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Estrus-Associated Decrements in a Water Maze Task are Limited to Acquisition

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FRYE, C. A. *Estrus-associated decrements in a water maze task are limited to acquisition.* PHYSIOL BEHAV **57**(1) 5–14, 1995.—To ascertain whether gonadal hormones have activational influences on spatial ability, the relationship between estrous cycle, sex differences and water maze performance was examined in two studies. In the first study, the performance of females at different cycle phases was compared within females and to that of males. All animals were naive to the task. Similar to other studies, females had longer latencies and distances to reach the water maze platform than males. This sex difference was statistically significant only in comparisons of estrous females and males, not in comparisons of diestrous females and males. To determine whether estrus-associated decrements in acquistion of the water maze task extended to postacquisition performance, a second study assessed performance of ovariectomized rats—trained to criterion in the task—whose cycle phases were mimicked by exogenous hormones. In the initial trial, "estrous" animals had longer latencies to reach the platform than "diestrous" and ovariectomized animals. In subsequent trials, no hormone-dependent differences in performance were observed. Taken together, the results indicate a modest association between phase of estrous cycle, acquisition, and postacquisition performance when the task is novel. These findings suggest estrus-associated decrements in acquisition may account for previous discrepancies among studies of sex differences in spatial ability.

Hormones Estrogen Progesterone Sex differences Spatial ability Memory
Activational effects of steroids

IN people, the sex difference in performance of spatial tasks, although small, is one of the more reliable individual differences in cognitive ability (29,32). Similarly, among rats, males tend to perform better on spatial mazes than do females (reviewed in 2). This sex difference, at least in rats, has been attributed to the organizational effect of sex hormones during the perinatal period. For example, female rats treated neonatally with testosterone showed improved performance in a Morris water maze task compared to nonandrogenized, control females (44). Compared to control animals, neonatally castrated males had impaired radial maze performance; while females, androgenized as a result of neonatal injections of estradiol benzoate, had improved performance (52). Studies of people exposed to atypical levels of sex hormones in utero—due to a medical condition (e.g., congenital adrenal hyperplasia) or exposure to exogenous hormones via the mother—indicate that similar factors may contribute to the sex difference in spatial ability in people (reviewed in 31,42).

Sex hormones may have some activational effects on cognitive function in people. There are reports of subtle differences in perceptual-spatial performance across the menstrual cycle (31,42), in comparisons of performance during and postpregnancy (54), and in comparisons of surgically menopausal women receiving replacement therapies (47). Androgen levels in adulthood were related to performance on spatial tests in some studies (8,19,33,46), but not in others (24,34). One possible explanation is that the relationship between androgens and spatial ability, at least in people, is curvilinear (33).

Animal studies may clarify the acute relationship between sex hormones and spatial ability. To date, there are few studies which have investigated the activational effects of sex hormones on spatial performance in rats. Gonadectomies in adulthood did not significantly influence performance on spatial tasks (27,52). Paradoxically, administration of testosterone to adult males decreased performance in a water maze (20).

In the present study, the possibility of an activational influence of sex hormones on spatial performance of female rats was examined by comparing the performance of females at different phases of the estrous cycle to the performance of males in a Morris water maze.

METHODS

Experiment 1

Subjects and housing. Female (n = 31) and male (n = 6) Long–Evans rats were obtained from Charles River Laboratories (Wilmington, MA) at approximately 70 days of age. Animals were individually housed in hanging stainless steel cages (24 × 18 × 19 cm) in a temperature-controlled room (21 ± 1°C) and

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were maintained on a 12:12 h light-dark cycle (lights on at 8:00 am). All rats had constant access to Purina Chow and tap water in their cages.

Estrous Cycle Determination

Samples of vaginal epithelium were obtained daily by lavage approximately 1 h prior to lights off and examined using low power light microscopy. Rats whose vaginal epithelium was characterized by the presense of primarily large round nucleated cells that characterize proestrus smears, a few cornified cells, and who responded to manual palpation with a lordosis rating (23) of at least 1 on 3 attempts, were considered in behavioral estrus (13). These rats which compromised the estrous group were actually tested on the evening of proestrus, which coincides when mating normally occurs and correlates with peaks of estradiol and progesterone, as well as hippocampal spine and synapse density (56). Rats who were 2 days away from being in behavioral estrous, whose vaginal epithelium did not contain nucleated or cornified cells homogeneously, and who responded to manual palpation uniformly with lordosis ratings of 0, were considered in diestrus. Behavioral testing began approximately 1 h after lights off, to coincide with increased activity. All rats demonstrated 4 consistent, 4-5 day estrous cycles prior to their inclusion in this experiment.

Water Maze Testing

A version of the original Morris Water Maze was utilized because this method has previously been used as a discriminable assay of spatial memory (35). The tank was 200 cm in diameter and 71 cm deep. The platform was made of wire mesh (5.3 × 5.3 × 33.5 cm) and painted white. There were many constant visual cues on the walls of the testing room. The experimenter, counters, video camera and computer equipment also served as constant visual cues. The video camera, which was situated above the water tank, was connected to both a computer tracking system (Multitracker, San Diego Instruments) and a video monitor. The tracking program automatically measured the latency and distance of the path each rat takes to reach the platform.

