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Post-training estrogen enhances spatial and object memory consolidation in female mice

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Abstract

The present study was designed to determine if post-training injections of a water-soluble form of 17β-estradiol could enhance spatial and object memory consolidation in young female mice. Young ovariectomized female mice were trained in Morris water maze and object recognition tasks, injected with 0.1, 0.2, or 0.4 mg/kg cyclodextrin-encapsulated 17β-estradiol or cyclodextrin-conjugated vehicle, and then re-tested after a delay. In the water maze, mice were trained in eight consecutive trials, injected, and memory for the platform location was re-tested after 24 h. All mice learned to find the platform on Day 1, but only mice receiving 0.2 mg/kg estradiol remembered the platform location on Day 2. In the object recognition task, mice were first presented with two identical objects, injected, and then presented with a familiar and novel object after a 24- or 48-h delay. For both delays, the 0.2 and 0.4 mg/kg doses enhanced memory for the familiar object. These data demonstrate that a 0.2 mg/kg dose of estradiol can enhance multiple types of memory consolidation in female mice, and suggest a narrower effective dose range for spatial memory than for object memory.

Keywords: Spatial memory; Water maze; Object recognition; Post-training; Estradiol; Mouse; Cyclodextrin

1. Introduction

Interest in the effects of sex-steroid hormones, such as estrogen, on memory has been increasing in recent years due, in part, to the potential detrimental impact on memory produced by the loss of these hormones during menopause (Sherwin, 2005). Much of the research aimed at understanding how hormones modulate memory has been conducted in young adult female rodents. In these animals, considerable evidence has demonstrated that estrogen has a profound influence on regions of the brain involved in memory, including the hippocampus and basal forebrain. For example, in the female hippocampus, estrogen has been shown to increase CA1 dendritic spine density (Frick et al., 2004; Woolley and McEwen, 1992, 1993) and synaptic proteins (Stone et al., 1998), reduce GABAergic inhibition of CA1 pyramidal neurons (Murphy et al., 1998), and enhance long-term potentiation (Foy et al., 1999; Warren et al., 1995) and neurogenesis (Tanapat et al., 1999). In the basal forebrain, estrogen enhances the functioning of cholinergic neurons that innervate the hippocampus (Gibbs and Aggarwal, 1998; Gibbs and Gabor, 2003; Wu et al., 1999). Together, these findings indicate that estrogen beneficially modulates hippocampal and basal forebrain function in females.

The prediction from these findings is that estrogen should enhance learning and memory in females. Indeed, numerous studies have shown that estrogen treatment given to ovariectomized female mice improves spatial reference memory in the Morris water maze (Heikkinen et al., 2002; Rissanen et al., 1999) and non-spatial memory in object recognition and avoidance tasks (Farr et al., 1995; Gresack and Frick, 2004; Vaucher et al., 2002). In female rats, estrogen improves spatial working memory in the T-maze (Fader et al., 1998; Miller et al., 1999), radial arm maze (Bimonte and Denenberg, 1999; Luine et al., 1998), and water maze (O'Neal et al., 1996; Sandstrom and Williams, 2001, 2004). Estrogen also appears to promote

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the use of a spatial strategy in memory tasks (Korol and Kolo, 2002; Korol et al., 2004). However, not all studies report a memory enhancing effect of estrogen (Chesler and Juraska, 2000; Galea et al., 2001; Holmes et al., 2002; Wide et al., 2004). For example, our lab recently reported that estrogen failed to improve memory in female rats tested in the water maze (Frick et al., 2004). An analysis of hippocampal spine synapse density from these rats showed that, unlike in behaviorally naïve rats, estrogen did not increase CA1 spine synapse density in rats tested in the water maze. These data suggest that the measurement of estrogen-induced changes in the brain can be significantly influenced by behavioral experience.

