

Research report

Sex differences in the dynamics of cue utilization and exploratory behavior

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Abstract

The purpose of the present study was to investigate the effects of sex and estrous cycle on exploratory behavior, as well as the degree to which reliance on environmental cues changes with training. Fischer 344 rats were placed three times in an open field box that contained three objects (two identical bottles and a cylinder). During the *initial* exposure to the environment all females showed higher activity levels and explored a larger region of the environment compared to males. However, upon subsequent exposure to the same environment, these sex differences disappeared. During the third and final session, the locations of the bottle and the cylinder were switched. The estrous females and to a lesser degree male rats, responded to the relocation of objects with a renewal of exploration and activity; proestrous females did not show this response. The rats were then trained on a four-arm radial maze reference memory task. The correct arm could be located by its relation to extra-room cues, a large distal white panel, or to local inserts on the maze. Once the animals consistently chose the goal arm, a probe session was conducted to determine which cues the animals were using to solve the task. During the probe trial both the white panel and the local inserts were rotated 90° clockwise and counterclockwise respectively and the animals' choice of arm recorded. During the first probe, females tended to rely on all three types of cues in solving the task. With additional training there was a shift towards predominantly using the distal visual information. In contrast, male rats did not show this shift; by the first probe session the males were predominantly using the distal visual information to solve the task. The findings indicate: (1) sex differences in the initial use of environmental cues; (2) the usage of environmental information is dynamic and changes with additional exposures to the environment. The results are related to previous findings on sex differences and estrous cycle effects, with an emphasis on the implications for hippocampal processing. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Sex differences in spatial processing have been reported in humans [8,15]. However, the degree to which these differences are innate or reflect differential experience is a matter of debate [31]. One approach to addressing these issues is to turn to animal studies where the environment is better controlled. Sex differences in spatial ability are found in rodents despite the attempt to maintain an equal and constant environment

for all animals [2,49,65]. The reported differences on spatial tasks could be the consequence of sex differences in activity levels [1,5], emotionality [1], or reflect a difference in the manner in which female and male rats explore their environment. For example, when rats are placed in an open field, males tend to defecate more and ambulate less than females [1,5].

One approach to address the issue of sex differences in spatial ability is to investigate which aspect the animals are attending to in the environment. Sex differences in cue use during navigation tasks have also been found in humans [50] and in rats [64,65].

The first study was designed to examine the effects of sex and estrous cycle on exploratory behavior. The

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experiment allowed for the assessment of how an animal explores a new environment, what aspects of the environment it attends to, and how this behavior changes with subsequent exposures to the environment. The second study was designed to examine for sex differences in the reliance on a distal white panel, local sensory cues, or extra-room sources of information to find a designated food reward. Additionally, the study examined whether animals change their reliance on these different types of environmental cues with extended training.

2. Experiment 1

The purpose of the present study was to investigate differences in activity and exploration. Both sex and estrous cycle effects were examined during initial and subsequent exposures to a given environment.

2.1. Materials and methods

2.1.1. Subjects

Twenty-two female and ten male (approximately 7 months old) Fischer 344 retired breeders (Harlan Sprague–Dawley, IN) were used in the experiment. Rats were singly housed in transparent plastic tubs, in a room with a 12:12 h light:dark cycle (lights off at 19:00 h). All animals were extensively handled before testing (3 weeks handling; 2 weeks lavaged). The females received daily vaginal lavages, between 13:00 and 15:00 h, to assure they were showing a regular cycle. The lavages were examined under a light microscope to identify the proportion of cornified epithelial cells, nucleated epithelial cells, and leukocytes [53]. As a handling control, the males were also held supine and anal probed with a cotton swab daily.

Testing was conducted between 14:00 and 17:00 h across a 5 day period. Ten of the females were tested during proestrus and 11 were tested during estrus. Verification of the animal's cycle stage was conducted both on the day of testing and on the following day.

2.1.2. Apparatus

The object exploration box was made of clear Plexiglas ($71 \times 71 \times 30 \text{ cm}^3$), with two identical glass bottles and one red cylinder in the box (Fig. 1). The testing room ($2.1 \times 2.7 \text{ m}^2$) was painted black except for a dim light in the southeast corner of the room and a white panel ($123 \times 243 \text{ cm}^2$) located on the south (S) wall of the box.

2.1.3. Procedure

After the animal was anal probed (male) or vaginally lavaged (female), the rat was taken from its colony room to a dimly lit outer room. The rat was removed

from its cage, carried into the testing room, and placed into the exploration box facing the southwest corner (Fig. 1a). The experimenter left the room and after approximately 5 s started to record the animal's position every 5 s, for 4 min. At the end of the 4 min the rat was taken out of the exploration box and placed back into its home cage, in the outer room. At this time, the box and the objects were cleaned with 30% isopropyl alcohol. After an inter-trial interval of approximately 60 s, the rat was placed back into the exploration box for a second session, and the same procedure followed. After the second session, the box was re-cleaned and the 'southeast' bottle and the 'northwest' red cylinder were switched (Fig. 1b). The rat was then placed back into the box for a final 4 min session.

2.1.4. Data collection and analysis

The animals were video taped via an overhead camera during the three sessions in the exploration box. The exploration box was projected onto a television screen where it was divided into a 7×7 grid matrix, consisting of 49 areas (each $10 \times 10 \text{ cm}^2$). Two observers recorded the animal's location every 5 s during the experiment. The first was present in the outer room at the time of behavioral testing, the second examined the videotape. Activity, exploration level, and defecation were calculated for each animal per session.

2.1.4.1. Activity level. Activity level refers to the percent of time the animal was moving. The animal's head position was recorded every 5 s for each session (each session 4 min). The object exploration box was divided into 49 squares of equal area. If the animal changed position from the previous square, it was defined as moving during those 5 s. Consequently, this measure could include repetitive visits into the same area during a session. Movement was totaled for each session. Activity was calculated by dividing the number of movements by the total amount of time bins by 100.

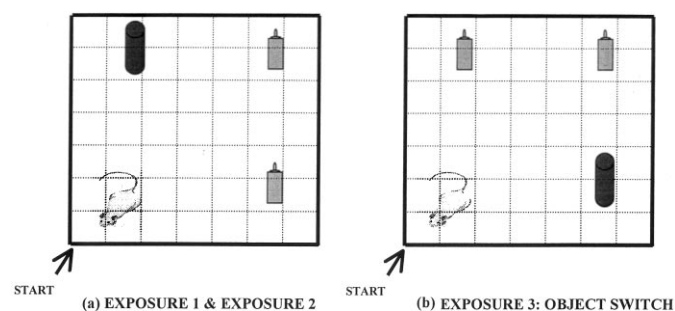


Fig. 1. Exploration box. (a) The box was made of clear plexiglas material ($71 \times 71 \times 30 \text{ cm}^3$) which contained two glass bottles and one red cylinder. The animal was placed in the southwest corner of the box and given three 4 min sessions to explore the environment. (b) During the third 4 min exposure, the locations of the northwest red cylinder and the southeast bottle were switched.