The tank was filled with 20–22°C water 36 cm deep. The water was colored white by adding a small quantity of nondairy creamer. The opaqueness of the water enabled the platform (situated 30 cm from the side of the tank) to be concealed approximately 2.5 cm below the surface of the water. Pairs of rats were taken in their home cages to the room containing the water maze and were tested by an experimenter naive to the rats' phase of the estrous cycle. Each rat was tested for 6 trials. Each trial was initiated at one of 3 starting positions. The starting position for a given trial was assigned randomly by the tracking program. Positions A, B, and C were located respectively at 43°, 198°, and 253° clockwise from the platform.

Rats were placed in the water facing the wall of the tank at the designated starting location and the experimenter initiated the tracking program. If the rat did not reach the platform within 120 s, the experimentor guided the rat to the platform. Once reaching the platform the rat was allowed to remain there for 45 s to allow orientation to visual cues. After completing the trial, rats were placed in their cages for an intertrial interval of 3 min. Trials were alternated between 2 rats until 6 trials per rat were completed.

Each rat was tested in the water maze on 2 separate days, which were a minimum of 2 days and a maximum of 5 days apart. Rats were tested based on their phase of cycle to complete the following groups (a) EE: estrus on both testing days 1 and 2, (n = 5); (b) ED: estrus on testing Day 1 and diestrus on Day 2, (n = 12); (c) DE: diestrus on day 1 and estrus on day 2, (n = 8); (d) DD: diestrus on both testing days 1 and 2, (n = 6); and (e) males, (n = 6).

Statistical Analysis

Two-factor, (5 ×12: condition by trials), repeated measures ANOVAs were performed for latency and distance to platform across trials 1–6 on Day 1 and 2 of testing. ANOVAs were followed by Duncan New Multiple Range post hoc comparisons to ascertain groups differences. Only groups different at the $p < 0.05$ are reported as significantly different.

Subsequent analyses considered test Day 1 and test Day 2 separately. Because of the heterogeneity of observations for the 2 day classification schema the performance of animals was assessed solely as a function of the phase of the estrous cycle on a single test day, (i.e., without consideration of cycle phase for the other test day). For example, no difference in performance would be expected between EE and ED groups, or between DE and DD groups, on Day 1. Thus, the groups considered were Estrus, Diestrus and Males. For Day 2, it is possible that no differences would be found between EE and DE groups or between DD and ED groups; however, exposure to the test during the Day 1 cycle phase could interact with the Day 2 cycle phase and affect Day 2 performance.

RESULTS

Latency to Platform

The two-factor ANOVA of performance for both test sessions indicated that the factor of group was significant $F(4, 32) = 2.87$, $p < 0.005$, as was the repeated factor of trials $F(11, 352) = 22.23$, $p < 0.0001$. There was also a significant interaction of these two factors $F(44, 352) = 1.418$, $p < 0.05$. Refer to Fig. 1 for mean latency to platform of the groups for each day. The ordering of mean latencies was related to cycle phase on a given day (as opposed to group membership considering both days) on both Day 1 and Day 2. That is, on both test days, estrous females ($\bar{X}$ = 35.6 ± 3.7) tended to show the longest latencies, followed by diestrous females ($\bar{X}$ = 27.5 ± 3.6), and males showed the shortest latencies ($\bar{X}$ = 21.1 ± 3.4). All animals showed a decrease in latencies across trials. Therefore, subsequent analyses examined Days 1 and 2 separately.

A two-factor, repeated measure ANOVA for Day 1 latency to platform indicated that the factor of group was significant $F(2, 34) = 4.17$, $p < 0.03$, as was the factor of trials $F(5, 34) = 18.04$, $p < 0.0001$. There was no significant interaction of the two factors. Posthoc analysis indicated estrous females (X = 54.0 + 4.4) had significantly ($p < 0.05$) longer latencies than males (X = 28.1 + 4.2) across trials (see Fig. 1a). Diestrous females (X = 39.0 + 4.4) were not significantly different from either estrous or male animals. One way ANOVAs followed by posthoc comparisons of groups for each trial on Day 1 revealed that on trial 3, estrous females took significantly longer to reach the platform than diestrous females or males. On trial 5, estrous females again took significantly longer than males.

Group appeared to have much less of an effect on performance on Day 2. The factor of group was not significant, however the factor of trials was significant $F(5, 34) = 9.430$, $p < 0.0001$. As Fig. 1b illustrates, latencies decreased significantly after the first trial. One way ANOVAs followed by posthoc comparisons of groups for each trial on Day 2 revealed that on trial 4, estrous females took significantly longer to reach the platform than males.