Similarly, estrogen-induced alterations in memory can be significantly influenced by estrogen-induced changes in numerous non-mnemonic aspects of behavioral task performance. In all of the aforementioned studies, hormones were administered prior to training, which precludes a distinction between effects on memory and other psychological processes. The learning of any task involves numerous non-mnemonic factors such as motivation, attention, and sensorimotor function. Hormone administration prior to training can influence any of these variables, in addition to memory (McGaughy and Sarter, 1999; Morgan and Pfaff, 2001; Pfaff et al., 2002). In contrast, if hormones are given after training (termed "post-training"), their specific effects on memory consolidation can be examined in the absence of these non-mnemonic confounds (McGaugh, 1989). Estrogen administered post-training to young female rats has been shown to enhance spatial reference memory consolidation in the Morris water maze (Packard, 1998). Rats were first trained in 8 consecutive trials to find a hidden escape platform in the water maze and then, immediately after the last trial, were injected with vehicle or 0.1, 0.2, or 0.4 mg/kg 17β-estradiol (Packard and Teather, 1997b). Memory for the platform location was measured 24 h later. The type of estrogen used in this design differs from conventional preparations dissolved in sesame oil in that it can be dissolved in saline and is metabolized within 24 h (Packard and Teather, 1997b; Pitha and Pitha, 1985); thus, it does not remain in the circulation during the 24-h re-test. Because estradiol is not present during training or retention testing, only memory consolidation can be affected. During training, all rats learned to find the platform. However, at re-test, all rats but those receiving 0.2 mg/kg estradiol took significantly longer to find the platform than during the last training trial (Packard and Teather, 1997b). The memory facilitation induced by 0.2 mg/kg estradiol is time-dependent, as injection of this dose 2 h post-training did not affect memory consolidation (Packard and Teather, 1997b). Further, this effect is likely mediated by the hippocampus, as post-training intrahippocampal infusions of 5 μg cyclodextrin-encapsulated 17β-estradiol also enhance spatial memory consolidation in the water maze (Packard and Teather, 1997a).

The distinction between estrogen effects on memory and other psychological processes is important for the use of hormone therapy in menopausal women; if motivational or affective changes alone are responsible for hormone therapy-induced improvements in memory tasks, then treatments that directly target these processes, rather than memory, could be used instead of hormones. Thus, it is critical to determine if the post-training findings can be replicated and generalized to other species or types of memory. Furthermore, by establishing dose–response curves for various types of memory, low doses of estrogen can be identified that may be effective when used in conjunction with additional variables (e.g., diet and exercise), thereby minimizing the potential health risks of hormone therapy.

To this end, the present study first sought to replicate these findings in another rodent species, mice. Establishing if post-training estradiol injections can facilitate memory consolidation in mice would allow future studies using estrogen receptor knockout mice to investigate the underlying molecular mechanisms by which estrogen modulates memory. Young ovariectomized female mice were trained in a hippocampal-dependent spatial Morris water maze task (Morris et al., 1982), immediately injected with 0.1, 0.2, or 0.4 mg/kg 17β-estradiol (E_2) or vehicle, and then re-tested after a 24-h delay as in Packard and Teather (1997b). Based on this work, we predicted that only the 0.2 mg/kg dose would enhance spatial memory consolidation. The present study also sought to extend previous findings by examining if post-training estradiol treatment could enhance memory in a non-aversively motivated task. Object memory was selected for study because the object recognition task used does not involve the aversive motivation inherent to the water maze or nutrient restriction utilized for many learning tasks, and depends solely on internal motivation to explore the objects. Thus, this task significantly minimizes confounds due to the stress of behavioral testing that may interfere with estrogen-induced alterations in the brain or behavior (Frick et al., 2004). Furthermore, the hippocampus has been shown to be critically involved in performance of this task (Baker and Kim, 2002; Clark et al., 2000). One recent study in female rats found that post-training injections of a synthetic estrogen enhanced memory for the identity and location of objects 4 h after injection (Luine et al., 2003). In adult female mice, we have shown that 0.2 mg/kg, but not 0.1 mg/kg 17β-estradiol, enhances object memory consolidation tested after 48 h (Gresack and Frick, 2004). Thus, the present study tested these doses and the 0.4 mg/kg dose of 17β-estradiol on object recognition using a 48-h delay. In order to test the hypothesis that the 0.1 and 0.4 mg/kg doses may be effective at a shorter delay, a 24-h delay was also tested. Based on our previous findings at the 48-h delay, we predicted that the 0.2 mg/kg dose would be beneficial at both delays. For the other doses, it is possible that a shorter delay would reveal either a beneficial effect or the same inverted U-shaped dose–response curve observed in the water maze (Packard and Teather, 1997b).

