 1978; Sutherland and Rudy, 1989). More recently, it has been shown that in female rats there are periodical morphological changes that are concomitant with the hormonal changes across the estrous cycle (Gould et al., 1990; Woolley and McEwen, 1992). Specifically, the estradiol level peak during the proestrus stage is accompanied by an approximate 30% increase in synaptic density on the apical dendrites of the pyramidal cells in the CA1 region of the hippocampus (Woolley and McEwen, 1992). During estrus, both estradiol levels and spine density return to basal levels. Electrophysiological changes are also observed in hippocampus. During proestrus, there is an increase in hippocampal plasticity and long-term potentiation (Cordoba Montoya and Carrer, 1997; Good et al., 1999; Warren et al., 1995), and a decrease of the seizure threshold (Terasawa and Timiras, 1968).

Consequently, rather than simply look for sex differences in navigation, it is important to consider the female rats' stage of the estrous cycle. Previous studies (Frye, 1995; Markus and Zecevic, 1997; Warren and Juraska, 1997) showed that female rats performed better on spatial tasks during low estrogen phases of the estrous cycle than during proestrus phase, when levels of estrogen are high. Other studies failed to find a difference in performance across estrous cycle (Berry et al., 1997; Schwegler et al., 1993; Stackman et al., 1997). There are also studies that show that proestrus animals are better at spatial tasks (Frick and Berger-Sweeney, 2001; Korol and Kolo, 2002). One possible source of the discrepancies may be related to the type of task used (Desmond and Levy, 1997; Dohanich, 2002). Generally, studies using stressful/aversive tasks (i.e., water maze, fear conditioning) show a detrimental effect of ovarian hormones on performance (Chesler and Juraska, 2000; Frye, 1995; Markus and Zecevic, 1997; Warren and Juraska, 1997). Conversely, studies using appetitive motivation report enhanced performance associated with high levels of ovarian hormones (Daniel and Dohanich, 2001; Daniel et al., 1997). Another possible factor is the type of memory task used. Estrogen has been shown to improve performance on working memory in the radial arm maze (Dohanich, 2002; Fader et al., 1999; Luine et al., 1998), T-maze (Fader et al., 1998), and water maze (Sandstrom and Williams, 2001), while having no effect on reference memory (Dohanich, 2002; Fader et al., 1999; Luine et al., 1998), or having a detrimental effect on reference memory in the water maze (Daniel et al., 1999; Frye, 1995; Warren and Juraska, 1997). However, neither the type of reinforcement nor the type of memory tested can account for all the data. The present study examines whether the source of these discrepancies is differences in the degree to which different landmarks are attended to.

The current experiments compare how male and female rats use landmark information when navigating through an environment. Three experiments were conducted, employing a water maze task and manipulating the spatial location of a salient intramaze landmark in relation to the goal. The manner in which the location of the landmark, sex, and the hormonal stage affected cue use was examined.

Methods

Subjects

Fifty-seven female and twenty male 344 Fischer rats (Harlan Sprague-Dawley, IN) were included in the study. All rats were 5 months old at the beginning of the study. They were single-housed in clear Plexiglas cages and the colony was maintained on a 12-h light/dark cycle (lights on at 06:30). Water and food were available ad libitum. The behavioral testing was performed between 14:00 and 18:30. Because of the constraints imposed on the available testing time by the estrous cycle, Experiment 1 was run in 3 batches and Experiment 2 was run in 2 batches. All procedures performed in this study were approved by the University of Connecticut Institutional Animal Care and Use Committee.

Apparatus

The water maze was a round blue polyethylene tank, 77.5 cm height, 122 cm diameter at the water level. The tank was filled to a depth of 48 cm with water (19–21°C). In Experiment 1, a white circular platform (15 cm diameter) with an attached perpendicular white rectangle (14 × 5 cm) was placed in the tank, 0.5 cm above the water level, in the center of one of the four quadrants. In Experiments 2 and 3, a transparent circular platform (15 cm diameter) was placed in the center of an adjacent quadrant in the tank, 1.5 cm below the water level.

All experiments were run in the same large room (size 4.5 × 5 m), with many extramaze cues available (door, cabinets, computer, lamp, posters on the walls). For Experiments 2 and 3, the pool was moved to a different corner of the room and the room was made to look different by adding a large white partition (174 × 170 cm) and changing the pattern and source of lighting. The new location of the platform was shifted from its previous location in relation to the real world, as well as in relation to major cues in the room. A camera (Panasonic) was placed above the center of the pool at each location and sent images to a SMART (Panlab, Spain) tracking system for behavioral analysis.

Procedure

Before behavioral testing, females were lavaged daily for at least 2 complete cycles. During the experiment, females

continued to be lavaged every day. On the days when they were tested on the water maze, females were lavaged both pre- and post-testing to ensure that they did not change over to the next estrous cycle phase during testing. Males received similar handling (flipped to expose the perineal region, and the scrotum was tapped with a Q-tip). All animals took part in Experiment 1. Subsequently, about half of the rats were tested in Experiment 2, and the others in Experiment 3.

Lavage

Vaginal smears were analyzed under a light microscope (10× objective) to determine the phase of the estrous cycle. The relative proportions of leukocytes, nucleated cells, and cornified cells were recorded. The smears were classified as proestrus phase if they had at least 50% nucleated cells with no more than 30% leukocytes, and as estrous phase if they had at least 70% cornified cells. Only females with at least 2 consecutive 4–5 days cycles before each behavioral testing session were included in the study; as a result, a total of 12 females with irregular cycles were excluded. Additionally, one proestrus female was dropped from the study because she changed to estrus during testing in Experiment 1. Data from two females were excluded (one from Experiment 2 and one from Experiment 3) because they stopped cycling after being tested in Experiment 1. Data from three males were dropped due to technical problems.

Experiment 1

Seventeen males, twenty-one proestrus females, and twenty-three estrous females were included in the analysis. All subjects received 8 trials in 1 day. Each block of 4 trials consisted of 1 trial from each of 4 different starting locations, and the pseudo-random starting sequence was the same for all animals. Rats were placed in the water facing the wall of the tank. After reaching the platform, rats were allowed 10 s on the platform. If an animal did not reach the platform within 60 s, the animal was placed on the platform for 10 s. The animals were then removed and placed in opaque cages on heating pads for 3 min. The ninth trial was a probe in which the platform was removed and the rats were allowed to swim in the pool for 30 s (see Fig. 1A).

Experiment 2

All rats were first tested on Experiment 1, after which they were tested in either Experiment 2 or Experiment 3. There was no difference between the animals chosen to participate in Experiment 2 or 3 in their learning curves or on their performance in the probe trial.