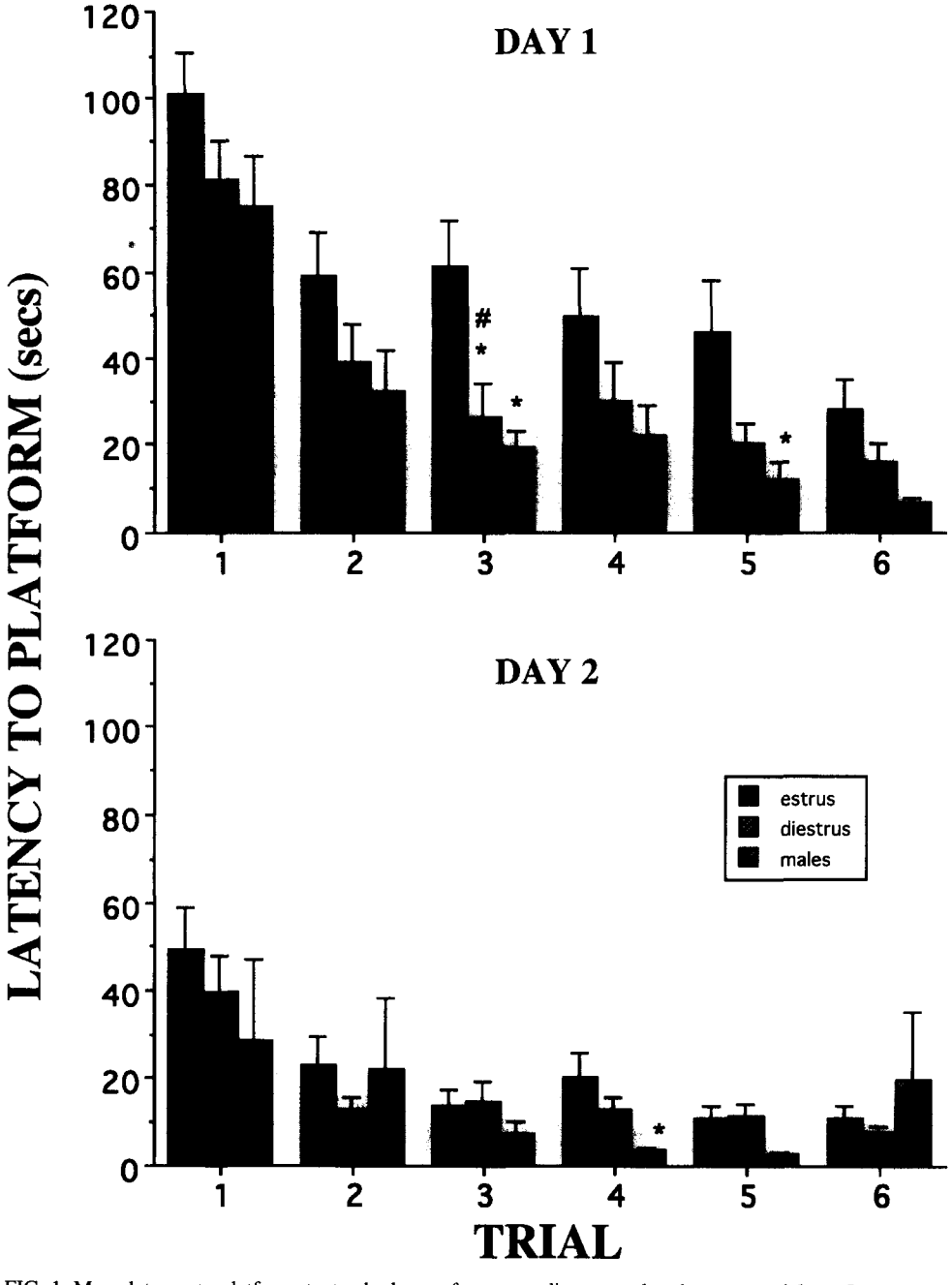

FIG. 1. Mean latency to platform + standard error for estrus, diestrus and males across trials on Day 1 (Top Panel A) and 2 (Bottom Panel B) of testing. * significantly different from estrous females when all intra- and intercycle differences are considered. # significantly different from estrous animals when intracycle only differences are examined ($p < .05$).

Distance to Platform

The distance to platform results were similar with the results for latency. The two-factor ANOVA indicated the repeated factor of trials $F(11, 352) = 25.82$, $p < 0.0001$, was significant, but there was no effect of group, $F(4, 32) = 1.78$, $p < 0.15$. There was no significant interaction of the two factors. See Fig. 2 for mean distance to platform of the groups for each trial.

When trials on Days 1 and 2 were considered separately, a two-factor, repeated measures ANOVA for Day 1 distance to platform indicated that the factor of group was significant $F(2, 34) = 3.06$, $p = 0.05$. Again, as Fig. 2a illustrates, estrous animals ($X = 114.1 + 8.0$), had significantly longer distances than males ($X = 73.4 + 11.4$), although not longer than diestrous animals ($X = 97 + 10$). The factor of trials was significant $F(5, 170) = 20.4$, $p < 0.0001$; distance decreased for all groups across trials.