2.1.4.2. Exploration level. Exploration was defined as the percent of movement into new areas. Exploration was calculated by dividing the number of visits into new areas (i.e., locations not visited before) by 49 (total possible visits) and multiplying the number by 100. This measure was able to distinguish between an animal that was only moving back and forth in a very limited portion of the box and one that explored a larger region.

2.1.4.3. Statistical analyses. Data analysis was conducted for both activity level and exploratory behavior for the three sessions. In addition, paired *t*-tests compared performance (activity and exploration) of the males, proestrous females, and estrous females, in the first two sessions and the final two sessions.

2.1.4.4. Defecation. Defecations were monitored for all three sessions.

2.1.4.5. Inter-observer reliability. All the data were coded by both observers and the observations were highly correlated on all measures (i.e., activity level and exploratory behavior) and across all of the sessions (range $r = 0.83–0.92$).

2.2. Results

Activity and exploration during the three sessions are depicted in Figs. 2 and 3, respectively.

2.2.1. First exposure

An ANOVA revealed significant differences in activity ($F(2, 30) = 7.55, P < 0.01$) and in exploration ($F(2, 30) = 16.34, P < 0.001$) among the three groups during the first exposure. Post hoc analysis of activity revealed a significant difference between the males and estrous females (Tukey, $P < 0.01$) and the males and proestrous females (Tukey, $P < 0.01$). Similarly, there were significant differences in exploration between the males and estrous females (Tukey, $P < 0.001$) and the males and proestrous females (Tukey, $P < 0.001$). Females tested during proestrus and estrus showed similar levels of both activity (Tukey, $P > 0.10$) and exploration (Tukey, $P > 0.10$).

Analysis of the defecation data revealed that 50% (5/10) of the males but only 5% (1/21) females (a proestrous female) defecated during the first session.

2.2.2. Second exposure

An ANOVA conducted for the second session revealed there were no significant differences in activity levels ($F(2, 30) = 1.47, P > 0.10$) or exploration ($F(2, 30) = 1.57, P > 0.10$) among the three groups.

Further analysis indicated that activity levels for males had a tendency to decline between the first two

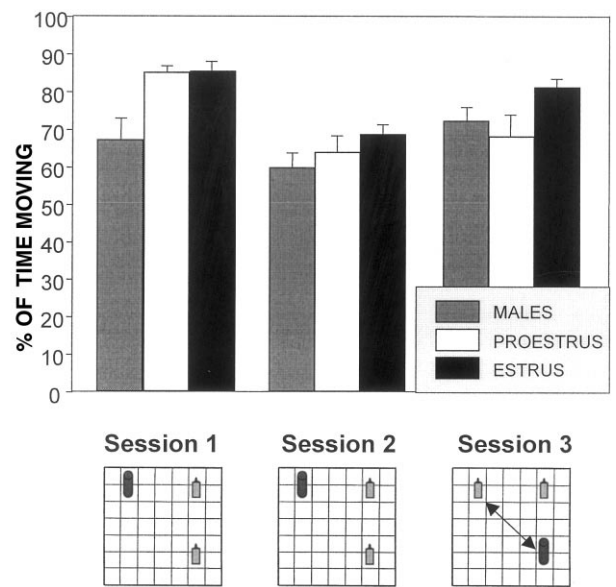


Fig. 2. Activity levels. The percent of time the males, proestrous females, and estrous females spent moving during each of the three sessions. There were significant differences among the groups during the first exposure ($P < 0.01$). Males were less active than proestrous females (Tukey, $P < 0.01$) and estrous females (Tukey, $P < 0.01$) during the first exposure but not the second exposure (Tukey, both $P > 0.10$). During the final exposure (object switch), there was no significant differences among the groups ($P = 0.08$). However, both males and estrous females showed an increase in activity levels between the second and third session (paired *t*-test, both $P < 0.05$).

sessions ($t(9) = 2.12, P = 0.06$). However, there were no differences in exploration between the first two sessions ($t(9) = -1.41, P > 0.10$). In contrast, proestrous females show a decrease in activity level ($t(9) = 4.57, P < 0.01$) and in exploration ($t(9) = 3.59, P < 0.01$) between Session 1 and Session 2. Similarly, estrous females show a decrease in activity ($t(10) = 6.56, P < 0.001$) and in exploration ($t(10) = 5.57, P < 0.001$) between the first two sessions.

In the second session, 50% (5/10) males, but only 14% (3/21) females (all estrous females) defecated.

2.2.3. Third exposure: object location switch

During the final session (after the switch of objects), there were no significant differences among the three groups in activity levels ($F(2, 30) = 2.83, P = 0.08$). However, there were differences in exploration ($F(2, 30) = 3.65, P < 0.05$). Post Hoc analysis of exploration revealed an estrous cycle effect. Females tested during estrus explored more than rats tested during proestrus (Tukey, $P < 0.05$).

Analysis of performance in the final two sessions showed that males increased activity between the last two sessions ($t(9) = 2.47, P < 0.05$), with a tendency ($t(9) = 1.85, P = 0.097$) to explore more new areas in the third session. Proestrous females did not show any change in activity ($t(9) = 0.59, P > 0.10$) nor explo-

ration ($t(9) = -0.55$, $P > 0.10$) between the last two sessions. However, unlike the proestrous females, estrous females showed an increase in both activity ($t(10) = 3.14$, $P < 0.05$), as well as in exploration ($t(10) = 3.00$, $P < 0.05$) between the final two sessions.

All animals showed similar levels of defecation during the final session, 20% (2/10) of males and 19% (4/21) of females (two estrous and two proestrous females).

2.3. Discussion

The purpose of the present experiment was to investigate whether there are behavioral differences between male and cycling female rats in exploring a new environment. Initial exploration was examined, as well as subsequent exposure to the same environment, and the response to a change in object locations. Activity, exploration, and defecations were examined. The results showed both sex differences and effects of the estrous cycle.

