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Effects of estrogen and progesterone on spatial memory consolidation in aged females

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Abstract

Interpretation of data illustrating that estrogen, with or without progestin, is detrimental to memory in post-menopausal women is complicated by the fact that little is known about the effects of progestins on memory. The present study examined if estrogen, alone or with progesterone, affects spatial memory consolidation in ovariectomized aged female mice. Mice received eight training trials in a spatial Morris water maze followed immediately by injection of water-soluble 17β-estradiol (E_2; 0.2 mg/kg) or vehicle. Mice were re-tested 24 h later. All mice learned to find the platform on Day 1. On Day 2, the performance of control, but not E_2 mice, deteriorated, suggesting that E_2 enhanced memory for the platform location. In a second experiment, mice were injected with E_2 and 10 or 20 mg/kg water-soluble progesterone. The 10 mg/kg dose of progesterone did not affect estrogen's ability to enhance spatial memory consolidation, but 20 mg/kg blocked this effect. These data indicate that estrogen can improve spatial memory consolidation in aged females and that this effect can be attenuated by progesterone.

Keywords: Spatial memory; Reference memory; Water maze; Aging; Post-training; Ovarian hormones; Progestin; Mouse; Cyclodextrin

1. Introduction

The substantial loss of estrogen experienced by menopausal women has been linked to memory loss in both normal aging and dementia. Estrogen treatment in healthy menopausal women has been shown to improve spatial working memory [3], object memory [4,19], and verbal memory [3,35]. Other studies, however, have reported little or no mnemonic benefits from estrogen treatment in women [14,45]. Indeed, recent data from the Women's Health Initiative Memory Study (WHIMS) indicates that estrogen, given alone or with a synthetic form of progesterone, actually increases the risk of cognitive decline in post-menopausal women [36,37]. These data sharply contrast with previous reports that estrogen benefits memory function in menopausal women, and highlights the need to understand the ways in which ovarian hormones modulate memory in aging females.

Animal models have been instrumental in elucidating the effects of ovarian hormones on the brain and behavior, and thus, can help shed light on this issue. In female rodents, estrogen has profound effects on brain regions that are critical to learning and memory, such as the hippocampus. For instance, elevated estrogen levels in young female rats have been associated with enhanced hippocampal long-term potentiation [42], neurogenesis [39], and CA1 dendritic spine density [43,44]. The most biologically active form of estrogen, estradiol, also increases dentate gyrus spine density in aged female rats [21] and synaptic protein levels in aged female mice [9]. Furthermore, estradiol improves spatial memory in aged male [17] and female [11] rats. In middle-aged and aged female mice, chronic estradiol treatment significantly improves performance in spatial reference memory and object memory tasks, and increases hippocampal levels of the presynaptic protein synaptophysin [6,9]. Although these data suggest that estradiol improves memory in aging rodents, this evidence

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is confounded by the fact that treatments were given prior to behavioral training. As such, these treatments may have affected non-mnemonic aspects of task performance, such as arousal, motivation, or sensorimotor function, in addition to memory. In contrast, administering estradiol after training allows specific effects on memory consolidation to be observed in the absence of these non-mnemonic confounds. Post-training peripheral administration of 0.2 mg/kg estradiol improves spatial memory consolidation in young female rats [29], but such treatment has never been attempted in aging females. Thus, it remains unclear whether estradiol treatment specifically improves memory in aged females.

Despite the ability of estradiol to consistently improve performance in memory tasks among aged rodents, studies examining the effects of progesterone or of hormone therapy consisting of estradiol plus progesterone have produced more equivocal results. For example, acute estradiol and progesterone administered to young female rats impairs spatial reference memory in the Morris water maze [2]. Similarly, young female mice receiving estradiol learned a footshock avoidance task significantly faster than mice receiving estrogen plus progesterone or progesterone alone [5]. When administered alone, progesterone and its metabolite, allopregnanolone, have also been shown to impair spatial working and reference memory in young rats [10,15]. On the other hand, treatment with estrogen and progesterone has been shown to reduce spatial reference and working memory impairments induced by the cholinergic agonist scopolamine [38] and protect against memory impairments induced by intra-hippocampal administration of the neurotoxin colchicine [41]. Furthermore, in middle-aged and aged female rats, chronic estradiol plus progesterone improves spatial reference and working memory [11,20]. As is the case for estradiol, all of these studies administered progesterone prior to training, and thus, it is unclear if the effects of this hormone are due to specific changes in memory consolidation or to non-mnemonic performance factors. This issue is particularly important for progesterone because this hormone binds to GABA-A receptors and may, therefore, reduce arousal [16].