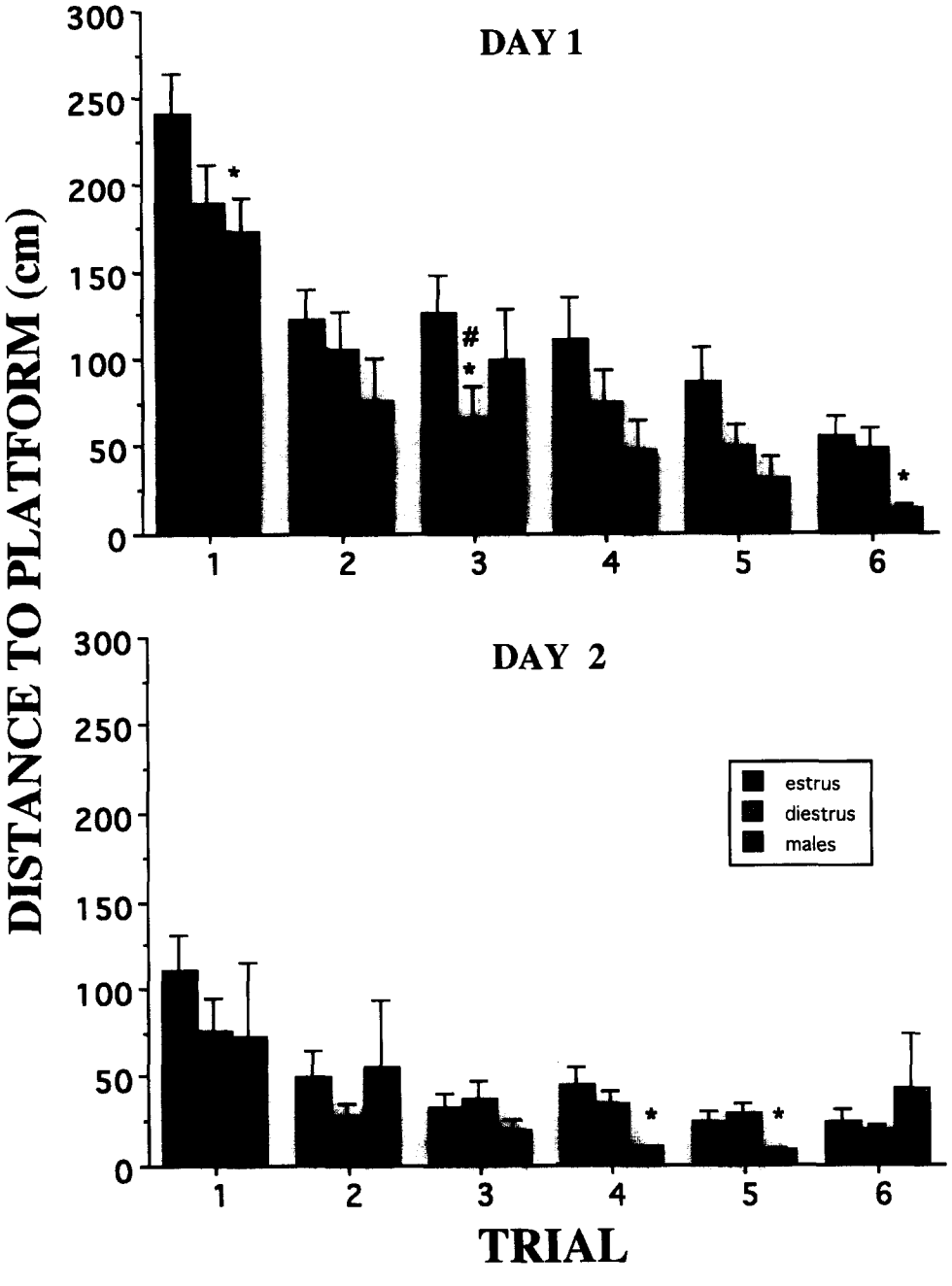

FIG. 2. Mean distance to platform + standard error for estrous, diestrous, and male groups across trials on Day 1 (Top Panel A) and Day 2 (Bottom Panel B). * significantly different from estrous females when all intra- and intercycle differences are considered. # significantly different from estrous animals when intracycle only differences are considered ($p < .05$).

There was no significant interaction of the two factors. A one way ANOVA comparing groups across each trial revealed that on trial 1 and 6 estrous females had significantly increased distances compared to males. On trial 3, estrous females also had longer distances than did diestrous females and males.

When assessing distance to platform for Day 2, the factor of group was not significant, but the factor of trials was $F(5, 170) = 8.783$, $p < 0.01$. The interaction of the two factors was not significant. The significant effects of trials is due to the average distance travelled decreasing as the number of trials increased. One-way ANOVA comparing groups across each trial revealed that on trials 4 and 6 estrous females had significantly increased distance compared to males (see Fig. 2b).

To assess intracycle differences, males were removed and the data were reanalyzed. With respect to latency, there was a trend for group $F(1, 29) = 3.551$, $p < 0.06$, and an effect of trial $F(5,$

145) = 16.13, $p < 0.001$; there was an interaction between these variables for Day 1 performance $F(5, 145) = 2.63$, $p < 0.02$. These effects can be attributed to estrous animals having longer latencies than diestrous animals on trial 3. No differences were noted for estrous and diestrous animals latencies on Day 2. Similar findings were noted for distance--there was no effect of group, however, there was an effect of trial $F(5, 145) = 19.47$, $p < 0.001$ and there was an interaction between these variables for Day 1 performance $F(5, 145) = 2.24$, $p < 0.05$. The interaction was due to estrous animals travelling longer distances than diestrous animals on trial 3. No differences were noted for estrous and diestrous animals distances on Day 2.

DISCUSSION

The results indicate that sex differences in performance on the water maze task are limited to differences between estrous females and males; there are no differences between diestrous females and males. This pattern of results was most prominant on the first test day. On the second test day, both estrous and diestrous females performed more poorly than males; however, the estrous-associated decrements in performance were less dramatic than those observed on Day 1.

Experiment 2

As estrus-associated decrements in spatial ability were most pronounced when the task was novel (Day 1), it was of interest whether gonadal hormones would affect performance of animals who had already acquired criterion performance in the water maze. That is, does phase of estrous cycle influence performance beyond the acquisition of a task?