2.3.1. Response to a novel environment

Both estrous and proestrous females were more active and defecated less than the males during the first exposure to the novel environment. Similar results have been reported in the past. Archer [1] showed that

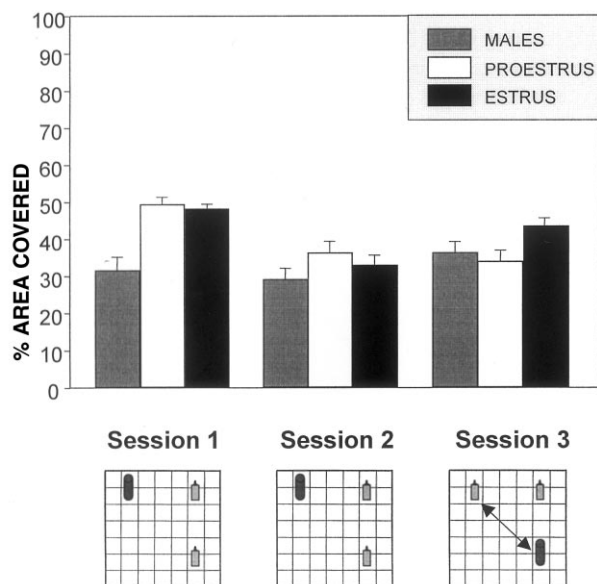


Fig. 3. Exploration levels. The percent of new area the males, proestrous females, and estrous females spent exploring across the three sessions. There were significant differences in exploration ($P < 0.001$). Males explored less than the proestrous females (Tukey, $P < 0.001$) and estrous females (Tukey, $P < 0.001$) during the first exposure but not the second exposure (Tukey, both $P > 0.10$). During the final exposure (object switch), estrous females explored more than proestrous females (Tukey, $P < 0.05$). Only estrous females showed an increase in exploration between the second and third session (paired t -test, $P < 0.05$).

females have a tendency to ambulate more and defecate less than males when placed in an open field. Female rats also enter the open arms of an elevated plus maze more readily than male rats [18]. Similarly, Leret et al. [27] showed that females were more active (higher number of entries into the open arms of an elevated plus maze), as well as less anxious (longer time spent on the open arms) than males.

An analysis of exploration conducted by Joseph et al. [19] showed that females explored more than males did when placed in an open maze field. In this task, the animal had to cross from one end of a field to the other. However, barriers were set up to create alleys that the animal could explore. Results showed that females had a greater tendency than males to enter these alleys. These data are in agreement with present findings in which there is a sex difference in both activity and exploration for initial exposure to an environment. Taken together the data suggest that males may be less active during an exploration task because they are more anxious when placed in an open environment.

2.3.2. Subsequent exposure to a 'novel' environment

Unlike many prior studies, the present study exposed the animals to the same environment multiple times. The results of the present study showed that during the second exposure to the same environment, the sex difference in activity diminished. Females appear to habituate to the context, showing a decrease in both activity and exploration level similar to the male levels. Joseph et al. [19] found that when animals were placed back into the same testing environment, both males and females decreased their exploratory behavior. Notably, males made fewer entries into the alleys compared to the females during the first placement in the open maze. In addition, in the current study, by the third exposure, male defecation levels were similar to females.

2.3.3. Response to object relocation

Animals without a hippocampus are impaired in the ability to respond to changes in object location made in an open field environment [52]. Interestingly, only the estrous females and to a lesser degree male rats, responded to the relocation of objects with a renewal of exploration and activity. This was not found in the proestrous females. This finding is in contrast with reports of higher activity levels in a running wheel for females in proestrus [61]. Thus, analysis of activity levels seems to depend on the type of activity being measured, running wheels versus exploration.

Similar to the present results, Joseph et al. [19] showed that when a new exploratory field (i.e. realignment of barriers) was introduced, only females increased their exploratory behavior. The stage of the females' estrous cycle was not accounted for in that

study. However, findings from the present study support and extend on their results suggesting that the *estrous females* were more sensitive to spatial changes made within an environment and increase their exploratory behavior.

Rather than examine for differences in sensitivity to cue manipulations within a given environment, the next experiment examined specifically which aspects the animals are attending to in their environment.

3. Experiment 2

The purpose of the second experiment was to investigate differences in cue use between male and female rats. Rats can use landmarks, local sensory information, distal information, and ‘Dead Reckoning’ to guide their behavior [38,51,59]. In their natural environment rats show flexibility in their mode of navigation, and can navigate successfully even in the absence of any observable landmark cues [7], or rely on landmark cues when geometric cues are made unavailable [57].

Preventing rats from using extra-room information impairs learning of a spatial radial arm maze task. This result was shown in both male [37] and female rats [12], indicating extra-room cue utilization in both sexes.

The amount of training has also been found to affect how an animal solves a task. With extended training, rats shift from displaying location based behavior to motor response based behavior [16,44,47] and an increased reliance on the distal visual information [59]. In addition, an animal’s biases in using environmental information can be obtained from experiments where different types of cues provide conflicting information to the animal. The animal’s response provides an index of which cues are most influential in guiding its behavior [58,59]. Williams et al. [64] used both male and female rats to examine the use of different types of extra-maze cues during navigation in a spatial radial-arm-maze task. The findings revealed that male rats used the shape of the environment (geometric cues), while females used both the shape of the environment and landmark cues to locate the food reward.

The present study differed from the Williams et al. [64] study in several aspects. The Williams et al. [64] study only investigated for differences between distal cues (objects around the room) and geometric information. In the current study, the geometry of the room was kept constant during both training and probe sessions and the focus was on the different types of landmark cues animals can use. Additionally in the present study, the dynamics of cue reliance

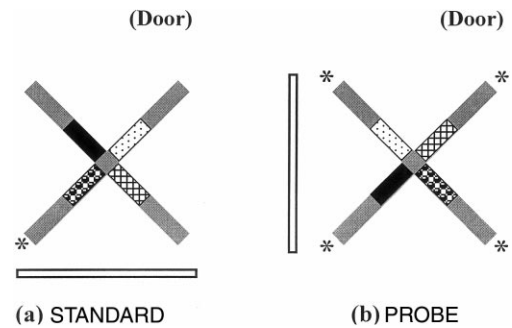


Fig. 4. Standard and Probe configuration of maze and testing room. (a) the room was completely black except for an illuminated white panel located alongside the south wall. Each maze arm had a distinct insert. During the standard sessions, only the southwest arm with the rubber insert was baited. (b) During the probe sessions, the inserts were rotated 90° counter-clockwise, the white panel rotated 90° clockwise, and all of the arms were baited. In both the standard and probe conditions, the animals were always brought in from the same entryway (door). * Depicts a baited arm.

was examined to investigate changes in cue use over time.

3.1. Materials and methods

3.1.1. Subjects

A subgroup of the rats from Experiment 1 was used for Experiment 2. Ten 7 month old females and nine 7 month old male Fischer 344 retired breeders (Harlan Sprague–Dawley, IN), were singly housed in transparent plastic tubs, in a room with a 12:12 h light:dark cycle (lights off at 19:00 h). All animals underwent extensive handling during the month preceding training and were exposed to multiple environments.

3.1.2. Apparatus

The maze consisted of four black Plexiglas arms ($10.5 \times 51 \text{ cm}^2$) forming a symmetrical ‘+’ (Fig. 4a). It should be noted that while the same room was used as in the first experiment, the configuration of cues was changed. A curtain was added to the testing room changing it into a square ($2.1 \times 2.1 \text{ m}^2$) and a large illuminated white panel ($123 \times 243 \text{ cm}^2$) was located on the south (S) wall, approximately 53 cm from the edge of the two closest maze arms. The initial segments of the arms differed in terms of their tactile, visual and possibly olfactory characteristics. Each of the arms had an insert: a black piece of rubber ($20 \times 10 \text{ cm}^2$), black wire mesh ($25 \times 10 \text{ cm}^2$), white contact paper ($25 \times 10 \text{ cm}^2$) and the fourth was black with a sanded surface. Extra-room cues included the single entryway and possible auditory stimuli (e.g. computer fans) from outside the maze room. Throughout the experiment only the southwest (SW) arm, with a rubber insert, was baited with chocolate sprinkles. The maze was cleaned with alcohol between rats.