2. Methods

2.1. Subjects

Female C57BL/6 mice were obtained from Taconic (Germantown, NY). Mice were ovariectomized by Taconic at 7 weeks of age and arrived at Yale at 8 weeks of age. They were housed up to 5 per shoebox cage in a room with a 12 : 12 h light/

dark cycle (lights on at 07:00), with all testing performed during the light phase. Ad libitum access to food and water was provided. Mice were handled for 5 min/day at least five times prior to testing to habituate them to being picked up by the experimenter. Behavioral testing began at 9 weeks of age. All procedures were approved by the Institutional Animal Care and Use Committee of Yale University, and conformed to the guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

An initial set of mice was tested in the Morris water maze and the object recognition task using a 24-h delay. Sample sizes for this group were as follows: Control ($n=10$), 0.1 mg/kg E_2 ($n=6$), 0.2 mg/kg E_2 ($n=10$), or 0.4 mg/kg E_2 ($n=6$). Object recognition testing for the 24-h delay took place approximately 2 weeks after the completion of water maze testing. To prevent interference resulting from prior exposure to object recognition testing, a new set of mice ($n=10$/group) was tested using a 48-h delay (Gresack and Frick, 2004).

2.2. Hormone treatment

Mice received intraperitoneal (i.p.) injections of 17β-estradiol (E_2) conjugated to the solubility enhancer 2-hydroxypropyl-β-cyclodextrin (HBC) dissolved in physiological saline in one of three doses: 0.1 mg/kg, 0.2 mg/kg, or 0.4 mg/kg. The 0.2 mg/kg dose has previously been shown to enhance spatial memory consolidation in young female rats when injected immediately, but not 2 h, after training (Packard and Teather, 1997b). Controls were injected with HBC vehicle dissolved in an equal volume of saline, which contained the same amount of cyclodextrin present in the 0.2 mg/kg dose of cyclodextrin-encapsulated E_2. HBC is a solubility-enhancer for the normally hydrophobic steroid hormones and does not alter the bioefficacy of the hormones (Pitha and Pitha, 1985). These water-soluble hormones can successfully cross the blood-brain barrier because they rapidly dissociate from the circulating solution into the tissue while the HBC remains in the solution (Taylor et al., 1989). The primary advantage of this hormone preparation is that it is metabolized within 24 h (Pitha et al., 1986; Taylor et al., 1989) and is, therefore, not present in the circulation during retention testing. This allows retention to be assessed in the absence of hormone effects on non-mnemonic performance factors. Because we have found it exceedingly difficult to accurately measure hormone levels in mice, serum levels were not obtained in the present study. However, previous reports have shown that a 1 μg dose of β-estradiol in oil produces levels similar to those seen in estrus, and a 10 μg dose produces levels similar to those seen in proestrus (Akinci and Johnston, 1997). The mean bodyweight for mice in this study was 22 g, yielding approximate estradiol doses of 2.2, 4.4 and 8.8 μg. Thus, the doses used in the present study were within physiological levels.

2.3. Morris water maze

Testing took place in a white circular tank (97 cm in diameter) filled with water (24 °C±2 °C). The water was made opaque with white nontoxic paint and the maze was surrounded by various extramaze cues. Data were collected using an HVS 2020 (Hampton, England) automated tracking system.

Mice were shaped 1 day prior to testing using a four-trial procedure in which a smaller ring (55 cm) was placed inside of the larger ring (97 cm) to decrease the total swimming area. Mice were first placed on a visible 10×10 cm^2 platform (covered in red tape) for 10 s and then removed. They were then placed at three distances progressively further from the platform and allowed to swim to the platform. If the mouse did not find the platform within 60 s, then it was led to it by the experimenter. No data were collected during shaping.

Spatial water maze testing was conducted as in Packard and Teather (1997a,b). During testing, a transparent Lucite platform (10×10 cm^2) was submerged just underneath the surface of the water and remained in the same location for all trials. On Day 1 of testing, eight consecutive spatial trials were conducted. Each mouse was placed in one of four start positions, which varied for each trial. The mouse was given 60 s to find the platform in each trial. If she did not find it, then the experimenter led her to the platform and let her sit on it for 10 s. She was then placed in a holding cage under a heat lamp for an intertrial interval of 45 s. Immediately after the completion of trial 8, the mouse was removed from the platform and injected with hormone or vehicle. Twenty four hours later, mice were re-tested in the spatial task for one trial to examine spatial memory retention. Swim distance (cm) was the primary measure of memory. Swim speed (cm/s) was also recorded.

2.4. Object recognition

The object recognition task was conducted in a wooden open field box ($58\times58\times46$ cm^3) painted white and located in a quiet room under dim lighting. Three objects were used, all of which were approximately 5–7 cm in height and width. The objects were a triangular prism constructed of transparent plastic with colored edges, a half inch threaded gate valve consisting of a brass body with cast-iron handle, and an object constructed of three rectangular Lego pieces arranged such that two pieces formed a square and a third, centered above, connected them. A video camera was mounted on the ceiling above the box and connected to a video recorder, monitor, and computer in an adjoining room. Throughout testing, the door to the testing room was closed and mice were observed on the monitor.