The goal of the present study was to determine if estradiol alone or estradiol plus progesterone affect spatial memory consolidation in aged (22 months) ovariectomized female mice. In order to pinpoint effects of the hormones on memory, in the absence of confounding effects on motivation or sensorimotor function, water-soluble hormone preparations were given immediately after training (post-training) in a spatial Morris water maze task. Consolidation was tested by measuring retention of the platform location 24 h later. Because these water-soluble hormones are metabolized within 24 h [30,40], retention is tested in the absence of hormones. Mice in Experiment 1 were injected intraperitoneally with 0.2 mg/kg 17β-estradiol (E_2) or vehicle immediately after water maze training. In young female rats, 0.2 mg/kg E_2 injected immediately, but not 2 h after training, significantly improves retention of the platform location, suggesting that this dose enhances spatial memory consolidation in the water maze [29]. The 0.2 mg/kg dose also improves object memory consolidation and spatial working memory in young female mice [13]. Thus, we hypothesized that 0.2 mg/kg estradiol would also improve spatial memory consolidation in aged females. In Experiment 2, a different group of mice was injected after water maze training with 0.2 mg/kg E_2 combined with 10 or 20 mg/kg progesterone. Because this is the first study to utilize water-soluble progesterone, these doses were based on previous studies using progesterone dissolved in oil [44]. As in those studies, our low progesterone dose was 50 times that of estradiol. The higher dose was given to approximate levels similar to those during proestrus [1]. Given that levels of both estradiol and progesterone are greatly reduced by middle age in C57BL/6 mice [24], we hypothesized that the combination of both hormones would improve memory in aged females.

2. Methods

2.1. Subjects

Subjects were 22 months old female C57BL/6 mice obtained from the National Institutes on Aging colony at Harlan Sprague Dawley (Indianapolis, IN). Mice were housed up to five per shoebox cage in a room with a 12:12 light/dark cycle (lights on at 07:00), with all testing performed during the light phase. Mice had ad libitum access to food and water. Animals were handled for 5 min/day at least five times prior to ovariectomy surgery to habituate them to being picked up by the experimenter. 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.

2.2. Ovariectomy

Although the estrous cycles of female mice have typically ceased by 22 months of age [8,25], all mice were ovariectomized to ensure similar endogenous levels of estrogens prior to treatment. Surgeries were conducted one month prior to behavioral testing as described previously [6,13]. Briefly, mice were anesthetized using 2% isoflourane gas in 100% oxygen. Bilateral dorsal incisions were made at the level of the pelvis, and the ovaries and tips of the uterine horns were isolated and removed. Uteri were then placed back into the body cavity, the muscle wall was sutured, and the skin closed with wound clips. Analgesia was provided by 300 mg/kg children's acetaminophen in the drinking water for one week post-surgery.

2.3. Morris water maze

Testing took place in a white circular tank (97 cm in diameter) filled with water (24 ± 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 one 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 cm × 10 cm 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 let to it by the experimenter. No data were collected during shaping.

Spatial water maze testing was conducted as in [29]. During testing, a transparent Lucite platform (10 cm × 10 cm) 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 (see below). Twenty-four hours later, mice were re-tested in the spatial task for four consecutive trials to examine spatial memory consolidation. As in previous studies [28,29], spatial memory consolidation can only truly be measured in the first trial of Day 2 because re-exposure to the platform in this trial serves as a powerful reminder. The remaining trials serve to determine if mice can re-learn the platform location. Swim distance (cm) was the primary measure of memory. Swim speed (cm/s) was also recorded.

2.4. Hormone treatment

2.4.1. Experiment 1

Mice were randomly assigned to groups receiving intraperitoneal (i.p.) injections of 0.2 mg/kg 17β-estradiol (E_2, $n = 10$) conjugated to the solubility enhancer 2-hydroxypropyl-β-cyclodextrin (HBC) dissolved in physiological saline. This dose has previously been shown to enhance spatial memory consolidation in young female rats [29]. Estradiol-treated mice were compared to control mice receiving HBC vehicle. Controls were tested in both Experiment 1 ($n = 4$) and Experiment 2 ($n = 7$) and combined into one group for data analyses.

HBC is a solubility-enhancer for the normally hydrophobic steroid hormones and does not alter the bioefficacy of the hormones [31]. 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 [40]. The primary advantage of this hormone preparation is that it is metabolized within 24 h [30,40] 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 such as arousal, attention, or motivation. Because we have found it exceedingly difficult to accurately measure hormone levels in aged 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 [1]. The mean bodyweight for mice in this study was 35.5 g, yielding an approximate estradiol dose of 7.1 μg.