This experiment allowed for greater control over the number of intervening days between test days 1 and 2 and over the number of animals in each hormonal condition. Two modifications were made for this experiment. First, in Experiment 1, EE and DD animals had had their test days separated by a longer period than animals in the ED and DE conditions. Although this would not be of consequence for Day 1 results, varying delays may have affected performance on Day 2, thus only the data from Day 1 can be interpretted with utmost confidence. Because of this confound, inherent to within subject comparisons of behavior on different days of the estrous cycle, the number of days intervening between test days was made uniform in Experiment 2. Second, the hormonal status of the animals was controlled by ovariectomizing them and replacing their endogenous hormones with exogenous ones which mimicked the estrous cycle. This insured greater uniformity of the number of observations in each group.

METHODS

Experiment 2

Subjects and housing. Female Long–Evans rats, ($n = 35$), at approximately 55 days of age were obtained from Charles River Laboatories (Wilmington, MA) and housed as described in Experiment 1. All animals were ovariectomized within 1 week of arrival under sodium pentobarbitol anesthesia (50 mg/kg or to effect). Following ventral incisions, the ovaries were isolated, ligated, and removed. Ten days later, all received training in the water-maze until criterion (under 10 s latency for 3 consecutive trials). Although it may have been optimal to train rats to criterion and test them in the same hormonal condition, rats were all trained when ovariectomized, thus insuring a homogeneous number of trials for all animals to achieve criterion performance. Six weeks following training the test phase was begun. The test phase consisted of treating these animals with sex hormones to mimic either "estrous" or "diestrous" phase, or with vehicle in the ovariectomized (OVX) control condition, prior to testing in the water maze. Performance of hormone-treated animals was compared with that of OVX control animals.

Test phase hormone regimen. Under ether anesthesia, 28 of the animals each received 2 silastic implants SC (50). One implant (0.062 ID, 0.125 OD) contained estradiol-17benzoate (EB: Sigma, St. Louis, MO, BETA-E2 3-Benzoate, 30 μg/ml; 10 mm/100 g BW), and one implant (0.132 ID, 0.183 OD) contained crystalline progesterone (Sigma, 4-Pregnene-3, 20,dione, 10 mm/animal). This treatment provides levels of E_2 and P (17) in the range observed during diestrus (6). As necessary, animals were brought into behavioral estrus, which was confirmed by manual palpation as detailed in experiment 1, by sex hormone injections (5). Briefly, on the day following silastic implantation, half of the animals (assigned to the "estrous" condition) received an SC injection of EB (30 μg dissolved in 0.1 ml sesame oil) and half received vehicle ("diestrous" condition). Twenty-four hours later, and 4 h prior to testing in the water-maze, animals who had been administered EB were injected SC with 2.5 mg P suspended in 0.2 ml, ("estrous" condition) while with the remaining again received vehicle ("diestrous" and OVX control condition). The day subsequent to the water-maze test, 7 of the previously "estrous" and "diestrous" animals were injected with EB, and 24 h later with P (EE and DE conditions, respectively). The remaining 7 previously "estrous"animals, 7 previously "diestrous" animals, and an additional 7 previously tested sham-implanted OVX control animals received vehicle according to the same schedule (ED, DD and OVX conditions, respectively). The animals were tested for a second time in the water-maze 4 h following the injection of P or vehicle.

RESULTS

Analyses Were Performed as Described in Experiment 1.

Latency to Platform

The overall results were consistent with those obtained from the study of endogenously cycling animals. A two-factor (group by trials) ANOVA indicated that overall the factor of group was significant, $F(4, 30) = 6.07$, $p = 0.01$, as was the repeated factor of trials, $F(11, 330) = 21.19$, $p < 0.0001$. There was also a significant interaction of the two factors, $F(44, 330) = 2.194$, $p < 0.0001$. Estrous-estrous animals ($\bar{X} = 24.8 \pm 3.7$) had significantly longer latencies than DD ($\bar{X} = 24.8 \pm 3.7$), DE ($\bar{X} = 11.5 \pm 2.2$), ED ($\bar{X} = 10.4 \pm 1.3$), and OVX ($\bar{X} = 10.7 \pm 2.1$) groups. Data not shown. Subsequent analyses examined animals grouped according to their condition on each test day separately.

As rats had been trained to criterion in the water maze task prior to the experiment when Day 1 and 2 were examined separately group effects were minimal. For latency group effects were limited to comparisons across groups for the individual trials which revealed estrous-associated decrements on the initial trial for both test days. For example, a two-factor, repeated measures ANOVA for latency to platform on Day 1 indicated that the factor of group was not significant but the repeated factor of trials was $F(5, 32) = 19.01$, $p < 0.0001$. There was no significant interaction of the two factors (see Fig. 3a). Although there was a decrease in Day 2 latency across trials, $F(5, 32) = 11.81$, $p < 0.001$, again there were no significant group differences. As Fig. 3b shows, there was a significant interaction between these factors, $F(10, 160) = 2.01$, $p < 0.05$. One way ANOVAs comparing groups for each trial indicated there was a trend showing that