Testing was conducted as described previously (Frick and Gresack, 2003; Gresack and Frick, 2004). Briefly, the task takes advantage of the natural affinity of mice for novelty. Three phases (habituation, sample, and choice) were conducted on separate test days. During habituation, each mouse was placed in the empty open field box and allowed to explore for 5 min. No data were collected in this phase. The next day, all mice completed the sample phase. Following a 1-min re-habituation to the box, two identical objects were placed in the northwest and northeast corners of the box (approximately 5 cm from the walls) and the mice explored the objects until they accumulated 30 s of exploration. Each mouse was then removed from the box, immediately injected with vehicle or E_2, and returned to its

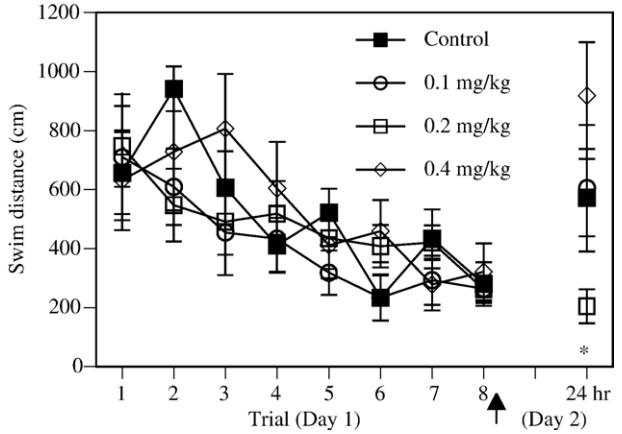

Fig. 1. The 0.2 mg/kg dose of estradiol significantly improved spatial memory retention in the Morris water maze. All groups exhibited similar swim distances in Day 1 of training (trials 1–8). Mice were injected immediately following trial 8 (arrow). On Day 2 (24 h), only 0.2 mg/kg mice retained memory for the platform location as indicated by maintenance of low swim distances. Mice receiving 0.2 mg/kg performed significantly better than controls or those receiving 0.4 mg/kg of estradiol (*p values <0.05).

home cage. Mice were tested in the choice phase 24 or 48 h after injection. Young female mice typically do not remember the familiar object in the choice phase after 24 h (Frick and Gresack, 2003), and rarely remember it after 48 h (Gresack and Frick, 2004) or 7 days (Frick and Gresack, 2003). During this phase, one familiar object (identical to that which was used in the sample phase) and one novel object were placed in the same corners of the box occupied during the sample phase. The location of the novel object was counterbalanced across mice in each group. Mice remained in the box until they accumulated 30 s of object exploration. If a mouse did not accumulate 30 s of exploration within 10 min during either the sample or choice phases, testing was discontinued and the mouse was excluded from the data analyses. During all phases of testing, the box and objects were cleaned with 70% ethanol between mice.

Time spent exploring each object was recorded during both the sample and choice phases using a video tracking system and a custom-written computer program. Object exploration was scored only when the mouse's nose or front paws touched the object. In addition, the time needed to accumulate 30 s of exploration (i.e., elapsed time) during both the sample and choice phases was recorded to control for potential hormone-induced differences in motivation, arousal, or motor function. Intact memory for the familiar object was demonstrated if the mouse exhibited a "preference" for the novel object in the choice phase. A preference was indicated if the mouse spent more time than chance with the novel object.

2.5. Data analysis

Water maze data from the eight trials of Day 1 were analyzed using one-way repeated measures analyses of variance (ANOVA) with Treatment as the independent variable and Trial as the repeated measure (SuperANOVA, Abacus Concepts; Berkeley, CA). One-way ANOVAs without repeated measures were performed on data from trial 8 of Day 1 to ensure that there were no significant group differences prior to hormone injection. Another one-way ANOVA was conducted on data from Day 2. Repeated measures ANOVAs were also conducted on data from trial 8 of Day 1 (last trial before injection) and trial 1 of Day 2 (trial after injection) to pinpoint hormone effects on memory consolidation. Fisher's Protected Least Significant Difference (PLSD) post-hoc tests were performed on all main effects of Treatment.

For the object recognition task, a preference for one object over another was assessed using one-sample t-tests to determine whether the time spent with each object differed significantly from the chance value of 15 s (Frick and Gresack, 2003; Gresack and Frick, 2004). This type of t-test was used because the times spent with each object are not independent; the total time exploring must equal 30 s, so time spent with one object reduces time spent with the other. t-tests were conducted for each group separately. For elapsed time, a one-way ANOVA was conducted with Treatment as the independent variable.