2.4.2. Experiment 2

A new set of mice was randomly assigned to groups injected with either HBC or with 0.2 mg/kg E_2 combined with 10 mg/kg ($n = 10$) or 20 mg/kg ($n = 10$) HBC-conjugated progesterone. Cyclodextrin-solubilized progesterone is reportedly metabolized in humans within 3 h [30]. Groups treated with estradiol plus progesterone will be termed 10 mg/kg $P + E_2$ and 20 mg/kg $P + E_2$. With an average bodyweight of 35.5 g, mice received approximately 0.36 and 0.71 mg of progesterone per injection in the 10 and 20 mg/kg groups. Previous work has demonstrated that progesterone doses of 0.1 mg and 1.0 mg produce hormone levels similar to those seen in estrus and proestrus, respectively [1]. Thus, both doses should be in the physiological range.

2.5. Uterine weights

In lieu of serum E_2 and P levels, uterine weights were recorded as a bioassay of the physiological effectiveness of hormone treatment. At least 2 weeks after behavioral testing, mice were injected with HBC or hormone and decapitated approximately 2 h later. Uteri were removed and weighed (g). Although 2 h is a rapid time frame in which to observe changes in uterine weights, this time point was selected because it was the latest time point within the 2 h window in which post-training estrogen affects spatial memory [29].

2.6. Data analysis

Water maze data 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, USA). Separate analyses were performed for the eight trials of Day 1 and the four trials of Day 2. 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. Repeated measures ANOVAs were also conducted on data from trial 8 of Day 1 (last trial before injection) and trial 1 of Day 2 (first trial after injection) to pinpoint hormone effects on memory consolidation. One-way ANOVAs without repeated measures were performed on uterine weights. Fisher's protected least significant difference (PLSD) post-hocs were performed a priori on all main effects of treatment to ensure that effects in one treat-

ment group were not obscured by a lack of effect in others. For Experiment 2, data were first analyzed comparing controls to both P + E_2 groups. In order to compare the P + E_2 groups to the E_2 group, separate ANOVAs were conducted for the most critical comparison, trial 8 of Day 1 versus trial 1 of Day 2, comparing each P + E_2 group to the control and E_2 groups. An alpha level of 0.05 was used to reject the null hypothesis.

3. Results

3.1. Experiment 1

3.1.1. Subjects

Upon arrival in the laboratory, all mice appeared in good health. Mice were randomly divided into hormone treatment groups before the start of behavioral testing. Half of the mice from each treatment group were tested in the water maze each day (it took 4 days to complete testing for Experiment 1). All mice completed behavioral testing. However, two controls and one E_2 mouse died of natural causes after testing. Thus, their uterine weights were not collected for analysis.

3.1.2. Uterine weights

Uterine weights in both experiments are illustrated in Fig. 1. Uterine weights were significantly increased by E_2 ($F(1,16) = 7.1$, $P < 0.05$), suggesting a significant physiological effect of E_2 administration.

3.1.3. Morris water maze

There were no significant group differences in swim distance or swim speed prior to injections on Day 1 ($Fs(1,19) =$ 1.6 and 3.7, respectively, $Ps > 0.05$). The rate of task acquisition on Day 1 was similar in both groups, as indicated by a significant main effect of trial for swim distance ($F(7,133) = 2.8$, $P < 0.009$; Fig. 2, Day 1), and swim speed ($F(7,133) = 14.3$, $P < 0.0001$; data not shown), in the absence of significant treatment × trial interactions for both measures ($Fs(7,133) = 1.0$, $Ps > 0.05$). By the last trial of Day 1, both groups performed similarly. A one-way ANOVA conducted on trial 8 revealed no significant effect of treatment on swim distance in this trial ($Fs(1,19) = 2.0$, $Ps > 0.05$), indicating that the groups did not differ cognitively prior to hormone treatment. Swim speeds were slightly, but significantly, faster in the E_2 group than in controls ($F(1,19) = 4.4$, $P = 0.0491$).

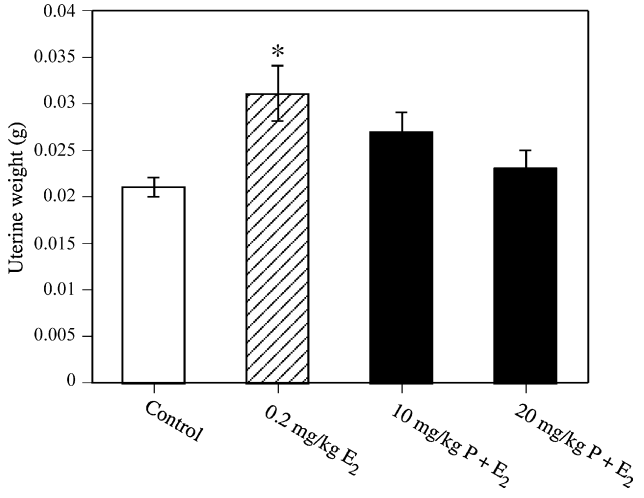

Fig. 1. Effects of hormone treatment on uterine weights. Each bar represents the mean ± standard error of the mean (S.E.M.) for each group. The 0.2 mg/kg E_2 group had significantly larger uteri than controls. When progesterone was added to this dose of E_2, uterine weights decreased. There was a significant difference in uterine weights between the 0.2 mg/kg E_2 and 20 mg/kg P + E_2 groups ($^*P < 0.05$ different from controls and 20 mg/kg P + E_2).