3.1.3. Procedure

Following Experiment 1, the animals were food deprived to 85% of their ad libitum weights and trained to go to the SW arm. Every morning the animals were transported from the animal room to the maze room in their home cage along the same pathway and through the same entryway. The rat was placed in the center of the maze, once it traversed down the baited arm, it was picked up and placed back on the center of the maze. In order to prevent the development of a motor response strategy, each time the animal was put on the maze it was placed facing a different arm¹. After the visit to the baited arm, the food cup was re-baited and this procedure was repeated five times or until 10 min elapsed. The maze was cleaned with 30% isopropyl alcohol between animals. The females were always tested before the males. In order to minimize any lingering odor, all of the inserts were removed from the maze and soaked in alcohol before the males were run.

From the seventh day onwards, the morning session was terminated early if the animal visited an incorrect arm. Thus in a given morning session a rat was scored from 0 to 5 depending upon its behavior (0, error on first arm visited, 5, consistently visited the correct arm on all five trials). To constitute a visit, the rat's front forelimbs had to pass 24 cm (i.e. about half way) down the arm. All animals were also re-tested in an afternoon session, in which the animal was taken off the maze after visiting a single arm regardless of its correctness (i.e. the rat was rewarded only if it visited the SW arm). The afternoon sessions were conducted in order to familiarize the animals with a single trial procedure.

On day 15, a probe session was conducted in which the afternoon session was modified. The local inserts were rotated 90° counterclockwise, the illuminated white panel was rotated 90° clockwise [58,59] and all four arms baited. The animals were placed on the maze facing the NE arm (Fig. 4b). This allowed for a dissociation among animals predominantly relying on the local inserts (SE arm), the distal white panel (NW arm), and extra-room information (SW arm), in guiding their behavior. The choice of the NE arm could indicate that the animal was using some other means to guide its behavior (e.g. simply continued down the arm it was facing). The animals were previously familiarized with the single trial afternoon procedure and their choice was reinforced regardless of the arm selected. Therefore, the probe session was designed to provide minimal disruption to ongoing performance. Regular morning and afternoon sessions continued for six more days at which time the probe session was repeated.

¹ This, as well as the location of the experimenter, was varied in a pseudo-random manner.

3.2. Results

After food deprivation to 85% of ad libitum body weight, the females' cycles became irregular. The effect of food deprivation on the estrous cycle is an important issue since it poses a serious confound for research using appetitive tasks [35]. Consequently, the data were analyzed for only sex differences.

A repeated measures ANOVA showed a main effect for learning over training days ($F(20, 340) = 41.93$, $P < 0.001$), no sex differences ($F(1, 17) = 1.22$, $P > 0.10$), and no sex by training day interaction ($F(20, 340) = 1.45$, $P = 0.095$) during the morning sessions. After approximately two weeks, the rats reached asymptotic levels of performance (consistently visiting the correct arm). Thus, the animals were reliably choosing the baited arm regardless of how they were placed on the maze. Importantly, this level of performance was maintained even after the first afternoon probe (Fig. 5).

Similar to the morning sessions, after about 2 weeks the animals were proficient during the afternoon sessions. Virtually no errors were committed on the afternoon sessions during the three days preceding each of the probes. Over the three days before the first probe, female rats were choosing the correct arm on average 96.7% and the males were at 100%. 3 days before the second probe, females were choosing the correct arm 100% of the time, while the males were at 88.9%. In addition, as can be seen in Fig. 6 the animals' response was quite rapid, with animals making their choices within approximately 5–10 s. In contrast, during the probe sessions the rats took much longer to choose an arm in comparison to the standard sessions, both on the first probe (ANOVA, $F(1, 37) = 8.3$, $P < 0.01$) and second probe (ANOVA, $F(1, 37) = 4.6$, $P < 0.05$). A

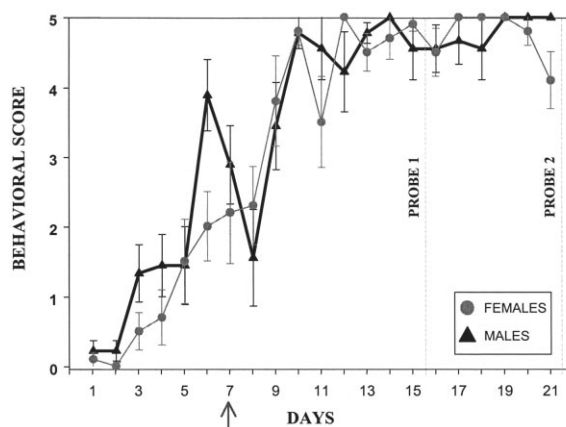


Fig. 5. Behavioral score for the daily morning sessions. The number of times each rat chose the baited arm before making an error (maximum of five trials per session by training day). Both males and females exhibit significant learning. The black arrow designates the change in training procedure, from day 7 animals were removed from the maze if they chose the wrong arm.

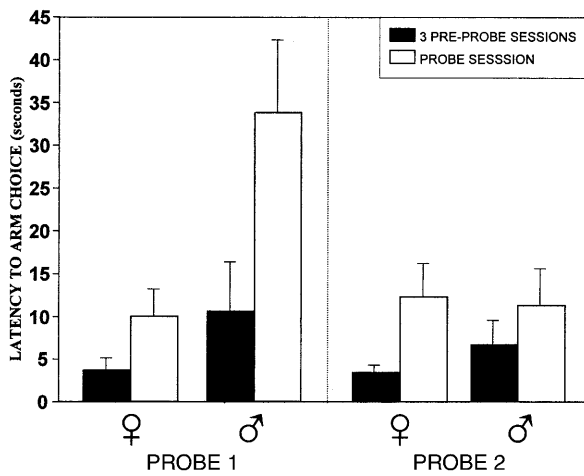


Fig. 6. Latency to arm choice on afternoon sessions. The average amount of time (seconds) before the animal selected an arm during the three standard afternoon sessions preceding the probe session (pre-probe) and for the probe sessions (probe 1 and 2). Both males and females took longer to choose an arm on the probe sessions. There was also a sex difference in latency for the first probe, with males taking longer to choose an arm. This sex difference was absent by the second probe.

sex difference was found for latency to arm choice for the first probe session, with males taking a longer time to make a choice, both on the pre-probe and probe sessions (ANOVA, $F(1, 37) = 9.0$, $P < 0.01$), with no interaction (ANOVA, $F(1, 37) = 2.7$, $P > 0.10$). By the second probe session, this sex difference in choice latency was not present (ANOVA, $F(1, 37) = 0.13$, $P > 0.10$).