3. Results

3.1. Subjects

All mice completed Morris water maze testing. However, two mice (one each in the 0.1 and 0.4 mg/kg groups) were excluded from the analyses of the 24-h delay in the object recognition task because they failed, during the sample phase, to accumulate 30 s of exploration within 10 min.

3.2. Morris water maze

All groups learned to find the platform similarly on Day 1, as indicated by a significant Trial effect for swim distance [$F(7,196)=8.9$, $P<0.0001$] in the absence of significant Treatment [$F(3,28)=0.7$, $P>0.05$] or Treatment × Trial [$F(21,196)=1.0$, $P>0.05$] effects (Fig. 1). Swim distances among the groups also did not differ during the last trial of Day 1 prior to hormone injection [$F(3,28)=0.1$, $P>0.05$]. Swim speeds (Fig.

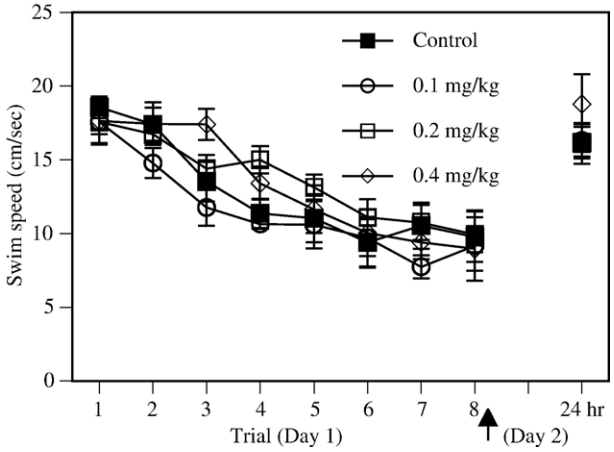

Fig. 2. Estradiol did not affect swim speeds during either Day 1 or Day 2. Mice were injected immediately following trial 8 (arrow).

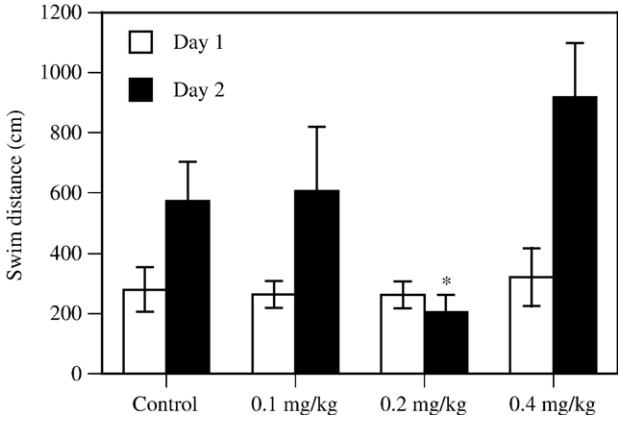

Fig. 3. Swim distance in trial 8 of Day 1 and the retention trial of Day 2. Only the 0.2 mg/kg group maintained similar swim distances from Day 1 to Day 2; distances in all other groups increased overnight. The 0.2 mg/kg group exhibited significantly shorter swim distances than the control or 0.4 mg/kg groups (*p values <0.05).

2) decreased throughout testing [$F(7,196)=35.7$, $P<0.0001$], but were not affected by estradiol [Treatment effect: $F(3,28)=0.9$, $P>0.05$; Treatment × Trial interaction: $F(21,196)=1.1$, $P>0.05$]. Swim speeds did not differ among the groups during trial 8 [$F(3,28)=0.07$, $P>0.05$].

In contrast to Day 1, the groups differed significantly on Day 2, as indicated by the main effect of Treatment for swim distance [$F(3,28)=4.5$, $P<0.02$; Fig. 1, 24-h time point]. Post-hoc tests indicated that the swim distances of the 0.2 mg/kg group were significantly lower than those of the Control and 0.4 mg/kg groups (P values <0.05). Swim speeds did not differ among the groups on Day 2 [$F(3,28)=0.7$, $P>0.05$; Fig. 2, 24-h time point], indicating that changes in swim speed did not contribute to the estradiol-induced alteration in swim distance.