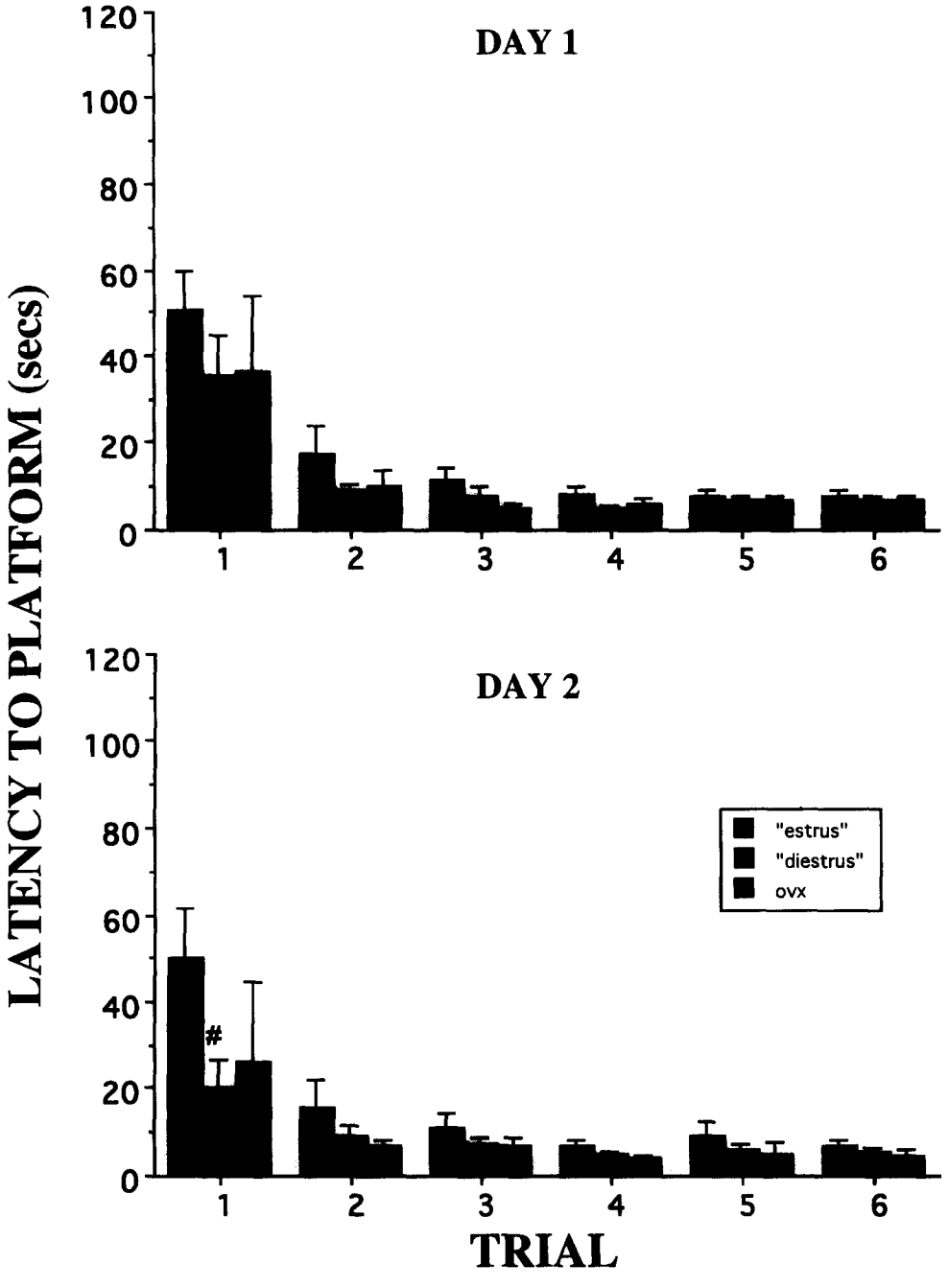

FIG. 3. Mean latency to platform + standard error for "estrous," "diestrous," and ovariectomixed (OVX) groups across trials on Day 1 (Top Panel A) and Day 2 (Bottom Panel B) of testing. # significantly different from "estrous" animals when only "estrus" and "diestrus" animals are considered ($p < .05$). Overall, animals in "estrus" on both days had significantly longer latencies than diestrous or ovariectomized animals; and estrus animals improved most from trials 1 to 2 on both test days.

"estrous" animals had a longer latency to reach the platform in the first trial on Days 1 and 2 than did other animals.

Distance to Platform

In general, the distance to platform results were also consistent with the results from Experiment 1. The overall two-way ANOVA indicated that the factor of group was significant $F(4, 30) = 4.046$, $p < .01$ as was the repeated factor of trials $F(11, 330) = 18.553$, $p < .0001$). There was also a significant interaction of the two factors $F(44, 330) = 1.532$, $p < 0.05$). All groups improved in terms of distance from Day 1 to Day 2, but the EE animals ($\bar{X} = 46.3 \pm 7.3$) had significantly longer distance than DD ($\bar{X} = 24.8 \pm 2.7$, DE ($\bar{X} = 32.9 \pm 4.6$), ED ($\bar{X} = 31.5 \pm 3.3$), and OVX ($\bar{X} = 26.1 \pm 4.4$).