Ten males, nine proestrus females, and twelve estrous females were included in the analysis of Experiment 2. The

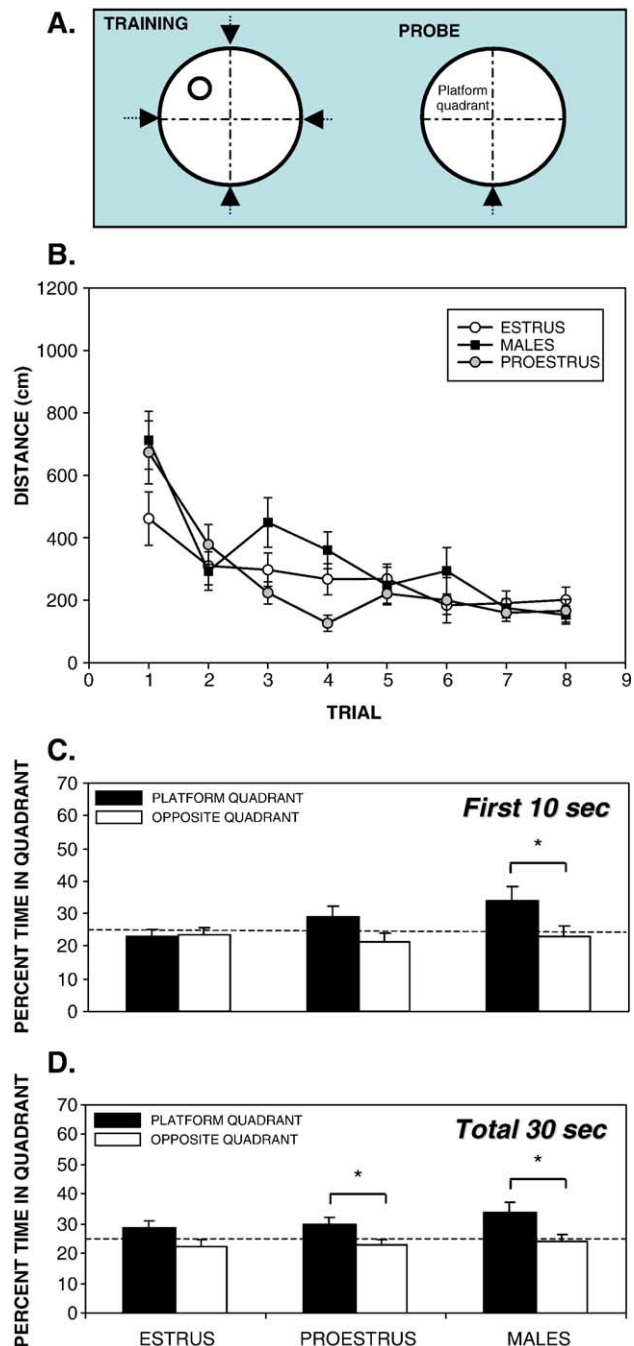


Fig. 1. (A) Schematic representation of the apparatus used in Experiment 1. Training trials configuration. The full circle represents the location of the visible platform. Probe trial configuration: the quadrant where the platform was located during training trials is called "Platform quadrant". Arrows represent starting locations. (B) Distance swam to reach the platform during training trials in Experiment 1. Each point represents the mean group performance ($\pm$ SEM) during each trial. (C) Probe trial performance during the first 10 s in Experiment 1. (D) Probe trial performance during the total 30 s in Experiment 1. For C and D, each bar represents percentage of time ($\pm$ SEM) spent in the correct quadrant. Asterisks represent significant differences between quadrants ($P < 0.05$). Estrous females ($n = 23$), proestrus females ($n = 21$), males ($n = 17$).

time interval between Experiment 1 and Experiment 2 was 2–3 complete cycles (8–15 days) for females, with a similar time interval for the males. In addition, females were tested at the same stage of the estrous cycle as they were during Experiment 1. Experiment 2 consisted of 16 training trials from 4 different starting locations, followed by a probe trial, all administered on the same day. Each block of 4 trials consists of 1 trial from each starting location, pseudo-randomly assigned within a block. The starting sequence was the same for all animals in the group. Rats were placed in the water facing the wall of the tank. A large white panel (35 cm high $\times$ 48 cm wide, covering 36.6° of the pool's circumference) was placed behind the platform on the pool wall (Fig. 2A). After reaching the platform, rats were allowed 10 s on the platform. If an animal did not reach the platform within 60 s, it was placed on the platform for 10 s. Animals were then removed and placed in opaque cages on heating pads for 3 min.

At the end of 16 trials, a probe trial was conducted. The hidden platform was removed from the water and the white panel was rotated 90° clockwise from its previous location (Fig. 2A). The rats were allowed to swim freely for 30 s.

Experiment 3

Seven males, ten proestrus females, and eleven estrous females were included in the analysis of Experiment 3. Procedure and methods for Experiment 3 were similar to those used in Experiment 2. The only difference was that the white panel was placed on the pool wall at a location 90° from the platform (see Fig. 3A).

Data analysis

The performance during the training trials was quantified based on the distance traveled from the starting point to the platform. Distance traveled was preferred over travel time as a measure of spatial ability. This eliminated potential confounds due to sex differences in swimming speed. The distance swum to reach the platform was analyzed by two-factor (group $\times$ trial) repeated-measures ANOVA. On probe trials, the platform was removed and the animals' behavior was examined over 30 s in the following manner:

1. Initial swim trajectory. Percentage of time spent in the goal quadrant (platform quadrant) was compared with percentage time spent in the opposite quadrant (for Experiment 1) or in the landmark quadrant (for Experiment 2 and 3) during the first 10 s of the probe using paired samples *t* tests.
2. Overall time spent in the goal quadrant versus time spent in the opposite quadrant (for Experiment 1) or the landmark quadrant (for Experiment 2 and 3) using paired samples *t* tests.
3. Overall time spent in the goal quadrant compared between groups, using one-way ANOVA.