The beneficial effect of the 0.2 mg/kg dose of estradiol is further illustrated by a repeated measures ANOVA on the swim distance data comparing performance in trial 8 of Day 1 and the retention trial from Day 2. In this analysis, the main effects of Treatment [$F(3,28)=3.2$, $P<0.05$] and Trial [$F(1,28)=20.2$, $P<0.0001$] were significant, as was the Treatment × Trial interaction [$F(3,28)=4.5$, $P<0.02$]. This interaction, illustrated in Fig. 3, is driven by the fact that swim distances in all groups but the 0.2 mg/kg group were higher on Day 2 than in the last trial of Day 1. Thus, these data indicate significant retention only in the 0.2 mg/kg group. In contrast to swim distance, swim speed did not differ among the groups from Day 1 to Day 2 [Treatment effect: $F(3,28)=0.1$, $P>0.05$; Treatment × Trial interaction: $F(3,28)=0.9$, $P>0.05$]. Swim speeds in all groups increased from Day 1 to Day 2 [Trial effect: $F(1,28)=71.8$, $P<0.0001$; Fig. 2].

3.3. Object recognition

3.3.1. 24-h delay

The time each group spent with the objects in the sample and choice phases is illustrated in Fig. 4. During the sample phase (Fig. 4A), the 0.1 mg/kg [$t(4)=3.8$, $P<0.03$] and 0.2 mg/kg [$t(9)=2.3$, $P<0.05$] groups spent significantly more time with the identical object in the northwest corner of the open field than chance (15 s). This inherent preference was minimized in the choice phase by counterbalancing the side on which the novel object was placed. In the choice phase (Fig. 4B), no preference for either object was shown by the Control or 0.1 mg/kg groups, but a significant preference for the novel object was demonstrated by the 0.2 and 0.4 mg/kg groups. Both of these groups spent more time than chance with the novel object [0.2 mg/kg: $t(9)=2.4$, $P<0.05$; 0.4 mg/kg: $t(4)=4.6$, $P<0.02$].

Elapsed time in both phases is presented in Table 1. Estradiol did not affect elapsed time in either the sample [$F(3,26)=0.3$, $P>0.05$] or the choice [$F(3,26)=1.4$, $P>0.05$] phases.

3.3.2. 48-h delay

Fig. 5 illustrates the time spent with the objects in the sample and choice phases for the 48-h delay. No groups showed a preference for either object in the sample phase (Fig. 5A). In contrast, the 0.2 and 0.4 mg/kg groups showed a significant preference for the novel object in the choice phase [0.2 mg/kg: $t(9)=2.4$, $P<0.04$; 0.4 mg/kg: $t(9)=5.1$, $P<0.001$; Fig. 5B]. These doses also produced significant preferences for the novel object at the 24-h delay.

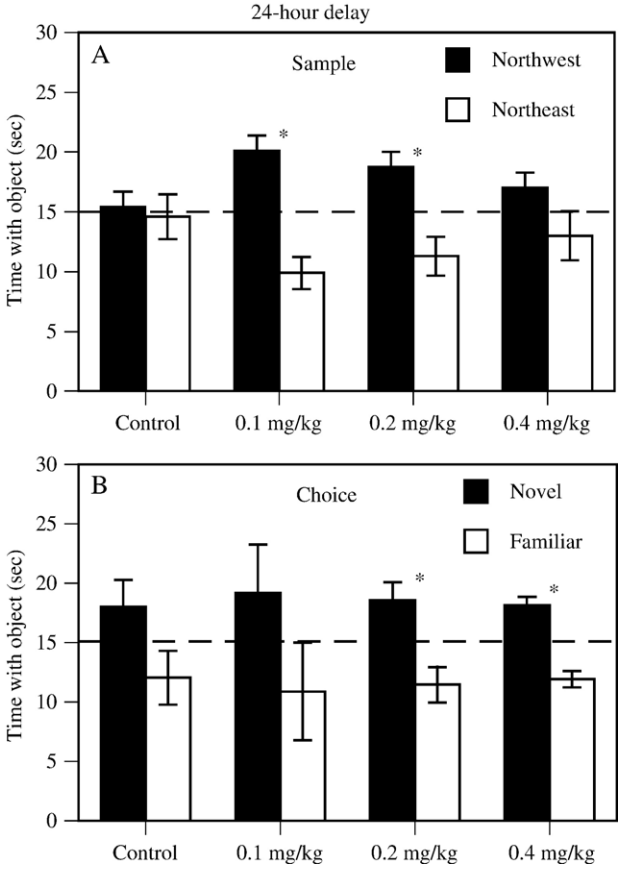

Fig. 4. (A) During the sample phase, the 0.1 mg/kg and 0.2 mg/kg groups spent significantly more time than chance (indicated by the dotted line at 15 sec) with the left object and less time than chance with the right object (*p values <0.05 for both left and right objects). (B) During the choice phase, only the 0.2 mg/kg and 0.4 mg/kg groups spent significantly more time than chance with the novel object (*p values <0.05 for both novel and familiar objects).