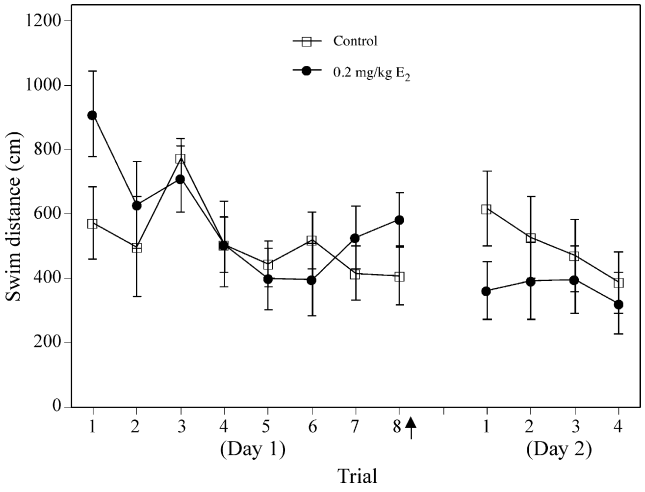

Fig. 2. Spatial memory as assessed by swim distance during eight spatial water maze training trials on Day 1 and four retention trials on Day 2. Each point represents the mean ± S.E.M. for each group. Swim distance on Day 1 declined in both groups across trials and only remained low during the first trial of Day 2 in the 0.2 mg/kg E_2 group. Controls performed worse on the first trial of Day 2 compared to the last trial of Day 1. The filled arrow indicates when injections were given.

The effect of post-training injections of estradiol on retention is shown in Figs. 2, 3, and 6. A repeated measures ANOVA including the last trial of Day 1 and the first trial of Day 2 indicated significant differences in swim distance between the groups. Although the effects of treatment ($F(1,19) = 0.2$, $P > 0.05$) and trial ($F(1,19) = 0.002$, $P > 0.05$) were not significant, the treatment × trial interaction ($F(1,19) = 4.7$, $P = 0.04$) indicated a differential pattern of change from Day 1 to Day 2. Swim distances in the E_2 group were reduced by 37.6% from trial 8 of Day 1 to trial 1 of Day 2, whereas swim distances of controls increased 51.8% overnight (Fig. 6). These data suggest that memory for the platform location deteriorated in controls overnight, and that E_2 enhanced memory for the platform location. Swim speeds increased from Day1 to Day 2 (trial effect, $F(1,19) = 2.92$, $P < 0.0001$), but there was no significant main effect of treatment ($F(1,19) = 0.8$, $P > 0.05$) or treatment × trial interaction ($F(1,19) = 0.5$, $P > 0.05$). Swim speeds for the control and E_2 groups on trial 8 of Day 1 were 8.5 ± 1.2 and 11.6 ± 0.7, and

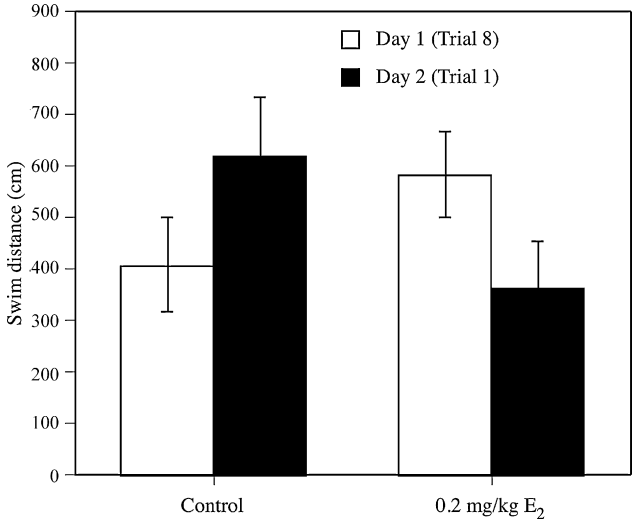

Fig. 3. Control mice exhibited longer swim distances on the last trial of Day 1 relative to the first trial of Day 2, suggesting a decline in their memory the platform location. In contrast, the 0.2 mg/kg E_2 group swam shorter distances on Day 2 relative to Day 1, suggesting enhanced memory for the platform location. Each bar represents the mean ± S.E.M. for each group. This differential pattern of change from Day 1 to Day 2 was supported by a significant treatment × trial interaction ($P = 0.04$).