The maze was thoroughly cleaned between animals and the experimenter counterbalanced the order of the rats within each sex group. However due to the fact that the females were tested before the males, an examination for the effect of order was conducted. Based upon an a priori decision, the average latencies of the first three male rats run were compared to the latencies of the last three male rats tested. The average latencies during the 3 days prior to the two probe sessions were computed. There was no significant effect of order before the first probe (ANOVA, $F(1, 5) = 0.68$, $P > 0.10$) or the second probe (ANOVA, $F(1, 5) = 0.74$, $P > 0.10$). During the first probe, the mean (\pm SEM) latency for the three days was 5.00 ± 1.95 s and 3.22 ± 0.91 s (first three animals tested, last three animals tested, respectively). During the second probe, the mean (\pm SEM) latency for the first three days was 4.00 ± 0.88 s and for the last 3 days was 2.89 ± 0.95 s (first three animals tested, last three animals tested, respectively). Thus, on a given day, the males run immediately after the females did not differ in latencies from the males run later.

A χ^2 analysis was performed for both afternoon probe sessions in order to determine whether there was

a significant tendency to use a particular type of cue in guiding behavior. On the first probe session female rats showed no significant preference among the three cue strategies ($\chi^2_{(2, N=8)} = 3.25$, $P > 0.10$). However, by the second probe, there were significant differences ($\chi^2_{(2, N=10)} = 10.4$, $P < 0.01$), in which most female rats relied on the distal white panel to guide their behavior (Fig. 7a). An examination of individual animals showed that four of the five rats using the distal cue on the first probe continued to do so on the second probe. In addition, four of the five rats using other cues on the first probe switched to using the distal cue on the second probe.

In contrast, males were already predominately using the distal white panel to guide their behavior by the first probe ($\chi^2_{(2, N=8)} = 6.25$, $P < 0.05$), Fig. 7(b). While on the second probe, there were no significant differences among the three cue strategies ($\chi^2_{(2, N=8)} = 3.25$, $P > 0.10$). An examination of the individual choices of the male rats showed that four of the six rats using the distal cue on the first probe stayed with that choice on the second probe. Further, one of the three rats using other cues on the first probe switched to relying on the distal cue on the second probe.

3.3. Discussion

Male and female rats were trained on a reference memory task that could be solved in three different manners. The correct arm could be located by its relation to the 'outside world', a large distal white panel, or to local inserts on the maze. The animals learned the task within 2 weeks and were consistently

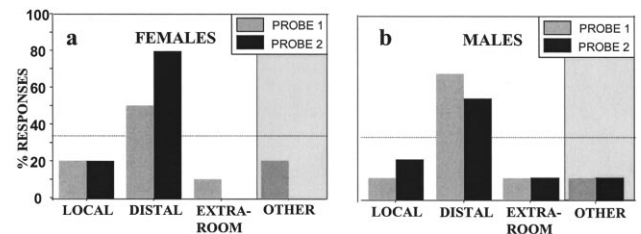


Fig. 7. Afternoon probe sessions. The percent of animals that chose arms aligned with the local, distal visual, extra-room cues, or other cues on the first and second probe sessions. (a) Female probe sessions: there was no significant preference among the three cue strategies for the first probe. On the second probe session, there were significant differences among the three cue strategies, with an overall bias for the distal visual cue. (b) Male probe sessions: on the first probe, there were significant differences among the three cue strategies, in which males were predominately relying on the distal visual cue. There were no significant differences among the three cue strategies for the second probe. The dashed line (at 33%) is the expected proportion of responses if animals rely on all three types of cues to an equal degree. The shaded region represents the percent of animals choosing the NE arm, an arm not predicted by any of the three strategies. The response of 'other' was not included in the statistical analysis.

choosing the goal arm regardless of how they were placed on the maze. This level of performance was stable and unaffected by the afternoon probes.

During the probes the three types of cues were dissociated, each aligned with a different 'correct' goal arm. Notably during the probes the rats took much longer to make a decision, supporting the interpretation that there was a conflict among these different strategies for the animals.

A prior study [59] conducted in the same testing room, showed that females had no significant preference for any specific type of cue during the first probe. The present study replicated this finding, showing that during the first probe there was no significant preference for the female rats to rely on any of the three types of cues. However, similar to Tropp and Markus [59], there was a tendency early on to rely on the distal visual information in the room. During the second probe this tendency became more pronounced with a majority of female rats relying on the distal white panel in choosing the goal arm.

These data suggest that during early exposure to a novel environment female rats attend to and use a wide array of information. However, with additional experience there is a tendency to focus primarily on distal visual landmarks. Electrophysiological recordings show a similar dynamic. With extended training, the thalamic directional system tends to become aligned with a salient visual cue card [14,24]. Additionally, Maaswinkel and Whishaw [30] showed that female rats can be trained to switch to different navigational strategies, however, in well trained animals, there is a preference for the use of visual distal information. The current results indicate that even when rats are not trained to solve a problem in a specific manner, they will develop a preferred way of solving the task. These data support the idea of a hierarchy in spatial navigation, in *which under the current conditions* distal visual information prevails. Clearly the relative salience of the different types of cues plays a critical role in the degree to which an animal will rely on them [46]. The present results indicated that even in a stable environment the use of cues changed with experience. Presumably, another configuration of more or less salient cues could have had a different effect on the animals.

Male rats exhibit a different dynamic. By the first probe, the males are predominately relying on the distal visual information to find the food reward. The males also differ in the learning of the task with more variability in their performance from day to day. Similarly, the latency to choose an arm both on regular training days as well as probe days differed between the males and females. Males had a longer latency before they made a decision both on regular sessions and the first probe session. However, by the second probe session the response time of the males was similar to the

females. Thus, male rats seem to be more affected than the females by changes in the environment. They also develop a reliance on the distal visual information faster than the females. Kanit et al. [21] have also shown that male and female rats use different strategies when confronted with a change in a familiar water maze. Females relied on the local visual cue to find the platform, where as males did not. Interestingly this effect was found only on the initial trial of the test day, on subsequent trials this sex difference was gone.

Other researchers have found sex differences in the reliance on different types of extra-maze cues [64,65]. Male and female gonadectomized rats were trained on a 12-arm-radial maze, in which eight of the arms were always baited with food. After reaching high levels of performance probes were conducted. Results from these test sessions revealed that only the alteration of geometric cues (shape of the environment) disrupted performance of the males. While females relied both on the geometric cues and the landmark cues (objects in the room). The current study used intact rather than gonadectomized rats. Differences in the hormonal milieu of adult rats influences navigational behavior [34], as does neonatal cryoanesthesia [40], either manipulation could potentially influence the use of certain types of cues.

In the present study, the geometry of the room was kept constant, and the reliance on the different types of 'landmarks' examined. Despite the procedural differences the results of the current experiment are similar to the findings of Williams et al. [64] indicating more flexibility in female than male rats. In addition to showing a more flexible use of cues, the current study indicates that the sex differences in cue utilization are diminished with repeated exposure to the environment.