Table 1
Elapsed time in the object recognition task

Group	24-h delay		48-h delay	
	Sample	Choice	Sample	Choice
Control	178.6±38.9	151.9±16.8	181.9±20.2	232.0±23.2
0.1 mg/kg	151.6±51.1	203.4±25.7	149.4±13.5	231.1±22.1
0.2 mg/kg	213.9±57.6	238.5±31.7	143.5±15.8	188.5±18.2
0.4 mg/kg	150.7±61.8	258.9±99.7	144.8±29.5	190.2±17.0

Values are expressed as mean±S.E.M.

Values for elapsed time in both phases are presented in Table 1. Similar to the 24-h delay, estradiol had no effect on elapsed time in either phase [sample: $F(3,36)=0.8$, $P>0.05$; choice: $F(3,36)=1.4$, $P>0.05$].

4. Discussion

Data from the Morris water maze clearly demonstrate that 0.2 mg/kg estradiol, but not 0.1 or 0.4 mg/kg estradiol, enhances spatial memory consolidation in young female mice. As such, this study exactly replicates the findings of Packard and Teather (1997b) and demonstrates that the beneficial effect of 0.2 mg/kg estradiol demonstrated in their study is sufficiently robust to generalize among rodent species. Although we only administered this dose immediately after training and did not include a delayed-injection group, the fact that two previous studies have shown that injections of estradiol 2 h after training have no effect on memory consolidation (Luine et al., 2003; Packard and Teather, 1997b) suggest that the effect of estradiol on memory in this study was likely limited to the 2-h period immediately after injection. Together, these findings indicate that estrogen can specifically affect spatial memory consolidation, and may suggest that the beneficial effects of pre-training estrogen treatment on spatial memory (Bimonte and Denenberg, 1999; Fader et al., 1998; Heikkinen et al., 2002; Luine et al., 1998; Miller et al., 1999; O'Neal et al., 1996; Rissanen et al., 1999; Sandstrom and Williams, 2001, 2004) result from changes in memory rather than in non-mnemonic aspects of task performance.

In the object recognition task, the 0.2 and 0.4 mg/kg doses of estradiol improved object memory consolidation at both delays, suggesting a robust and reliable effect on object memory. These data are consistent with previous findings that a synthetic estrogen can improve object memory consolidation in female rats after a 4-h delay (Luine et al., 2003). Although both delays in the present study revealed a beneficial effect of the 0.2 and 0.4 mg/kg doses, the data from the 48-h delay are more compelling. In the 24-h delay, two groups showed a significant preference for one identical object during the sample phase, and even the control and 0.1 mg/kg groups showed a modest (although non-significant) preference for the novel object during the choice phase. This later point suggests that the 24-h delay was not quite long enough for the familiar object to be forgotten. In contrast, with the 48-h delay, no clear preferences were shown by any group during the sample phase or by the control and 0.1 mg/kg groups in the choice phase.

It is interesting, however, that the 0.4 mg/kg dose was as beneficial at both delays as the 0.2 mg/kg dose. This dose–response relationship is different from that seen in the water maze, where the 0.4 mg/kg dose was completely ineffective (indeed, this group performed the worst of all groups on Day 2). This might suggest a narrower therapeutic window for spatial memory facilitation than for object memory, such that memory in the water maze is affected by only an optimal dose of estradiol, whereas object memory can be modulated by several doses of estradiol. This notion is supported by data from middle-aged female rats in which three doses of 17β-estradiol administered orally over several months significantly improved object recognition, but not spatial memory (Fernandez and Frick, 2004). However, the water maze and object recognition tasks differ in many ways, so direct comparisons of the effectiveness of each dose would be best made with more information about the conditions in which these doses are effective. For example, testing different delays in the water maze and higher doses of estradiol (e.g., 0.6 or 0.8 mg/kg) in the object recognition task to more fully examine the upper range of the dose–response curve would help to address this issue. If this work supports the dose–response relationships shown in the present study, then this may indicate that object memory deficits in menopausal women (Duka et al., 2000) could be easier to alleviate with hormone therapy than spatial memory deficits (Duff and Hampson, 2000; Duka et al., 2000).