When Day 1 and 2 were examined separately group effects were not apparent for distance as they had been for latency. A two-factor, repeated measures ANOVA for Day 1 distance to platform indicated that the factor of group was not significant (refer to Fig. 4a), but the factor of trials was, $F(5, 160) = 18.53$, $p < 0.0001$; distance decreased for all groups across trials. There was no significant interaction of the two factors. Again, for Day 2 distance to platform, the factor of group was not significant, (see Fig. 4b); but the factor of trials was significant, $F(5, 160) = 9.963$, $p < 0.0005$. Comparisons of groups for each trial revealed all animals showed a decrease in distance from the first to second trial. There was no significant interaction of the two factors.

To assess intracycle differences the data of ovariectomized, control animals were removed and the remaining reanalyzed. With respect to latency, there was an effect of group, $F(1, 26) = 4.08$, $p < 0.05$, an effect of trial, $F(5, 130) = 15.26$, $p < 0.001$, and an interaction between these variables for Day 2 performance, $F(5, 130) = 4.710$, $p < 0.01$. This is attributable to "es-

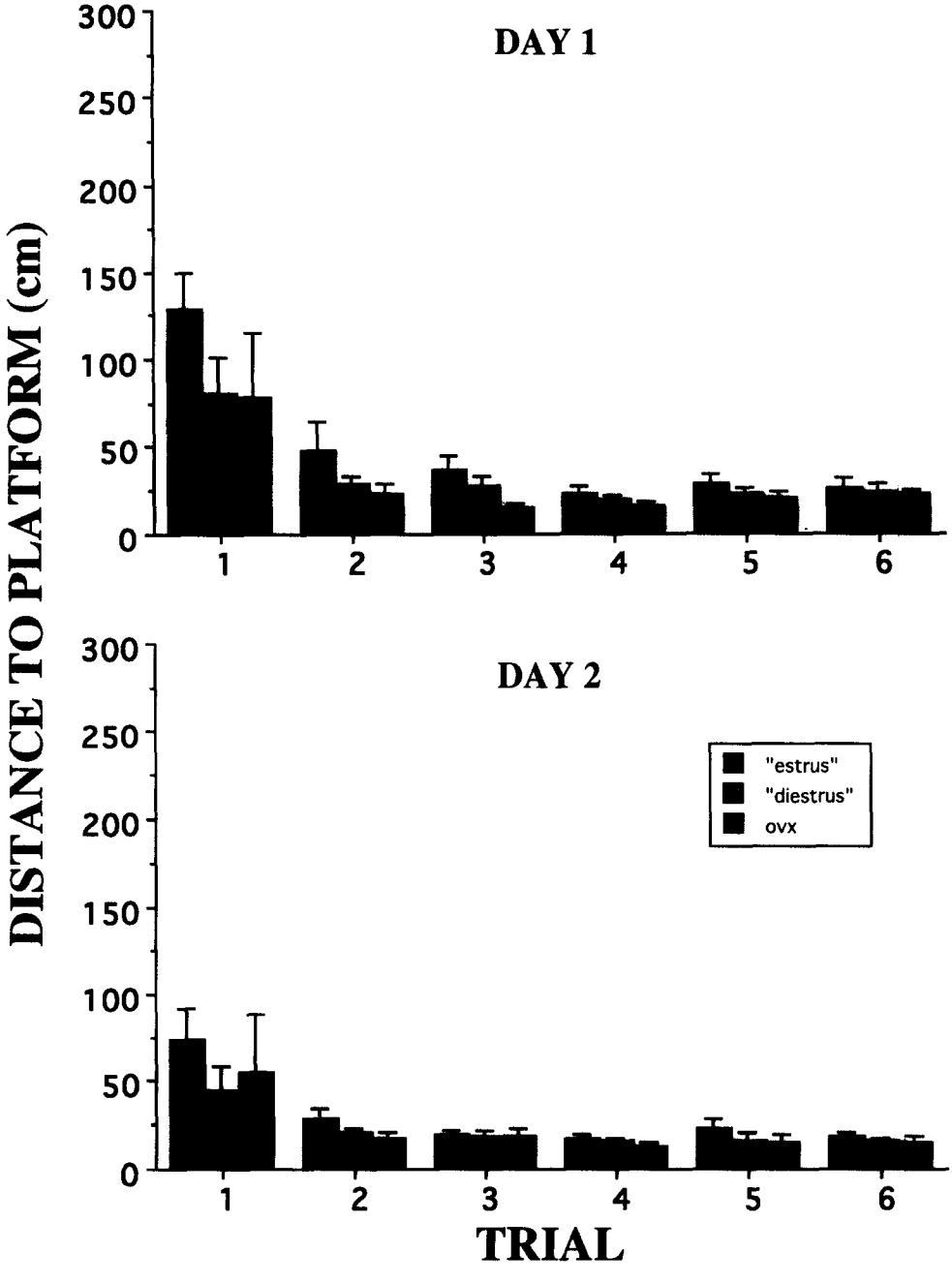

FIG. 4. Mean distance to platform + standard error for "estrous," "diestrous," and ovariectomized (OVX) groups across trials on Day 1 (Top Panel A) and Day 2 (Bottom Panel B). Over all trials, "estrous" animals had significantly longer distances and most improved performance from trial 1 to 2 than all others animals.

trous'' animals having a longer latency than ''diestrous'' animals in trial 1. For distance, there was no effect of group but there was an effect of trial, $F(5, 130) = 10.3, p < 0.001$, and an interaction between these variables for Day 1, $F(5, 130) = 2.36, p < 0.04$. This interaction was due to ''estrous'' animals exhibiting the most improvement. On Day 2, only a significant effect of trial was noted, $F(5, 130) = 1.63, p < 0.01$.