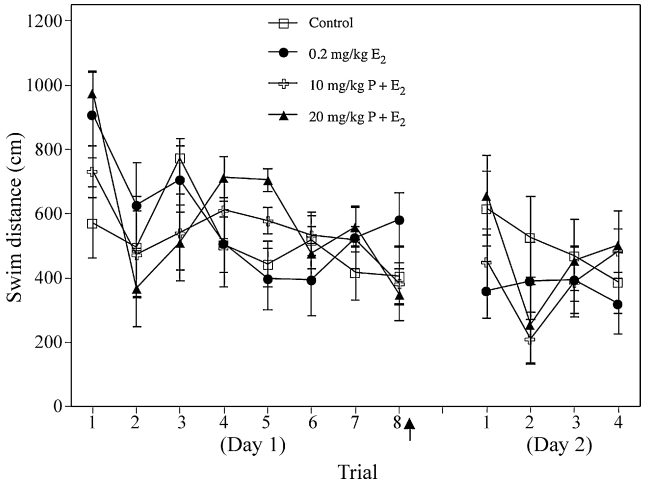

Fig. 4. Spatial memory as assessed by swim distance during eight spatial water maze training trials on Day 1 and four retention trials on Day 2. Each point represents the mean ± S.E.M. for each group. Swim distance during Day 1 declined in all groups across trials. Mice receiving 20 mg/kg P + E_2 exhibited longer swim distances on Day 2 relative to Day 1, indicating a decline in their memory the platform location. Data from the 0.2 mg/kg E_2 group are shown for comparison.

on trial 1 of Day 2 were 13.9 ± 1.5 and 15.8 ± 0.7, respectively.

After the first retention trial on Day 2, the control group was able to re-learn the platform location (Fig. 2). There were no significant differences in swim distance or speed in the four retention trials of Day 2 (treatment effect, $Fs(1,19) = 2.5$ and 1.1; trial effect, $Fs(3,57) = 0.6$ and 1.8; treatment × trial interaction $Fs(3,57) = 0.3$ and 0.6, respectively, $Ps > 0.05$).

3.2. Experiment 2

3.2.1. Subjects

The results of Experiment 1 suggested that 0.2 mg/kg E_2 improves spatial memory consolidation in aged females. Thus, this dose was combined with two doses of progesterone in Experiment 2 to determine if co-administration of progesterone would further benefit memory consolidation or would attenuate the effects of estradiol alone. Mice were generally healthy at the beginning of the experiment. Animals were randomly divided into hormone treatment groups before the start of behavioral testing. Half of the mice from each treatment group were tested in the water maze each day (it took 4 days to complete testing for Experiment 2). One mouse in the 20 mg/kg P + E_2 group was excluded from the water maze analyses due to cataracts. This mouse was also excluded from the uterine weight analyses.

3.2.2. Uterine weights

In contrast to Experiment 1, where 0.2 mg/kg E_2 significantly increased uterine weights, neither P + E_2 treatment increased uterine weights (main effect of treatment, $F(2,26) = 2.2$, $P > 0.05$). In order to compare uterine weights of the estradiol plus progesterone groups to the 0.2 mg/kg E_2 group, another ANOVA was conducted including the controls, both progesterone groups, and the 0.2 mg/kg E_2 group. Uterine weights in both P + E_2 groups were lower than those of the 0.2 mg/kg E_2 group ($F(3,34) = 3.4$, $P = 0.03$), and post-hoc tests revealed that this difference was significant for the 20 mg/kg P + E_2 group ($P = 0.02$, Fig. 1).

3.2.3. Morris water maze

On Day 1, there was a significant effect of trial ($Fs(7,189) = 5.3$ and 29.6, $Ps < 0.0001$), but not treatment ($Fs(2,27) = 0.8$ and 0.5, $Ps > 0.05$) for swim distance (Fig. 4) and swim speed (data not shown), respectively, indicating that all groups learned the platform location equally well. The treatment x trial interaction was also not significant for either measure ($Fs(14,189) = 1.7$ and 1.2, respectively, $Ps > 0.05$). There were no group differences in swim distance or swim speed during the last trial of Day 1 ($Fs(2,27) = 0.1$ and 0.9, respectively, $Ps > 0.05$). In order to enable comparisons of the P + E_2 groups with the E_2 group from Day 1 to Day 2, a one-way ANOVA including the control, E_2, and two P + E_2 groups was performed on the data from the last trial of Day 1. This analysis showed that the groups did not differ in trial 8 of Day 1 ($F(3,36) = 1.6$, $P > 0.05$). Swim speeds for the 10 and 20 mg/kg P + E_2 groups on trial 8 of Day 1 were 7.0 ± 0.9 and 8.7 ± 0.7, and on trial 1 of Day 2 were 14.7 ± 0.7 and 14.2 ± 1.4, respectively.