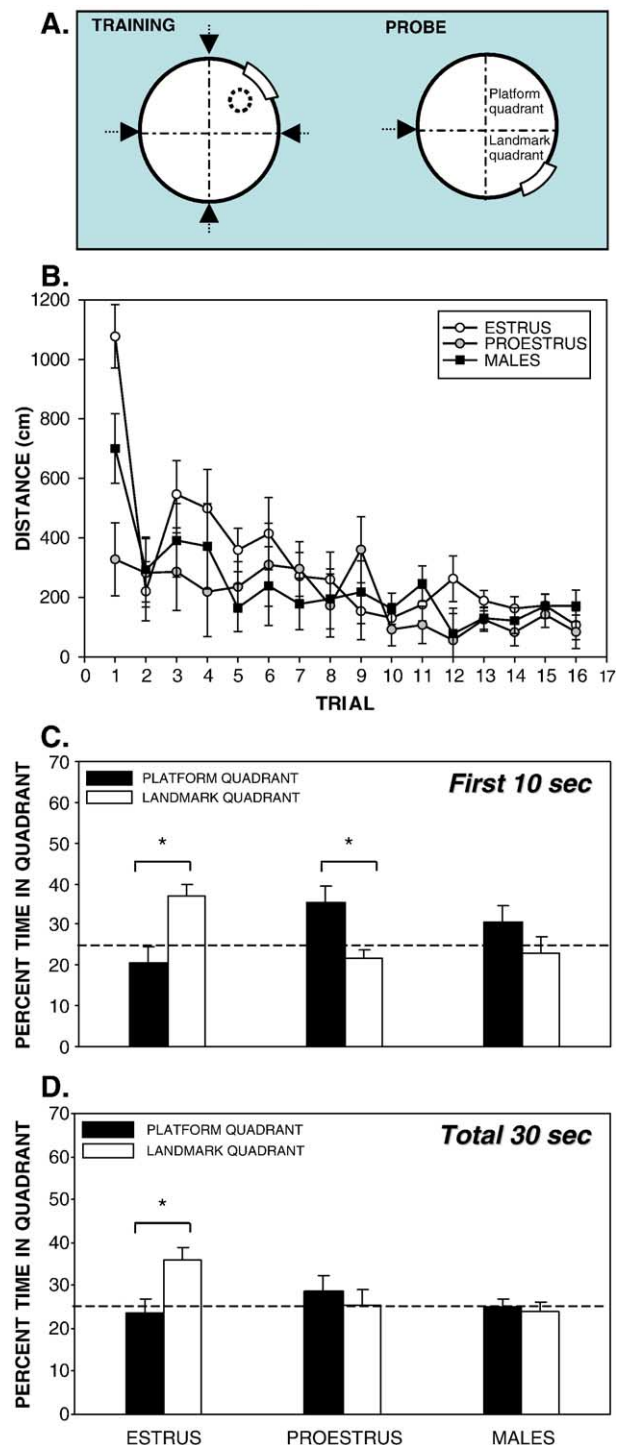


Fig. 2. (A) Schematic representation of the apparatus used in Experiment 2. Training trials configuration. The dotted circles represent the hidden platform. The white cue is placed on the pool wall behind the hidden platform. Probe trial configuration: the quadrant where the platform was located during training trials is called "Platform quadrant". (B) Distance swam to reach the platform in Experiment 2. Each point represents the mean group performance ($\pm$ SEM) during each trial. (C) Probe trial performance during the first 10 s in Experiment 2. (D) Probe trial performance over the total 30 s in Experiment 2. For C and D, each bar represents percentage of time ($\pm$ SEM) spent in the correct quadrant. Asterisks represent significant differences between quadrants ($P < 0.05$). Estrous females ($n = 12$) and proestrus females ($n = 9$) and males ($n = 10$).

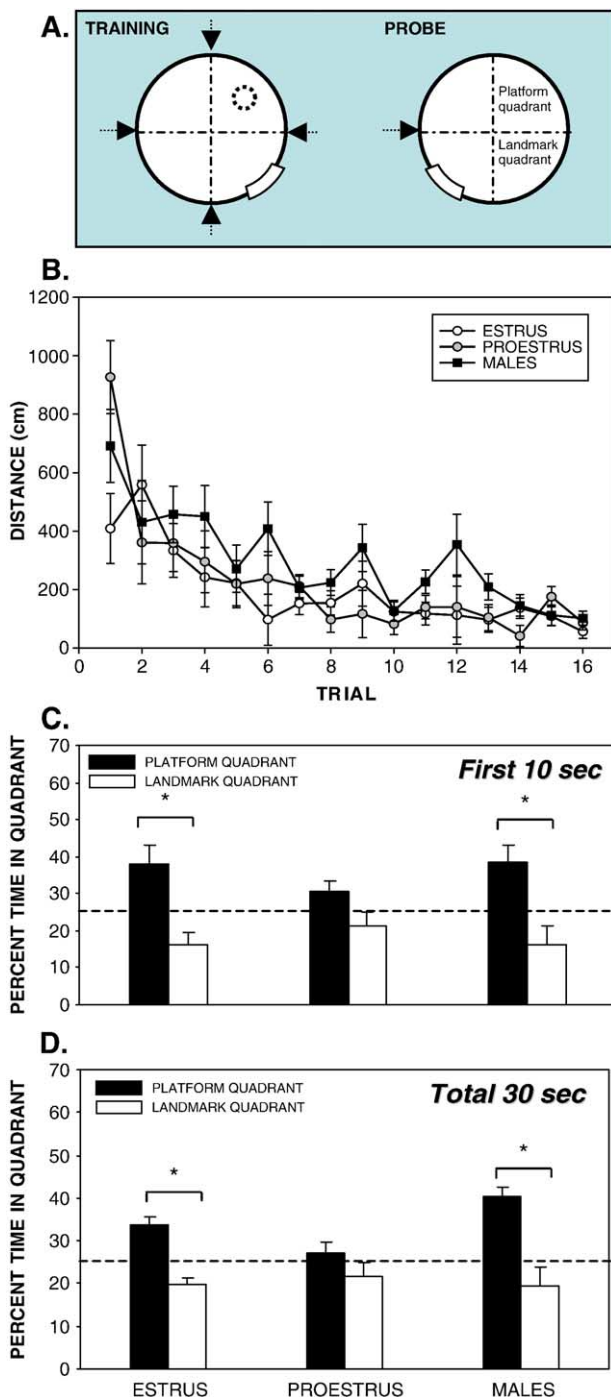


Fig. 3. (A) Schematic representation of the apparatus used in Experiment 3. Training trials configuration. The dotted circle represents the hidden platform. The white cue is placed on the pool wall, 90° shifted from the platform. Probe trial configuration: the quadrant where the platform was located during training trials is called "Platform quadrant". (B) Distance swam to reach the platform in Experiment 3. Each point represents the mean group performance ($\pm$ SEM) during each trial. (C) Probe trial performance during the first 10 s in Experiment 3. (D) Probe trial performance in Experiment 3. For B and C, each bar represents percentage of time ($\pm$ SEM) spent in the correct quadrant. Asterisks represent significant differences between quadrants ($P < 0.05$). Estrous females ($n = 11$), proestrus females ($n = 10$), males ($n = 7$).

Unless specified, for all non-significant results reported, P is greater than 0.1.