4. General discussion

The present study examined sex differences in both exploratory behavior as well as cue utilization in various environments. The first experiment examined the effect of initial and subsequent exposure to the same environment on behavior, as well as the animals' response to changes made within their environment. The second experiment allowed for the assessment of animals' utilization of different features in the environment, over the course of training. The results revealed that males and females differ on how they initially interact within their environment, however this difference diminishes with repeated exposures.

4.1. Sex differences in response to a new environment: anxiety measures

In the present experiment, males were less active than

females when placed in the exploration box for the first time. Similarly, during the early training on the plus maze, the male rats appeared to be more disrupted by changes and took longer to make decisions. These findings support and extend on previous research showing that males are more anxious and defecate more when placed in an open field [1]. In both experiments the sex difference in anxiety diminished with subsequent exposure, with the male stress response decreasing and approaching that of the females. This was evident both as a reduction in defecation in the exploration task as well as a decrease in choice latencies on the plus maze.

4.2. Sex differences in response to a new environment: cue utilization

Both experiments allowed for the examination of how changes made within the environment as well as overexposure to the same context affect behavior. Between the first and second probe sessions on the navigation task, it appeared that the reliance on the distal visual information got stronger for the females. In contrast, the male rats initially showed a strong reliance on the distal visual information. In the exploration task, both males and estrous females respond to changes in cue configuration. However, this response was stronger in the estrous females as evident by an increase in exploratory behavior. Notably, proestrous animals were not affected by the change in cue configuration, a hippocampus sensitive manipulation.

4.3. Differential effects of the estrous cycle on 'hippocampal tasks'

The hippocampus is important for processing the spatial layout and configural representation of an environment [32,33,41,42,56]. Animals with lesions produced in the hippocampus have deficits in spatial ability [17] and show an impaired response to changes in cue configuration in their environment [52]. This is of interest due to findings that hippocampal dendritic morphology fluctuates across the rat estrous cycle. Specifically during proestrus, there is an approximate 30% increase in synaptic density on the apical dendrites, in the CA1 region of the hippocampus [67].

Estrous cycle differences have been reported on a number of spatial memory tasks. Proestrous rats examined on a fear conditioning paradigm showed less retention of spatial context compared to both males and estrous females [36]. Similarly, proestrous females are impaired on finding a hidden platform in the water maze [13,26,63, however see 3]. In contrast, on the radial arm maze, these levels of estrogen do not seem to be detrimental for performance. Stackman et al. [54] found no difference in spatial working memory ability

across the natural estrous cycle. In addition, ovariectomized rats with estrogen replacement show better performance on 8-arm radial maze tasks than rats without estrogen replacement [4,10,29]. However, non-cycling rats may be impaired in relation to naturally cycling animals, since the cessation of the estrous cycle has detrimental effects on spatial ability [34]. Thus, examining the behavioral effects of estrogen in ovariectomized rats may be a better model of estrapause/menopause than of the natural estrous cycle.

4.4. Implications of the data: estrous cycle effects on hippocampal ability

The disparate findings regarding the effects of the estrous cycle have resulted in a number of hypotheses. It has been proposed that high levels of estrogen impair hippocampal function on aversive tasks but not on appetitively motivated tasks [36]. An alternative explanation of these findings may be related to different types of hippocampal processing [11]. The results of the present study have implications for these different hypotheses.

4.4.1. Differential effects of the estrous cycle on behavioral tasks

Stress has been shown to affect performance on behavioral tasks [e.g., 28,63]. The female stress response, measured by levels of the hormones, corticosterone and adrenocorticotrophin (ACTH), fluctuates across the estrous cycle. For example, Viau and Meaney [60] measured levels of these stress hormones after females received 20 min of restraint stress. Proestrous females had significantly higher levels of these hormones. Thus, findings of impaired performance for proestrous females on aversive tasks (e.g. water maze) may be due to the altered stress response during that stage of the cycle [13,63]. Consequently, the demands of a task and motivational component may alter performance during different stages of the estrous cycle.

In the present study proestrous and estrous females showed similar activity and exploration patterns when placed in a novel environment. Further, both proestrous and estrous females show similar defecation levels across the three sessions. Thus, despite similar stress levels only the estrous females respond to changes made to the configuration of cues. Consequently, it would seem that differences found on behavioral tasks are not solely due to estrous cycle changes in the stress response of these animals.

4.4.2. Differential effects of the estrous cycle on working and reference memory tasks

Proestrous rats show deficits in the water maze and fear conditioning, both reference memory tasks. In

contrast, no such effects are found on a radial maze working memory task [54]. The present study examined sensitivity to object relocation. This is a hippocampal task that does not easily fit into the working/reference memory dichotomy, however it may be viewed as a reference memory task since the environment is stable across sessions [43]. The fact that high estrogen was related to reduced sensitivity to object relocation (i.e., impaired hippocampal function) is in agreement with previous findings on reference memory tasks. Consequently, these results support the view that the differential effects of the estrous cycle may be related to task demands.

One potential source for this interaction with task demands is the possibility that different regions of the hippocampus subserve different types of information processing. This interpretation is supported by evidence that CA1 [67, see also 6] and dentate granule cells [39] are affected by fluctuations in hormone levels. However, the CA3 region of the hippocampal formation is unaffected [66]. Thus, it is possible that depending upon task demands, sub-regions within the hippocampus are involved to a greater or lesser extent.

Another interpretation of these data would be that these different ‘hippocampal tasks’ vary in their level of interdependence upon other non-hippocampal brain regions. This interpretation is supported by evidence of sex differences in dendritic morphology in the prefrontal cortex, in rats [25] as well as meadow voles [22], and the fact that these differences seem to be related to behavioral ability [22]. In addition, variations in environmental conditions have also been shown to have a differential effect on brain regions (e.g. cerebral cortex, hippocampus, and corpus callosum) of males and females [20]. Thus, it is possible that changes in other brain regions affect performance of animals tested on ‘hippocampal tasks,’ across the estrous cycle.

4.4.3. An alternative explanation of estrous cycle effects: previous exposure

One of the important findings of the present study is that there are changes in cue utilization with repeated exposure to a stable environment. A possible explanation for the inconsistent findings of hippocampal ability across the estrous cycle may be due to the amount of training the animals received. In the fear conditioning and water maze paradigms, the animals have limited exposure to the environment and/or task before behavioral assessment. In these tasks, high levels of estrogen have been related to impaired hippocampal function [13,63]. In contrast, on the radial maze tasks the animals receive many habituation trials before the initiation of the memory training [e.g. 54,55].

A careful examination of water maze studies also seems to suggest an important role for the amount of pre-exposure. In a study by Berry et al. [3], in which the animals received extensive pre-training on a separate water maze before testing, there were no estrous cycle effects. Similarly, when animals were re-examined six weeks after reaching criterion levels of performance, differences between proestrous and diestrous animals were less pronounced [13]. Taken together these findings suggest that differences in spatial performance across the estrous cycle may be limited to the early period of task acquisition.