The effect of post-training injections of vehicle, E_2, and P + E_2 on retention is shown in Figs. 4–6. A repeated measures ANOVA for swim distance including last trial of Day 1 and the first trial of Day 2 indicated no significant differences among the control, E_2, and P + E_2 groups, as suggested by a

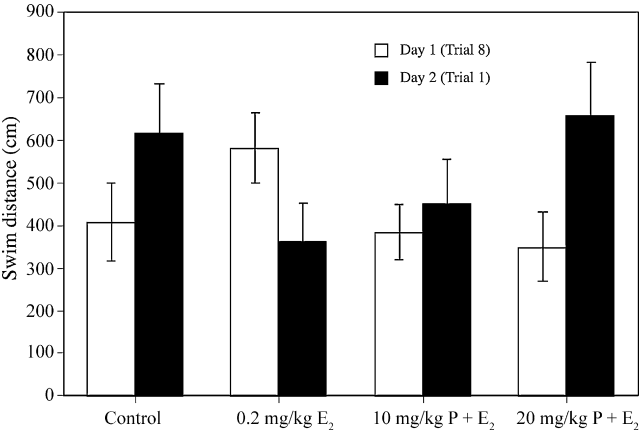

Fig. 5. Swim distances on the last trial of Day 1 and the first trial of Day 2. Each bar represents the mean ± S.E.M. for each group. The controls and both P + E_2 groups swam further on Day 2 than on Day 1. Data from the 0.2 mg/kg E_2 group are shown to illustrate the fact that the 20 mg/kg dose of progesterone reversed the beneficial effect of E_2 on spatial memory consolidation. The 20 mg/kg and control groups performed similarly, as indicated by a significant treatment × trial interaction between the 20 mg/kg dose, controls, and the E_2 alone group ($P = 0.04$). Thus, the significant treatment × trial interaction present between controls and E_2 mice in Experiment 1 remained. The treatment × trial interaction in the analysis of the 10 mg/kg dose, controls, and the E_2 alone group was not significant ($P > 0.05$), suggesting little reduction in the beneficial effect of E_2 by 10 mg/kg progesterone.

non-significant treatment effect ($F(2,27) = 0.7$, $P > 0.05$) and treatment × trial interaction ($F(2,27) = 0.6$, $P > 0.05$). The trial effect was significant ($F(1,27) = 4.8$, $P < 0.04$), suggesting similar changes in performance among the groups from Day 1 to Day 2. Swim distances in the 10 and 20 mg/kg P + E_2 groups increased 17.9% and 88.7%, respectively, from Day 1 to Day 2 (Fig. 6). To compare the effects of each P + E_2 dose to that of E_2 alone, each dose was separately compared to controls and the E_2 group. With the 10 mg/kg dose, the significant treatment × trial interaction present between controls and E_2 mice was no longer significant ($F(2,28) = 2.5$, $P > 0.05$). This finding may suggest that the 10 mg/kg P dose slightly reduces the beneficial effect of E_2 on memory consolidation. The treatment ($F(2,28) = 0.6$, $P > 0.05$) and trial ($F(1,28) = 0.06$, $P > 0.05$) effects were also not significant for this dose. The 20 mg/kg dose, however, completely blocked the beneficial effect of E_2. In this analysis, the significant treatment × trial interaction present between controls and E_2 mice remained present ($F(2,27) = 3.6$, $P = 0.04$), and inspection of Figs. 4 and 5 indicates that 20 mg/kg P + E_2 mice performed similarly to controls on both days. In fact, Fig. 6 illustrates that the percent increase from Day 1 to Day 2 was higher for the 20 mg/kg P + E_2 group (88.7%) than for controls (51.8%). These data contrast with the 37.6% reduction for the 0.2 mg/kg E_2 group.

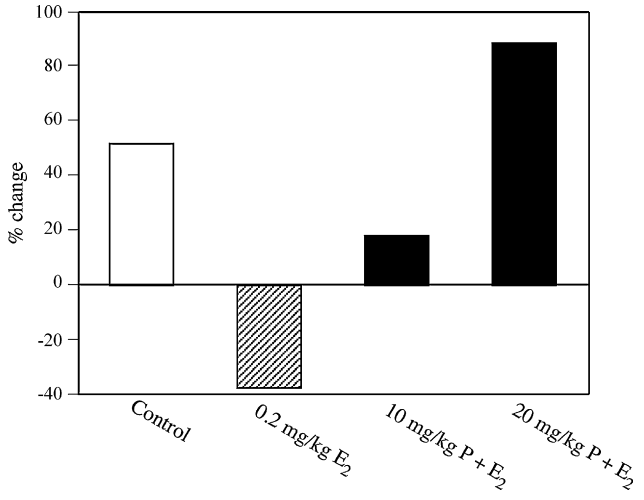

Fig. 6. Percent change in performance from the last trial of Day 1 to the first trial of Day 2 for both experiments. The values shown were calculated as swim distance on Day 2 minus swim distances on Day 1, divided by swim distance on Day 2, times 100. Swim distances increased from Day 1 to Day 2 in all but the 0.2 mg/kg E_2 group.