Results

Experiment 1

The rats in Experiment 1 were tested in 3 batches. Two batches were subsequently tested in Experiment 2, and the third batch was tested in Experiment 3. Performance of the rats was not different among the 3 batches (repeated-measures ANOVA for the distance swam to reach the platform during training trials). Therefore, the data from the 3 batches were pooled.

Training

During the training trials (Fig. 1B), all groups showed a decrease in the distance swam to reach the platform, as revealed by a two-factor (group by trial) ANOVA ($F(7,406) = 20.566$, $P < 0.001$). There was also a significant difference between groups ($F(2,58) = 4.648$, $P < 0.05$), and a significant group by trial interaction ($F(14, 406) = 2.005$, $P < 0.05$). Post hoc analysis (Tukey) indicated that males swam significantly longer distances than estrous females ($P < 0.05$) and proestrus females ($P < 0.05$). By the 5th trial though, all groups showed similar performance, swimming similar distances, as shown by repeated-measures ANOVA analysis of the last 4 trials.

Probe trial

If the subjects learned the task based on the extramaze cues, then during the probe they should show a spatial bias for the quadrant where the platform was located. If the subjects navigated only using the intramaze cue, once the visible cue was removed their performance in the platform quadrant should be at chance level.

An examination of the initial 10 s of the probe trial (Fig. 1C) revealed that the groups differed in the percentage of time spent in the platform quadrant ($F(2,60) = 3.26$, $P < 0.05$), with the males spending significantly more time in platform quadrant than the estrous females (Tukey, $P < 0.038$). For the other quadrants, there were no group differences. A preference for the platform versus the opposite quadrant during the first 10 s of the probe trial was significant for the males ($t_{16} = 2.313$, $P < 0.05$), with a similar trend for proestrus females ($t_{20} = 1.862$, $P = 0.077$).

An examination of the behavior across the whole 30-s probe (Fig. 1D) showed that proestrus females and males spent significantly more time in the platform quadrant compared with the opposite quadrant ($t_{20} = 2.099$, $P < 0.05$; $t_{16} = 2.416$, $P < 0.05$, respectively), while estrous females showed only a trend for significance ($t_{22} = 2.057$, $P = 0.052$). Moreover, independent-sample t tests showed that proestrus females and males spent significantly more than 25% (chance level) of their swim time in the target quadrant

($t_{20} = 2.228$, $P < 0.05$; $t_{16} = 2.292$, $P < 0.05$, respectively). Estrous females performed at chance levels. A comparison of the groups over the whole probe duration was not significant for any quadrant.

Experiment 2

The rats in Experiment 2 were run in 2 batches. A repeated-measures ANOVA showed that distance swum to reach the platform during training trials was not different between the 2 batches, so the data were pooled.

Training

The groups differed on the first trial ($F(2,30) = 10.655$, $P < 0.001$). A Tukey test revealed that estrous females swam a greater distance compared to proestrus females ($P < 0.001$). There was a trend for male swim distance to be intermediate between estrous and proestrus females ($P = 0.061$ and $P = 0.089$, respectively). An analysis of percentage of time spent in each of the 4 quadrants showed no difference among the 3 groups, with all of the groups spending a similar percentage of time in all quadrants (i.e., no spatial bias). Furthermore, this difference was limited to the initial trial. A repeated-measures ANOVA for the distance traveled to find the platform across all 16 training trials showed a significant main effect of trial ($F(15,420) = 9.020$, $P < 0.001$). There was no effect of group and no group by trial interaction (Fig. 2B).

Probe

In the probe trial, the platform was removed and the intramaze cue (white panel) was rotated 90° clockwise. A subject that learned the location of the hidden platform based on the extramaze environment should spend more time in the quadrant where the platform was previously located (“platform quadrant”). A subject that used the intramaze cue to navigate to the platform should spend more time in the new quadrant where the intramaze cue was located (“landmark quadrant”).

An examination of the initial 10 s of the probe trial (Fig. 2C) revealed the group differed in the percentage of time spent in the landmark and platform quadrants ($F(2,30) = 7.396$, $P < 0.01$; $F(2,30) = 3.438$, $P < 0.05$, respectively). Tukey comparisons showed that estrous females spent significantly more time in the landmark quadrant than males ($P < 0.05$) and proestrus females ($P < 0.01$), and significantly less time in the platform quadrant than proestrus females ($P < 0.05$). Comparing time spent in the landmark versus the platform quadrant during the first 10 s on the probe trial showed a preference for the landmark quadrant in estrous females ($t_{11} = 2.42$, $P < 0.05$). Conversely, proestrus females spent significantly more time in the platform than the landmark quadrant ($t_8 = 2.337$, $P < 0.05$).

An examination of the behavior across the whole 30-s probe (Fig. 2D) showed that estrous females spent more time in the landmark than in the platform quadrant ($t_{11} =$

2.225, $P < 0.05$). A one-way ANOVA showed that the percentage of time spent in the platform quadrant did not differ among groups. Conversely, the percentage of time spent in the landmark quadrant was significantly different among groups ($F(2,30) = 4.768$, $P < 0.05$). Tukey comparisons revealed that estrous females spent significantly more time than males ($P < 0.05$) and tended to spend more time than proestrus females ($P = 0.059$) in the landmark quadrant. No search bias was observed for the two other quadrants.

Experiment 3

Training

The distance swum on the first trial was different among groups ($F(2,30) = 4.512$, $P < 0.05$). A Tukey test revealed that estrous females swam less distance compared to proestrus females ($P < 0.05$). However, there was no difference in the percentage of time spent in either the cue or landmark quadrant.

Repeated-measures ANOVA for the distance traveled to find the hidden platform during the 16 training trials (Fig. 3B) showed an effect of trial ($F(15, 420) = 12.875$, $P < 0.001$) and of group ($F(2,28) = 3.374$, $P < 0.05$), with no group by trial interaction. A Tukey test revealed that males traveled longer distances to reach the platform than estrous females ($P < 0.05$). However, towards the end of training, all groups exhibited similar performance, and an analysis of the last 4 training trials showed no group difference.

Probe

In the probe trial, the hidden platform was removed and the intramaze landmark (white panel) rotated 90° clockwise to allow for an examination of cue usage. A subject that learned the location of the hidden platform based on the extramaze cues should spend more time in the quadrant where the platform was previously located (“platform quadrant”). A subject that used the intramaze landmark to navigate to the platform should spend more time in the quadrant 90° clockwise from where the platform had been located (“landmark quadrant”).