4.5. Implications of the data: sex differences in hippocampal ability

The importance of distinguishing between early and subsequent exposures to the environment has implications for interpreting the literature on sex differences in performance on hippocampal tasks. Notably there is substantial evidence indicating that the hippocampus plays only a transient role in spatial processing [9,23,62]. Thus, once the environment is highly familiar to the animal the hippocampus is no longer needed. An examination of previous research on spatial radial arm mazes show that males and females differ in performance during the acquisition of the task. Thus, males require fewer trials to reach criterion levels of performance in the radial arm maze [48]. Other studies have found a sex difference in spatial performance, however this sex difference disappears when the animals reached asymptotic levels of performance [64,65]. Similarly, Kanit et al. [21] showed a sex difference only on the initial but not subsequent trials of a ‘strategy switch’ water maze task. Further, prior training and familiarization with non-spatial components of a water maze task eliminated sex differences in spatial performance [45]. Thus, previous exposure to a task appears to have a strong impact on future performance and may account for some of the discrepancies reported in the literature.

In summary, the findings showed that there are initial behavioral differences related to anxiety, exploration and cue utilization. However, with additional exposures to the environment these differences are diminished. Thus, the results suggest that the period of greatest sex and estrous cycle effects are during the early stages of learning a spatial task. In future research it is critical to examine for sex and estrous cycle effects both during early acquisition as well as at asymptotic levels of performance. Additionally, behavioral tests should also include measures of both mnemonic and other abilities to assess whether sex differences are attributed to overall cognitive performance or differences in activity or anxiety levels.

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References

- [1] Archer J. Rodent sex differences in emotional and related behavior. *Behav Biol* 1975;14:451–79.
- [2] Beatty WW. Hormonal organization of sex differences in play fighting and spatial behavior. In: de Vries GJ, Debruin JPC, Uylings HBM, Corner MA, editors. *Sex differences in the brain*. Amsterdam: Elsevier, 1984:315–30.
- [3] Berry B, McMahan R, Gallagher M. Spatial learning and memory at defined points of the estrous cycle: effects on performance of a hippocampal-dependent task. *Behav Neurosci* 1997;111:267–74.
- [4] Bimonte HA, Denenberg VH. Estradiol facilitates performance as working memory load increases. *Psychoneuroendocrinology* 1999;24:161–73.
- [5] Blizard DA, Lippman HR, Chen JJ. Sex differences in open-field behavior in the rat: The inductive and activational role of gonadal hormones. *Physiol Behav* 1975;14:601–8.
- [6] Blum S, Moore AN, Adams F, Dash PK. A mitogen-activated protein kinase cascade in the CA1/CA2 subfield of the dorsal hippocampus is essential for long term spatial memory. *J Neurosci* 1999;19:3535–44.
- [7] Calhoun JB. The ecology and sociology of the Norway rat. Bethesda, MD, US Department of Health, Education, and Welfare, 1962.
- [8] Caplan PJ, Crawford M, Hyde JS, Richardson JTE. Gender differences in human cognition. New York: Oxford University Press, 1997.
- [9] Caramanos Z, Shapiro ML. Spatial memory and *n*-methyl-*d*-aspartate receptor antagonists apy and mk-801: memory impairments depend on familiarity with the environments, drug dose, and training duration. *Behav Neurosci* 1994;108:30–43.
- [10] Daniel JM, Fader AJ, Spencer AL, Dohanich GP. Estrogen enhances performance of female rats during acquisition of a radial arm maze. *Horm Behav* 1997;32:217–25.
- [11] Desmond NL, Levy WB. Ovarian steroidal control of connectivity in the female hippocampus: an overview of recent experimental findings and speculations on its functional consequences. *Hippocampus* 1997;7:239–45.
- [12] Dudchenko PA, Goodridge JP, Seiterle DA, Taube JS. Effects of repeated disorientation on the acquisition of spatial tasks in rats: dissociation between the appetitive radial arm maze and aversive water maze. *J Exp Psychol Anim Behav Process* 1997;23:194–210.
- [13] Frye CA. Estrus — associated decrements in water maze task are limited to acquisition. *Physiol Behav* 1995;57:5–14.
- [14] Goodridge JP, Dudchenko PA, Worboys KA, Golob EJ, Taube JS. Cue control and head direction cells. *Behav Neurosci* 1998;112:749–61.
- [15] Halpern DF. Sex differences in cognitive abilities. Hillsdale: Erlbaum, 1986.
- [16] Hicks LH. Effects of overtraining on acquisition and reversal of place and response learning. *Psychol Rep* 1964;15:459–62.
- [17] Jarrard LE. On the role of the hippocampus in learning and memory in the rat. *Behav Neural Biol* 1993;60:9–26.
- [18] Johnston AL, File SE. Sex differences in animal tests of anxiety. *Physiol Behav* 1991;49:245–50.
- [19] Joseph R, Hess S, Birecree E. Effects of hormone manipulations and exploration on sex differences in maze learning. *Behav Biol* 1978;24:364–77.
- [20] Juraska JM. Sex differences in ‘cognitive’ regions of the rat brain. *Psychoneuroendocrinology* 1991;16:105–19.
- [21] Kanit L, Taskiran D, Furedy JJ, Kulali B, McDonald R, Pogun S. Nicotine interacts with sex in affecting rat choice between ‘look-out’ and ‘navigational’ cognitive styles in the morris water maze place learning task. *Brain Res Bull* 1998;46:441–5.
- [22] Kavaliers M, Ossenkopp K, Galea LAM, Kolb B. Sex differences in spatial learning and prefrontal and parietal cortical dendritic morphology in the meadow vole, *Microtus pennsylvanicus*. *Brain Res* 1998;810:41–7.
- [23] Kim JJ, Fanselow MS. Modality-specific retrograde amnesia of fear. *Science* 1992;256:675–7.
- [24] Knierim JJ, Kudrimoti HS, McNaughton BL. Place cells, head direction cells and the learning of landmark stability. *J Neurosci* 1995;15(3):1648–59.
- [25] Kolb B, Stewart J. Sex-related differences in dendritic branching of cells in the prefrontal cortex of rats. *J Neuroendocrinol* 1991;3:95–9.
- [26] Korol DL, Unick K, Goosens K, Crane C, Gold PE, Foster TC. Estrogen effects on spatial performance and hippocampal physiology in female rats. *Soc Neurosci Abstr* 1994;20:1436.
- [27] Leret ML, Molina-Holgado F, Gonzalez MI. The effect of perinatal exposure to estrogens on the sexually dimorphic response to novelty. *Physiol Behav* 1994;55:371–3.
- [28] Luine V, Villegas M, Martinez C, McEwen BS. Repeated stress causes reversible impairments of spatial memory performance. *Brain Res* 1994;639:167–70.
- [29] Luine VN, Rentas L, Sterbank L, Beck K. Estradiol effects on rat spatial memory. *Soc Neurosci Abstr* 1996;22:1387.
- [30] Maaswinkel H, Whishaw IQ. Homing with locale, taxon, and dead reckoning strategies by foraging rats: sensory hierarchy in spatial navigation. *Behav Brain Res* 1999;99:143–52.
- [31] Maccoby EE, Jacklin CN. Psychology of sex differences. Palo Alto: Stanford University Press, 1974.
- [32] Maguire EA, Frackowiak RSJ, Frith CD. Recalling routes around London: activation of the right hippocampus in taxi drivers. *J Neurosci* 1997;17:7103–10.
- [33] Maguire EA, Gadian DG, Johnsrude IS, Good CD, Ashburner J, Frackowiak RSJ, et al. Navigation-related structural change in the hippocampi of taxi drivers. *Proc Natl Acad Sci* 2000;97(8):4398–403.
- [34] Markowska AL. Sex dimorphisms in the rate of age-related decline in spatial memory: relevance to alterations in the estrous cycle. *J Neurosci* 1999;19:8122–33.
- [35] Markus EJ, Tropp J. Strategies for assessing the effects of sex and estrous cycle on hippocampal ability. *Soc Neurosci Abstr* 1999;25:1627.
- [36] Markus EJ, Zecevic M. Sex differences and estrous cycle changes in hippocampus-dependent fear conditioning. *Psychobiology* 1997;25(3):246–52.
- [37] Martin GM, Harley CW, Smith AR, Hoyles ES, Hynes CA. Spatial disorientation blocks reliable goal location on a plus maze but does not prevent goal location in the Morris maze. *J Exp Psychol Anim Behav Process* 1997;23:183–93.
- [38] McNaughton BL, Chen LL, Markus EJ. ‘Dead Reckoning,’ landmark learning, and sense of direction: a neurophysiological and computational hypothesis. *J Cogn Neurosci* 1991;3:191–201.
- [39] Miranda P, Williams CL, Einstein G. Granule cells in aging rats are sexually dimorphic in their response to estradiol. *J Neurosci* 1999;19:3316–25.
- [40] Nunez JL, Koss WA, Juraska JM. Hippocampal anatomy and water maze performance are affected by neonatal cryoanesthesia in rats of both sexes. *Horm Behav* 2000;37:169–78.