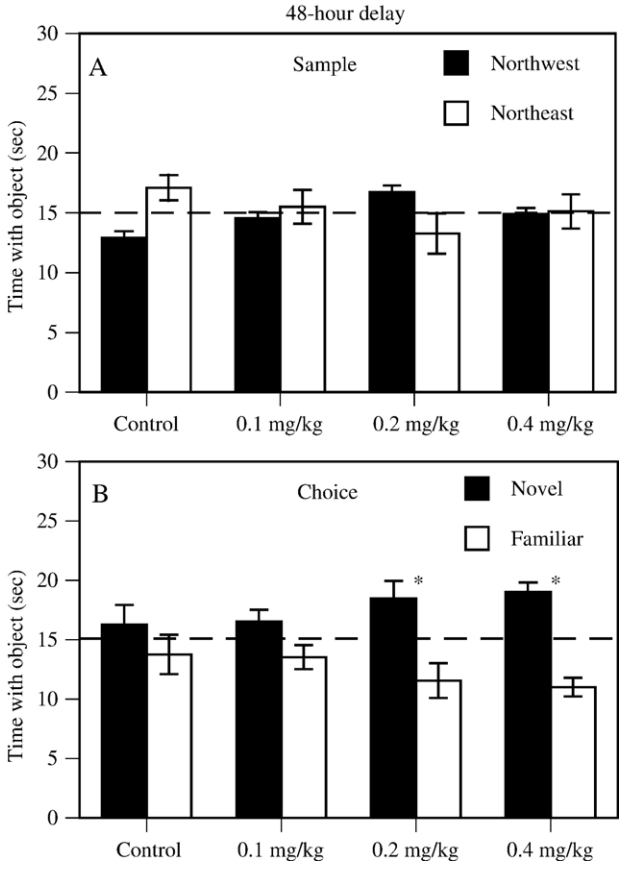

Fig. 5. (A) No groups showed a preference for either identical object during the sample phase. (B) During the choice phase, only the 0.2 mg/kg and 0.4 mg/kg groups spent significantly more time than chance with the novel object (*p values <0.05 for both novel and familiar objects).

The fact, however, that the 0.1 mg/kg dose was ineffective at both delays and in the water maze establishes this dose as a generally sub-effective dose for multiple types of memory. As such, future research could be aimed at combining this dose with other pharmacological or behavioral treatments as a way to reap the benefits of hormone therapy at lower doses. For example, concurrent post-training administration of the 0.1 mg/kg dose and a sub-effective dose of the cholinergic agonist oxotremorine enhanced spatial memory in the water maze as much as 0.2 mg/kg estradiol alone (Packard and Teather, 1997b). In contrast, combining the 0.1 mg/kg dose with a behavioral treatment such as environmental enrichment only very marginally improved spatial working memory in a radial arm maze relative to 0.1 mg/kg estradiol alone (Gresack and Frick, 2004). However, more work will be needed to more fully examine interactions between sub-effective doses of estradiol and behavioral modifications such as diet and exercise.

The present data suggest that the 0.2 mg/kg dose of estradiol is the optimal dose to use in future studies investigating the molecular mechanisms by which estrogen modulates memory consolidation. For example, this dose could be used to determine if estrogen affects memory via membrane-bound receptors linked to signal transduction pathways or rather by classical intracellular estrogen receptors (ERα and ERβ). The latter could be examined by administering 0.2 mg/kg estradiol post-training to ERα or ERβ knockout mice. The former could be studied by combining the 0.2 mg/kg dose with inhibitors of signal transduction. For example, our lab recently showed in young female mice that concurrent systemic or dorsal hippocampal administration of 0.2 mg/kg estradiol and an inhibitor of the enzyme that phosphorylates the molecule extracellular signal-regulated kinase (ERK) completely abolishes the beneficial effects of 0.2 mg/kg estradiol on object memory (Fernandez et al., 2005). These data demonstrate that estradiol facilitates object memory consolidation in young female mice by activating the ERK pathway in the dorsal hippocampus. Future work can address whether this activation involves classical estrogen receptors.

In conclusion, the present study demonstrates for the first time that post-training estradiol administration can significantly facilitate spatial and object memory consolidation in female mice. The fact that post-training administration allowed for memory to be examined in the absence of non-mnemonic confounds related to task performance suggests that estrogen specifically influences spatial and object memory formation. Furthermore, the data indicate different dose–response relationships for spatial and object memory, which may suggest distinct neural mechanisms in the hormonal regulation of these types of memory. As such, this study provides information about estrogenic modulation of memory that may have important implications for the development of hormone treatments for menopausal women.

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