An examination of the initial 10 s of the probe trial (Fig. 3C) showed no group difference in the percentage of time spent in any of the quadrants. Males and estrous females spent significantly more time in the platform quadrant than in the landmark quadrant during the first 10 s on the probe trial ($t_6 = 3.781$, $P < 0.05$; $t_{10} = 2.849$, $P < 0.05$, respectively).

As can be seen in Fig. 3D, over the full 30 s of the probe trial males and estrous females spent significantly more time in the platform quadrant than in the landmark quadrant ($t_6 = 4.611$, $P < 0.05$; $t_{10} = 4.333$, $P < 0.05$, respectively). Proestrus females performed at chance levels in both quadrants. A one-way ANOVA showed that the percentage of time spent in the platform quadrant was different among groups ($F(2,27) = 8.184$, $P < 0.01$). A Tukey test revealed

that males spent significantly more time than proestrus females in the platform quadrant ($P < 0.001$). There were no differences among groups with regard to the percentage of time spent in the landmark quadrant. No search bias was observed for the other 2 quadrants.

The rats subsequently run on Experiment 2 or 3 were similar in their performance in Experiment 1. Repeated-measures ANOVA showed no main effect of group (Experiment 2 and 3 rats) and no group $\times$ trial interaction for the distance swam during learning trials in Experiment 1, for either males or estrous and proestrus females. Moreover, t tests comparing the percentage of time spent in target quadrant in Experiment 1 showed no significant difference between males, estrous females, or proestrus females that were run in Experiments 2 and 3.

Discussion

The current series of experiments was designed to examine the relative influence of intramaze and extramaze information during navigation in the water maze in three different experiments. All rats were tested in Experiment 1, and then they were tested in either Experiment 2 or Experiment 3. The rats tested on the latter 2 experiments were similar in their performance on Experiment 1, and this allowed us to compare the results obtained in Experiments 2 and 3.

Experiment 1

The results indicate that despite the salient local cue, rats were also to-some-degree attending to extramaze information in the environment. This is similar to the findings in mice. Hauben et al. (1999) showed that saline-treated mice that received training in the visible platform version of a water maze task tended to spend more time in the quadrant where the platform was located, when administered a probe trial. The weak spatial learning in the current study precluded finding large differences among the groups; however, proestrus females and males showed a somewhat stronger influence of extramaze cues than estrous rats, with these groups initially swimming towards the correct quadrant and remaining there more than the estrous group.

Experiment 2

During training trials in Experiment 2, both the intramaze landmark and the extramaze environment predicted the location of the platform. During the probe trial, the rats were faced with a change in the spatial relation between the intramaze and extramaze cues. The probe trial in Experiment 2 encompassed a mismatch between the intra- and extramaze cues, this was different from the probe trial of Experiment 1, where the intramaze cue was simply removed. The proestrus females initially used an extramaze

search strategy followed by a search of all the quadrants, a fact reflected by chance level performance in both platform and landmark quadrants. This type of search behavior differs from that found during “normal” water maze tasks (Morris, 1984; Stoelzel et al., 2002), where the animals tend to continue searching in the quadrant where the platform had been. Male rats also showed a search of all quadrants (Fig. 4B). A different pattern was seen in estrous females. They showed a sustained search pattern for the platform in the intramaze-defined landmark quadrant (Fig. 4A). These estrous cycle effects are in agreement with recently published data showing an effect of estrogen on strategy selection in the water maze. Daniel and Lee (2004) had a visible landmark located 20 cm to the north of the hidden platform during training trials, a situation similar to our Experiment 2. Removal of the visible landmark during the probe trial impaired performance of ovariectomized females receiving control treatment, indicating that they relied on the landmark in order to locate the platform, similar to our estrous group. Conversely, removal of the visible landmark in the probe trial had no effect on ovariectomized females receiving estrogen replacement, suggesting that estrogen biased the animals against solely using a cue strategy, similar to our proestrus group.

Experiment 3

During training trials in Experiment 3, both the intramaze cue and the extramaze cues predicted the location of the platform. However, unlike Experiment 2, the intramaze cue was not directly behind the goal. During the probe trial, the rats were faced with a conflict between the intra- and extramaze information, a situation similar to Experiment 2. In this situation, the rats tended to use the extramaze cues more than the intramaze cue when searching for the goal. This was seen both in the initial swim path and over the full 30-s probe. The proestrus rats were an exception, they failed to initially swim to either goal location and over the 30 s of

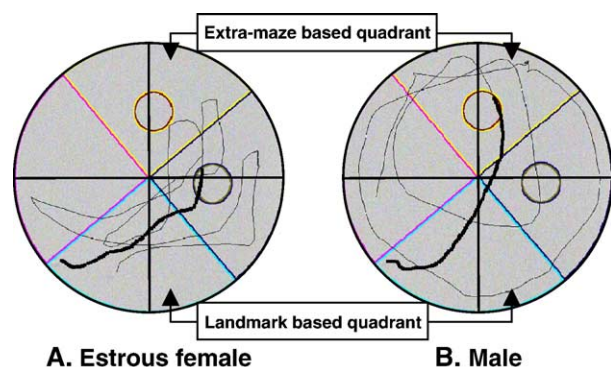


Fig. 4. Representative examples of paths traveled by an estrous female (A) and a male (B) during the probe trial in Experiment 2. The circles represent hypothetical locations of the platform. The initial swim path until reaching one of the two possible platform locations is shown as thick line.

the probe they did not show a preference nor alternate between the two possible goal locations.

The influence of intramaze cue location—males

The influence of the intramaze cue was related to its proximity to the goal. However, this was shown to differ among males and estrous and proestrus females. Most of the literature regarding cue use in navigation has been previously done on male rats; therefore, the discussion will first focus on the males. This will be followed by an examination of the sex/estrous cycle effects.

The data from the current study suggest that when both intra- and extramaze cues are available, the specific location of the intramaze cue in relation to the goal influences the strategy used to solve the task. When the intramaze landmark coincided with the goal (Experiment 1), it had a strong influence on behavior. The probe trial (removal of the visible cue) showed that male rats were only weakly influenced by extramaze cues. When the landmark was not at the goal but still situated in close proximity to it (Experiment 2), the influence of both the intra- and extramaze cues on rats' navigation was similar. In response to the conflicting information provided by these two types of cues, the rats proceeded to explore the whole maze. When the intramaze cue was situated on the wall 90° from the goal (Experiment 3), its influence on behavior was weak. During the probe session, the animals swam to the extramaze-defined location and seemed to ignore the intramaze cue. Taken together, the results of Experiments 2 and 3 indicate a reduction in the influence of the intramaze cue the further away it was situated from the goal.