GENERAL DISCUSSION

The results of the present studies indicate that phase of the estrous cycle is associated with differential performance on the Morris water maze test of spatial memory. Estrous females performed poorer as indicated by longer latencies and greater distances to the hidden platform. As in previous studies, (52), males showed better performance than both diestrous and estrous females. This sex difference was more marked in comparisons of estrous females and males than in comparisons of diestrous females and males. Estrus-associated decrements in performance were also observed in ovariectomized animals in which phases of the cycle were mimicked via exogenous administration of hormones. ''Estrous'' animals showed poorer performance on initial trials than ''diestrous'' and ovariectomized animals. The differences observed among the groups in Experiment 2 may have been less pronounced given that ovariectomized animals (control and hormone-replaced) were tested following acquisition of the spatial task and not when naive to the task as in Experiment 1. Taken together these findings suggest that gonadal hormones have a slight activational influence on spatial ability and that estrus-associated decrements in water-maze performance are modest and limited to the acquisition phase of a task.

Phase of the estrous cycle is associated with performance on other learning tasks such as conditioned avoidance (45), as well as with performance on a variety of other behavioral measures. For example, phase of cycle influences pain sensitivity (12,13), food intake (51), locomotor activity (3,7,16), and aggression (10,11,40). Additionally, sex differences are observed for the above behaviors (2). Thus, any of these factors may have contributed to the difference observed in water maze performance between estrous and diestrous animals. For example, it has been debated whether the sex difference in spatial maze performance is due to sex-related differences in activity as opposed to learning (49). A study of prairie voles examined the relationship between errors and activity in maze learning and found no support for the activity hypothesis (15). Examination of the present distance/seconds travelled revealed that although not statistically different in both experiments 1 (E = 2.1; D = 2.4 and Males = 2.6 cm/s) and 2 (EE = 1.8; DD = 2.2; DE = 2.8; ED = 3.0 and OVX = 2.4 cm/s) estrous animals consistently covered the least distance per set time. Thus, the possibilty that differential activity/strength contributed to the present variation observed, using a different spatial task and a different species than previously reported (15), cannot be ruled out.

In the perinatal period, sex hormones have an organizational effect on spatial performance (2). The results of the present study are consistent with the hypothesis that, in adulthood, sex hormones have a slight activational effect on spatial performance, at least in females. However, several endocrine and neural systems show fluctuations over the estrous cycle that potentially contribute to behavior variations in the water maze. For example, estrous cycle is associated with basal (9) and stress-related (50) levels of glucocorticoids and ACTH. The hormonal systems of the hypothalamic–pituitary–adrenal (HPA) axis may influence performance in the water maze task (37). Thus, another interpretation of these results may be that the sex-related and cycle-related variation are due to differential response of the groups to stress when the task being acquired.

A fact which lends support to the latter interpretation is that both spatial memory and HPA responsivity to stress are controlled in part by the hippocampus (25,36,38). In addition, cyclic changes have been observed in the rat hippocampus. The density of dendritic spines in CA1 hippocampal pyramidal cells varies over the estrous cycle (55,56), such that peaks in the density of dendritic spines would correlate with when both the normally cycling estrous animals (Experiment 1) and hormone replaced ''estrous'' rats were tested. Hippocampal physiology also changes across the estrous cycle of rats (28,48). Consistent with the possibility that estrous cycle fluctuations in the HPA may be involved in the difference in spatial performance are the high levels of Type I and Type II corticosteroid receptors in the hippocampus (14,43). In contrast, levels of sex hormone receptors are low in the hippocampus (30).

In our study, the differences among groups diminished over trials, possibly a result of habituation to the stressful nature of the task. Male and female rats perform differently in tests of anxiety (1). However, the direction of the differences depends upon the test used. This suggests that performance on these tests may be due to, or affected by, different variables in males and females (26). Sex differences in performance on behavioral tests may be mediated by physiological correlates of variation in endocrine status.

Causal interpretations of spatial performance variations are even more difficult to establish from studies of individual differences in people. Whereas some studies of women report differences in cognitive performance across the menstrual cycle (22,39), others found no differences (18). When differences were discovered they were attributed to the variations in levels of sex hormones which produce the menstrual cycle (21). It is likely that other variables associated with the menstrual cycle contribute to these cognitive differences given the influence of stress on cognitive performance (4,41,53).

Consistent with the previously mentioned studies of sex differences on spatial mazes, a marked sex difference in performance on the water maze spatial test was found. However, not all studies find a sex difference in spatial performance (49). In the present study, the sex difference in performance was more marked in comparisons of estrous females and males than in comparisons of diestrous females and males. That phase of the cycle is associated with performance on the water test may account for some of the inconsistency in the literature among studies of spatial performance in rats. Controlling for cycle phase may reduce variability in the data. It remains unclear as to which factor of test performance (e.g., learning, motivation, motoricity) is affected. It is a reasonable hypothesis to suggest that cyclic fluctuations in sex hormones have an activational influence on spatial performance given their organizational effects on spatial performance. However, two intriguing possibilities are that other covarying systems are involved, and that the factors influencing spatial performance are different for males and females. Thus the role of stress in inter- and intra-sex variation in performance in the water maze is currently under investigation.

ACKNOWLEDGEMENTS

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