- [41] O'Keefe J, Nadel L. The hippocampus as a cognitive map. Oxford: Clarendon Press, 1978.
- [42] Oler JA, Markus EJ. Age related deficits in the ability to encode contextual change: a place cell analysis. *Hippocampus* 2000;10(3):338–50.
- [43] Olton DS, Samuelson RJ. Remembrance of places passed: spatial memory in rats. *J Exp Psychol Anim Behav Process* 1976;2:97–115.
- [44] Packard MG, McGaugh JL. Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiol Learn Mem* 1996;65:65–72.
- [45] Perrot-Sinal TS, Kostenuik MA, Ossenkopp K, Kavaliers M. Sex differences in performance in the morris water maze and the effects of initial nonstationary hidden platform training. *Behav Neurosci* 1996;110:1309–20.
- [46] Rescorla RA, Wagner AR. A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. In: Black AH, Prokasy WF, editors. *Classical conditioning. II. Current research and theory*. New York: Appleton-Century-Crofts, 1972.
- [47] Ritchie BF, Aeschliman B, Pierce P. Studies in spatial learning. VIII. Place performance and the acquisition of place dispositions. *J Comp Physiol Psychol* 1950;43:73–85.
- [48] Roof RL. Neonatal exogenous testosterone modifies sex difference in radial arm and morris water maze performance in prepubescent and adult rats. *Behav Brain Res* 1993;53:1–10.
- [49] Roof RL, Havens MD. Testosterone improves maze performance and induces development of a male hippocampus in females. *Brain Res* 1992;572:310–3.
- [50] Sandstrom NJ, Kaufman J, Huettel SA. Males and females use different distal cues in a virtual environment navigation task. *Brain Res Cogn Brain Res* 1998;6(4):351–60.
- [51] Save E, Poucet B. Involvement of the hippocampus and associative parietal cortex in the use of proximal and distal landmarks for navigation. *Behav Brain Res* 2000;109:195–206.
- [52] Save E, Poucet B, Foreman N, Buhot M. Object exploration and reaction to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal formation. *Behav Neurosci* 1992;106:447–56.
- [53] Schwartz NB, Hoffman JC. Ovulation: basic aspects. In: Balin H, Glasser S, editors. *Reproductive Biology*. Amsterdam: Excerpta Medica, 1972:438–76.
- [54] Stackman RW, Blasberg ME, Langan CJ, Clark AS. Stability of spatial working memory across the estrous cycle of long-evans rats. *Neurobiol Learn Mem* 1997;67:167–71.
- [55] Stackman RW, Walsh TJ. Anatomical specificity and time-dependence of chlordiazepoxide-induced spatial memory impairments. *Behav Neurosci* 1995;109:436–45.
- [56] Sutherland RJ, Rudy JW. Configural association theory: the role of the hippocampal formation in learning, memory, and amnesia. *Psychobiology* 1989;17:129–44.
- [57] Suzuki S, Augerinos G, Black AH. Stimulus control of spatial behavior on the eight-arm maze in rats. *Learn Motiv* 1980;11:1–18.
- [58] Tanila H, Shapiro M, Gallagher M, Eichenbaum H. Brain aging: changes in the nature of information coding by the hippocampus. *J Neurosci* 1997;17(13):5155–66.
- [59] Tropp J, Markus EJ. Behavioral strategy shifts with training: rats on an elevated plus maze. *Psychobiology* 1999;27:480–5.
- [60] Viau V, Meaney MJ. Variations in the hypothalamic-pituitary-adrenal response to stress during the estrous cycle in the rat. *Endocrinology* 1991;129(5):2503–11.
- [61] Wade GN. Gonadal hormones and behavioral regulation of body weight. *Physiol Behav* 1972;8:523–34.
- [62] Ward MT, Oler JA, Markus EJ. Hippocampal dysfunction during aging I: Deficits in memory consolidation. *Neurobiol Aging* 1999;20:363–72.
- [63] Warren SG, Juraska JM. Spatial and nonspatial learning across the rat estrous cycle. *Behav Neurosci* 1997;111:259–66.
- [64] Williams CL, Barnett AM, Meck WH. Organizational effects of early gonadal secretions on sexual differentiation in spatial memory. *Behav Neurosci* 1990;104:84–97.
- [65] Williams CL, Meck WH. The organizational effects of gonadal steroids on sexually dimorphic spatial ability. *Psychoneuroendocrinology* 1991;16:155–76.
- [66] Woolley CL, Gould E, Frankfurt M, McEwen BS. Naturally occurring fluctuations in dendritic spine density on adult hippocampal pyramidal neurons. *J Neurosci* 1990;10:4035–9.
- [67] Woolley CS, McEwen BS. Estradiol mediates fluctuations in hippocampal synaptic density during the estrous cycle in the adult rat. *J Neurosci* 1992;12:2549–54.