This changing effectiveness of the cue cannot be explained by differences in size or color of the cue. The influence of the cue was weakest in Experiment 3; however, the same cue was more effective in Experiment 2. Furthermore, the intramaze cue was most effective in Experiment 1, even though the cue used in Experiments 2 and 3 (white panel) was almost 20 times larger. Consequently, the results indicate that the proximity of the intramaze cue to the goal was the decisive factor for how influential this cue was in navigation. The powerful influence of a cue at a goal was also shown by Kanit et al. (1998). After 3 days of visible platform training (4 trials/day), hiding the platform caused a substantial impairment in male search behavior (Figs. 1 and 4H in Kanit et al., 1998). These results contrast findings of goal-directed behavior in a circular high walled cylinder by Lenck-Santini et al. (2002). In their study, when the cues were rotated without the animal present, the rats used the intramaze cues regardless of their proximity to the goal. It is plausible that their results were due to the paucity of available extramaze information (a uniform circular curtain). Interestingly, when the intramaze cue rotations occurred in the presence of rats, they followed the cues only when they were adjacent to the goal. Furthermore, when there was a

conflict between an intramaze cue at the goal and a cue on the wall they tended to follow the cue at the goal (Lenck-Santini et al., 2002).

Many factors affect which stimuli are attended to (Rescorla and Wagner, 1972). The current results indicate that at least one important factor influencing the degree to which the intramaze cues are used in males is their proximity to the goal.

The influence of intramaze cue location—females

Manipulating the location of the intramaze cue also affected navigation patterns in female rats. The manner in which it was affected was related to the estrous cycle stage of the animal. Estrous females were strongly affected by the intramaze cue when it was at or adjacent to the goal. Thus taking away the cue at the goal (Experiment 1) resulted in a random search pattern, and rotating the cue adjacent to the goal (Experiment 2) resulted in a corresponding rotation of their search. Only when the cue was situated on the pool wall 90° from the goal did estrous females show an extramaze-based search pattern.

The results were quite different for the proestrus females. In all three experiments, the proestrus rats showed the influence of both the intra- and extramaze cues. Even when the cue was at the goal (Experiment 1), these animals showed a weak spatial bias in their search. When placing the cue adjacent (Experiment 2) or 90° (Experiment 3) from the goal, a few of the rats initially swam to either the intramaze-based or the extramaze-based location. However, most of the proestrus animals did not show an initial preference for either location and over the course of the probe displayed a random search.

Conflicting spatial and intramaze information resulted in a "new environment"

All animals showed significant learning of the platform location and were at an asymptotic level of performance before being tested on the probe. However, both males (Experiment 2) and proestrus females (Experiment 2 and 3) displayed random search patterns during probe sessions. The fact that after an initial search the rats started searching in all the quadrants is quite different from the search behavior "normally" found during probes in the water maze. Usually, after the animals have learned the location of a hidden platform, they tend to search in the location where the platform had been even after it has been removed (e.g., Morris, 1984; Stoelzel et al., 2002; Warren and Juraska, 2000). Furthermore, in the present study, the animals did not alternate between the two possible locations. Rather, once the platform was not initially found, the animals' search pattern in the maze showed no savings/effect of the local or distal cues, seemingly treating the probe situation as a new environment. Similarly, hippocampal recordings have

shown that a conflict between cues in a previously familiar environment can result in a new spatial representation of the environment (Knierim et al., 1995; Tanila et al., 1997).

A comparison of males and estrous and proestrus females

In our study, males were initially slower at learning two of the tasks (Experiment 1 and 3). Usually, males learn spatial tasks faster than females; however, this is not always the case (Markowska, 1999; Warren et al., 1990). Importantly, all groups of animals showed equivalent performance before being tested on the probe. Furthermore, the probe analysis focused on relative cue use and not on absolute performance.

Our results indicate a difference in cue utilization in males and proestrus and estrous females. Estrous females tended to be most affected by the dominant cue. When the intramaze cue coincided with or was adjacent to the goal, it affected their behavior more than the other groups of rats. When the intramaze cue was distant from the goal, the cue manipulation did not disrupt their ability to use the extramaze cues. In contrast, the proestrus females seemed to be sensitive to all the cues, both intra- and extramaze. Under all three conditions, they were affected by both the intramaze cue manipulation and the extramaze environment. The males were affected by the extramaze environment under all conditions and by the intramaze cue only when it was adjacent to the goal. Kanit et al. (1998) also showed sex differences in relative use of intra- and extramaze cues; however, they did not examine the female hormonal cycle.

It should be noted that despite the fact that performance in Experiment 1 was similar, there were some estrous cycle-related differences on the first trial of Experiments 2 and 3. This may indicate differences in the initial manner in which these females explore the maze, differences that could be related to their previous experience in Experiment 1. The current study was not designed to systematically examine the effect of previous experience on a new maze/room configuration. However, this is an interesting issue that should be addressed in future studies.

Navigation and the estrous cycle

An elegant series of studies by Korol and colleagues (Korol and Kolo, 2002; Korol et al., 2004) showed that estrous females tend to focus more on egocentric information (e.g., muscle response, path integration) than on extramaze information when solving a simple navigation task, while proestrus females are more affected by the extramaze information. Based on the idea of competition between brain systems (Devan and White, 1999; White, 2004), they proposed changes in the balance between the hippocampus and the striatum as the mechanism underlying these different styles of interacting with the environment. Specifically, estrogen enhances hippocampus-dependent

place learning, while impairing striatum-dependent response learning in ovariectomized rats given estrogen replacement (Korol and Kolo, 2002) and in naturally cycling female rats (Korol et al., 2004). While their data can explain some behavioral differences across the estrous cycle, it cannot address all the diverse results found for tasks dependent on hippocampus with no striatum-dependent alternative.

In most previous studies on navigation and hippocampal function, the animals were tested on standard hippocampus-dependent tasks and levels of performance compared. The results have been confusing, with conflicting findings from different labs. Some of the results seem to indicate that estrogen has different effects on working and reference memory tasks (see Desmond and Levy, 1997; Dohanich, 2002). While this may explain some of the different findings across tasks, it is less good at explaining conflicting results on a single task. Thus, natural cycling females have been shown to perform better on the water maze during estrous (low estrogen) than proestrus (high estrogen) by some (Frye, 1995; Warren and Juraska, 1997) but not others (Berry et al., 1997).