After the first retention trial, all three groups in Experiment 2 were able to re-learn the platform location during the remaining retention trials (Fig. 4). The main effect of trial was significant for swim distance for the four retention trials ($F(3,81) = 2.7$, $P < 0.05$), indicating overall re-learning of the platform location. The main effect of trial for swim speed was not significant ($F(3,81) = 0.3$, $P > 0.05$), indicating a more direct route to the platform without increased speed. Non-significant main effects of treatment ($Fs(2,27) = 1.0$ and 0.8, $Ps > 0.05$) and treatment × trial interactions ($Fs(3,81) = 1.0$ and 0.6, $Ps > 0.05$) for swim distance and swim speed indicated a similar rate of re-learning of the platform location among the groups.

4. Discussion

4.1. Effects of estradiol

The results of Experiment 1 indicate that post-training administration of 0.2 mg/kg E_2 can improve spatial memory consolidation in aged female mice. The present findings are the first to demonstrate that post-training estradiol injections enhance memory in aged females. These data are consistent with studies in which estradiol was given prior to training in aging rats [11,20] and mice [9]. More importantly, these findings are consistent with previous data from young female rats [29] tested in the same spatial water maze paradigm showing that immediate, but not 2-h delayed, injection of 0.2 mg/kg E_2 significantly improved spatial memory consolidation. Similar effects of immediate, but not 2-h delayed, post-training estradiol have been reported in female [28] and male [27] rats after intra-hippocampal infusion. Together, these studies suggest that post-training estradiol effects memory consolidation within 2 h after training. The beneficial effects of 0.2 mg/kg E_2 on spatial memory consolidation in aged female mice is also consistent with previous reports from our lab that this dose facilitates object memory consolidation and spatial working memory in young adult female mice [13]. Thus, the beneficial effects of this dose generalize to other types of memory, as well as between species and across ages.

The widespread effectiveness of this dose may be due to alterations in signal transduction in the dorsal hippocampus. For example, our lab recently showed in young female mice that this dose significantly increases phosphorylation of the molecule extracellular signal-regulated kinase (ERK) in the dorsal hippocampus one hour after injection [7]. Concurrent systemic or dorsal hippocampal administration of 0.2 mg/kg E_2 and an inhibitor of the enzyme that phosphorylates ERK completely abolishes the beneficial effects of 0.2 mg/kg E_2 on object memory and the estrogen-induced increase in pERK [7]. These data demonstrate that E_2 facilitates object memory consolidation in young female mice by activating the ERK pathway in the dorsal hippocampus. Although this study examined a different type of memory from the present study, the fact that this dose has similar effects on several types of memory consolidation would suggest a common molecular mechanism. We will further address this possibility in future studies.

Although the groups did not significantly differ in trial 8 of Day 1, it could be argued that the beneficial effect of 0.2 mg/kg E_2 is an artifact of their worse performance in this trial compared to controls. However, even if the E_2 group had performed identically to controls in trial 8 of Day 1, their performance on Day 2 would still have indicated a beneficial effect of this dose. Unlike the controls, whose swim distances were 51.8% higher on Day 2, swim distances of the 0.2 mg/kg E_2 mice on trial 1 of Day 2 were similar to (and even lower than) the distances controls achieved on trial 8 of Day 1. Thus, even if the 0.2 mg/kg E_2 group performed identical to controls on trial 8 of Day 1, only the 0.2 mg/kg E_2 group would have maintained that level of performance overnight. The fact that controls were able to relearn the hidden platform location on Day 2, suggests that the significant treatment × trial interaction observed between Days 1 and 2 was due to specific effects of estradiol on memory rather than on non-mnemonic aspects of task performance.

4.2. *Effects of estradiol plus progesterone*

In order to elucidate the effects of combined estradiol plus progesterone treatment on memory consolidation, mice in Experiment 2 were given post-training injections of 0.2 mg/kg E_2 in combination with two doses of progesterone. Both doses of progesterone tended to reduce estradiol's ability to facilitate spatial memory consolidation. The 20 mg/kg dose blocked estradiol's effects on memory, whereas the 10 mg/kg dose seemed to slightly reduce the beneficial effects of estradiol. Interestingly, uterine weights in the 20 mg/kg $P + E_2$ group were significantly lower than those of the 0.2 mg/kg E_2 group, perhaps suggesting that the memory data for this group reflected serum levels of estradiol and/or progesterone. This hypothesis seems to be supported by the fact that the 20 mg/kg $P + E_2$ group had the lowest uterine weights and the worst memory of the two $P + E_2$ groups.