Furthermore, simply comparing performance on what would seem to be hippocampal tasks can yield seemingly conflicting results even within the same lab. The results of Experiment 1 could be interpreted as proestrus rats being “more spatial” than estrous females, since proestrus animals showed a spatial search when the local cue was removed. This contrasts other results in our lab where it was the animals in estrus that showed the more spatial/hippocampus-based response. Estrous females showed a stronger response to manipulations of the position of a local landmark in an open field (Tropp and Markus, 2001) and showed stronger contextual fear retention (Markus and Zecevic, 1997) than proestrus animals. The findings from the current series of experiments suggest a different interpretation of the behavioral findings reported by our lab and others. We propose that proestrus animals tend to be affected by and attend to a wider range of stimuli, while animals in estrus are more affected by fewer salient/dominant stimuli. Thus, in the open field, estrous animals were more affected by changes in the dominant local cue than proestrus animals (Tropp and Markus, 2001). During contextual fear conditioning, there are many uncontrolled transient, irrelevant stimuli which are thought to have only a weak (if any) effect on the animal (e.g., changes in the whine of a fan, vibrations from doors closing, small changes in lighting). If proestrus animals show an increased attendance to these “minor-unreliable” contextual cues and estrous animals tend to focus more on the more salient contextual information, one would get better context retention in the estrous animals (Markus and Zecevic, 1997).

This interpretation can also explain the conflicting water maze results where the estrous females are sometimes better than proestrus females. It has been pointed out that the

impairment of proestrus females is countered by pre-training the animals on the task (Bannerman et al., 1995; Berry et al., 1997; Warren and Juraska, 1997). The current findings may indicate that the pre-training helps the proestrus animals focus only on the relevant environmental cues and be less distracted by other cues.

It had been hypothesized that estrogen influences the strategy selection in situations when several strategies are available (Korol et al., 2004). This hypothesis refers to the dynamics of interaction and/or competition between brain systems. Our data support the idea that high levels of ovarian hormones during proestrus biased females against using a single spatial landmark, but rather to use multiple sources of information. It is unclear to what degree this reflects flexibility solely within the hippocampal system or an interaction with other brain regions.

The idea that proestrus females are making many associations, even to irrelevant (to the experimenter) stimuli, is consistent with the neurobiological changes in the hippocampus across the cycle. Animals in proestrus have more synaptic spines (Woolley and McEwen, 1992) and show increased synaptic plasticity (Cordoba Montoya and Carrer, 1997; Good et al., 1999; Terasawa and Timiras, 1968; Warren et al., 1995).

Taken together, the biological data, previous behavioral studies, and the current results suggest that during proestrus females have an increased tendency to make associations to multiple stimuli. Depending on the specific task, procedure, and environment, in some situations this will manifest as improved performance while in others as impairment.

Acknowledgments

We thank Courtney Ellard, Christina Figueiredo, and Shaun Gruenbaum for their assistance with lavaging and data analysis. We also thank Dr. Jennifer Tropp for her suggestions in preparing the manuscript. A portion of the results was presented in a preliminary form as a Society for Neuroscience abstract (Sava et al., 2002).

References

- Bannerman, D.M., Good, M.A., Butcher, S.P., Ramsay, M., Morris, R.G., 1995. Distinct components of spatial learning revealed by prior training and NMDA receptor blockade. *Nature* 378, 182–186.
- Benhamou, S., Poucet, B., 1998. Landmark use by navigating rats (*Rattus norvegicus*): contrasting geometric and featural information. *J. Comp. Psychol.* 112, 317–322.
- Berry, B., McMahan, R., Gallagher, M., 1997. Spatial learning and memory at defined points of the estrous cycle: effects on performance of a hippocampal-dependent task. *Behav. Neurosci.* 111, 267–274.
- Cheng, K., 1986. A purely geometric module in rat's spatial representation. *Cognition* 23, 149–178.
- Chesler, E.J., Juraska, J.M., 2000. Acute administration of estrogen and progesterone impairs the acquisition of the spatial Morris water maze in ovariectomized rats. *Horm. Behav.* 38 (4), 234–242.
- Collett, T.S., Collett, M., 2002. Memory use in insect visual navigation. *Nat. Rev., Neurosci.* 3, 542–552.
- Cordoba Montoya, D.A., Carrer, H.F., 1997. Estrogen facilitates induction of long-term potentiation in the hippocampus of awake rats. *Brain Res.* 778, 430–438.
- Daniel, J.M., Dohanich, G.P., 2001. Acetylcholine mediates the estrogen-induced increase in NMDA receptor binding in CA1 of the hippocampus and the associated improvement in working memory. *J. Neurosci.* 21 (17), 6949–6956.
- Daniel, J.M., Lee, C.D., 2004. Estrogen replacement in ovariectomized rats affects strategy selection in the Morris water maze. *Neurobiol. Learn. Mem.* 82 (2), 142–149.
- Daniel, J.M., Fader, A.J., Spencer, A.L., Dohanich, G.P., 1997. Estrogen enhances performance of female rats during acquisition of a radial arm maze. *Horm. Behav.* 32 (3), 217–225.
- Daniel, J.M., Roberts, S.L., Dohanich, G.P., 1999. Effects of ovarian hormones and environment on radial maze and water maze performance of female rats. *Physiol. Behav.* 66 (1), 11–20.
- Devan, B.D., White, N.M., 1999. Parallel information processing in the dorsal striatum: relation to hippocampal function. *J. Neurosci.* 19, 2789–2798.
- Desmond, N.L., Levy, W.B., 1997. Ovarian steroidal control of connectivity in the female hippocampus: an overview of recent experimental findings and speculations on its functional consequences. *Hippocampus* 7, 239–245.
- Dohanich, G.P., 2002. Gonadal steroids, learning and memory. In: Pfaff, D.W., Arnold, A.P., Etgen, A.M., Fahrbach, S.E., Rubin, R.T. (Eds.), *Hormones, Brain and Behavior*, vol. 2. Academic Press (Elsevier Science), San Diego, pp. 265–327.
- Fader, A.J., Hendricson, A.W., Dohanich, G.P., 1998. Estrogen improves performance of reinforced T-maze alternation and prevents the amnesic effects of scopolamine administered systemically or intrahippocampally. *Neurobiol. Learn. Mem.* 69 (3), 225–240.
- Fader, A.J., Johnson, P.E., Dohanich, G.P., 1999. Estrogen improves working but not reference memory and prevents amnesic effects of scopolamine of a radial-arm maze. *Pharmacol. Biochem. Behav.* 62 (4), 711–717.
- Frick, K.M., Berger-Sweeney, J., 2001. Spatial reference memory and neocortical neurochemistry vary with the estrous cycle in c57bl/6 mice. *Behav. Neurosci.* 115, 229–237.
- Frye, C.A., 1995. Estrus-associated decrements in a water maze task are limited to acquisition. *Physiol. Behav.* 57, 5–14.
- Gallistel, C.R., 1990. *The Organization of Learning*. MIT Press, Cambridge, MA.
- Good, M., Day, M., Muir, J.L., 1999. Cyclical changes in endogenous levels of oestrogen modulate the induction of LTD and LTP in the hippocampal CA1 region. *Eur. J. Neurosci.* 11, 4476–4480.
- Gould, E., Woolley, C.S., Frankfurt, M., McEwen, B.S., 1990. Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J. Neurosci.* 10, 1286–1291.
- Greene, C.M., Cook, R.G., 1997. Landmark geometry and identity controls spatial navigation in rats. *Anim. Learn. Behav.* 25, 312–323.
- Hauben, U., D'Hooge, R., Soetens, E., De Deyn, P.P., 1999. Effects of oral administration of the competitive *N*-methyl-D-aspartate antagonist, CGP 40116, on passive avoidance, spatial learning, and neuromotor abilities in mice. *Brain Res. Bull.* 48, 333–341.
- Kanit, L., Takran, D., Furedy, J.J., Kulali, B., McDonald, R., Pögun, S., 1998. Nicotine interacts with sex in affecting rat choice between “look-out” and “navigation” cognitive styles in the Morris water maze place learning task. *Brain Res. Bull.* 46, 441–445.
- Knierim, J.J., Kudrimoti, H.S., McNaughton, B.L., 1995. Place cells, head direction cells and the learning of landmark stability. *J. Neurosci.* 15, 1648–1659.
- Korol, D.L., Kolo, L.L., 2002. Estrogen-induced changes in place and response learning in young adult female rats. *Behav. Neurosci.* 116, 411–420.
- Korol, D.L., Malin, E.L., Borden, K.A., Busby, R.A., Couper-Leo, J., 2004.

- Shifts in preferred learning strategy across the estrous cycle in female rats. *Horm. Behav.* 45 (5), 330–338.
- Lenck-Santini, P.P., Muller, R.U., Save, E., Poucet, B., 2002. Relationships between place cell firing fields and navigational decisions by rats. *J. Neurosci.* 22, 9035–9047.
- Luine, V.N., Richards, S.T., Wu, V.Y., Beck, K.D., 1998. Estradiol enhances learning and memory in a spatial memory task and effects levels of monoaminergic neurotransmitters. *Horm. Behav.* 34 (2), 149–162.
- Margules, J., Gallistel, C.R., 1988. Heading in the rat: determination by environmental shape. *Anim. Learn. Behav.* 16, 404–410.
- Markowska, A.L., 1999. Sex dimorphisms in the rate of age-related decline in spatial memory: relevance to alterations in the estrous cycle. *J. Neurosci.* 19, 8122–8133.
- Markus, E.J., Zecevic, M., 1997. Sex differences and estrous cycle changes in hippocampus-dependent fear conditioning. *Psychobiology* 25, 246–252.
- Morris, R.G.M., 1984. Development of the water-maze procedure for studying learning in the rat. *J. Neurosci.* 11, 47–60.
- O'Keefe, J., Nadel, L., 1978. *The Hippocampus as a Cognitive Map*. Clarendon Press, Oxford.
- Rescorla, R.A., Wagner, A.R., 1972. A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and non-reinforcement. In: Black, A.H., Prokasy, W.F. (Eds.), *Classical Conditioning II. Current Research and Theory*. Appleton-Century-Crofts, pp. 64–99.
- Sandstrom, N.J., Williams, C.L., 2001. Memory retention is modulated by acute estradiol and progesterone replacement. *Behav. Neurosci.* 115 (2), 384–393.
- Sava, S., Astur, R.S., Markus E.J., 2002. Spatial ability in rodents and humans: Cue utilization, sex differences and the influence of the natural hormone state. *Society for Neuroscience Abstracts*. 375.8.
- Schwegler, H., Mueller, G.G., Crusio, W.E., Szemes, L., Seress, L., 1993. Hippocampal morphology and spatially related behavior in Long–Evans and CFY rats. *Hippocampus* 3, 1–7.
- Sherry, D., Duff, S., 1996. Behavioural and neural bases of orientation in food-storing birds. *J. Exp. Biol.* 199, 165–172.
- Stackman, R.W., Blasberg, M.E., Langan, C.J., Clark, A.S., 1997. Stability of spatial working memory across the estrous cycle of Long–Evans rats. *Neurobiol. Learn. Mem.* 67, 167–171.
- Stoelzel, C.R., Stavnezer, A.J., Denenberg, V.H., Ward, M., Markus, E.J., 2002. The effects of aging and dorsal hippocampal lesions: performance on spatial and nonspatial comparable versions of the water maze. *Neurobiol. Learn. Mem.* 78, 217–233.
- Sutherland, R.J., Rudy, J.W., 1989. Configural association theory: the role of the hippocampal formation in learning, memory, and amnesia. *Psychobiology* 17, 129–144.
- Tanila, H., Sipila, P., Shapiro, M., Eichenbaum, H., 1997. Brain aging: impaired coding of novel environmental cues. *J. Neurosci.* 17, 5167–5174.
- Terasawa, E., Timiras, P., 1968. Electrical activity during the estrous cycle of the rat: cyclic changes in limbic structures. *Endocrinology* 83, 207–216.
- Tropp, J., Markus, E.J., 2001. Sex differences in the dynamics of cue utilization and exploratory behavior. *Behav. Brain Res.* 119, 143–154.
- Warren, S.G., Juraska, J.M., 1997. Spatial and nonspatial learning across the rat estrous cycle. *Behav. Neurosci.* 111, 259–266.
- Warren, S.G., Juraska, J.M., 2000. Sex differences and estropausal phase effects on water maze performance in aged rats. *Neurobiol. Learn. Mem.* 74, 229–240.
- Warren, S.G., Wilson, L.A., Nadel, L., 1990. Sexually dimorphic spatial abilities in the Morris water task. *Abstr.-Soc. Neurosci.* 16, 1321.
- Warren, S.G., Humphreys, A.G., Juraska, J.M., Greenough, W.T., 1995. LTP varies across the estrous cycle: enhanced synaptic plasticity in proestrous rats. *Brain Res.* 703, 26–30.
- White, N.M., 2004. The role of stimulus ambiguity and movement in spatial navigation: a multiple memory systems analysis of location discrimination. *Neurobiol. Learn. Mem.* 28, 216–229.
- Williams, C.L., Barnett, A.M., Meck, W.H., 1990. Organizational effects of early gonadal secretions on sexual differentiation in spatial memory. *Behav. Neurosci.* 104, 84–97.
- Woolley, C.S., McEwen, B.S., 1992. Estradiol mediates fluctuations in hippocampal synaptic density during the estrous cycle in the adult rat. *J. Neurosci.* 12, 2549–2554.