Although beneficial effects of estradiol on memory in rats and mice have been well documented [9,18,26], the effect of combined treatment with estradiol plus progesterone is unclear. Our 20 mg/kg $P + E_2$ data are consistent with previous reports in which young female rodents treated with estradiol plus progesterone were impaired in acquisition of footshock avoidance [5] and the spatial Morris water maze [2]. However, these findings are at odds with spatial memory-enhancing effects of acute estradiol plus progesterone in young female rats [34] and acute or chronic treatments in middle-aged [20] and aged [11] female rats. There are several possible reasons for this discrepancy, including the doses of hormones used and the way in which they were given (i.e., injected peripherally versus silastic capsules). Further, the present study utilized water-soluble hormones, whereas other studies used hormones dissolved in oil and incorporated into pellets. Differences in metabolism among these preparations may have affected the results. Certainly, the fact that we administered hormones post-training whereas previous studies treated rats prior to training may be a major contributor; progesterone may need to be in the circulation during training to be beneficial. As such, progesterone may largely affect performance factors in the water maze rather than memory. However, the post-training injections used in the present study should have allowed us to observe effects of progesterone on memory independent of these factors.

It is also possible that we only observed a detrimental effect of 20 mg/kg progesterone on memory because we administered progesterone with an optimal dose of estradiol. If 0.2 mg/kg E_2 improves spatial memory consolidation to maximal levels, then the addition of progesterone could not possibly have improved memory any further. Indeed, by co-administering progesterone with this optimal dose, it may not have been surprising that the only possible outcome was to interfere with this maximal effect. We selected the 0.2 mg/kg E_2 dose for use in Experiment 2 because of our initial hypothesis that the combination of both hormones would be as effective, or more effective, than estradiol alone. In contrast, it appears that the 0.2 mg/kg E_2 dose alone stimulates the aging brain (e.g., possibly by phosphorylating ERK) sufficiently to maximally consolidate spatial memories. Progesterone may positively affect memory consolidation if administered alone or in combination with a sub-optimal dose of estradiol. This possibility will need to be addressed in future studies.

The neuronal mechanisms underlying progesterone's effects on memory remain unclear. One hypothesis is that progesterone impairs memory by binding to GABA-A receptors, thereby promoting GABAergic activity [5]. Enhanced GABAergic activity may reduce arousal levels, which may lead to progesterone-induced impairments in memory [5]. However, the post-training injections given in the present study would have minimized the influence of progesterone-induced changes in arousal on memory because progesterone was not present in the circulation during either day of testing. Nevertheless, inhibition of the activity of GABAergic interneurons in the hippocampus is associated with an estradiol-induced increase in CA1 dendritic spine density [22] and progesterone blocks estradiol's effect on dendritic

spines [23]. Therefore, enhanced GABAergic activity immediately after injection as the result of progesterone binding to GABA-A receptors on GABAergic interneurons in the hippocampus may directly interfere with the ability of estradiol to increase dendritic spine density, and possibly, improve memory.

The effects of acute post-training administration of estradiol and progesterone reported in this study are relevant to humans because the data show that these hormones specifically affect memory, rather than other aspects of task performance such as arousal, attention, or motivation. If hormones affected performance in memory tasks by enhancing these factors, then other, less risky treatments could be used to affect these factors, and thus, improve memory. However, by demonstrating a specific effect on memory, these data indicate that hormonal modulation of memory is possible in aging females. Although some clinical studies in menopausal women report memory-enhancing effects of estrogens plus progestin [3,33], the present data are somewhat consistent with several reports in which estrogen and progestin treatment was detrimental to cognitive function in post-menopausal women [12,32,37]. However, because medroxyprogesterone acetate (MPA), rather than progesterone, was used in clinical trials reporting negative effects of progestin on cognitive function, and some trials reporting positive effects of hormone therapy used progesterone, it is unclear if the negative effects of progestins are specific to MPA or apply to all progestins. Given the effects of 20 mg/kg progesterone on memory consolidation in the present study, it is possible that natural progesterone combined with estrogen is also detrimental to cognition. This, however, would need to be confirmed with studies using chronic administration of estrogens and progesterone.

In conclusion, the present study is the first to demonstrate that post-training administration of 0.2 mg/kg estradiol can improve spatial memory consolidation in aged female mice, and that 20 mg/kg progesterone can reduce this beneficial effect. With the numbers of menopausal women increasing, it is imperative that the effects of progestins on the brain and cognition be well understood. These novel results lay an important foundation for future studies investigating progesterone's effects on cognitive function in aging females.

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