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Differential effects of acute progesterone administration on spatial and object memory in middle-aged and aged female C57BL/6 mice

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ABSTRACT

The present study examined the effects of acute progesterone administration on hippocampal-dependent memory consolidation in ovariectomized middle-aged (16 months old) and aged (22 months old) female mice. Spatial memory was tested in a 2-day Morris water-maze task and object memory was tested using an object recognition task with 24- and 48-h delays. Immediately after water-maze training, mice received i.p. injections of vehicle, or 5.0, 10.0, or 20.0 mg/kg of water-soluble progesterone. Twenty-four hours later, retention of the platform location was tested. No overnight forgetting of the platform location was observed in middle-aged vehicle-treated mice. Acute progesterone administration had no effect on spatial memory in middle-aged mice. However, aged vehicle-treated mice demonstrated impaired memory for the platform location on Day 2 relative to Day 1. Twenty mg/kg, but not 5 or 10 mg/kg, progesterone reversed these deficits, suggesting that 20 mg/kg progesterone can improve spatial memory in aged females. In the object recognition task, mice explored two identical objects and then immediately received vehicle or progesterone injections. In middle-aged mice, 10 and 20 mg/kg progesterone enhanced object memory consolidation, relative to chance, after 24-h, but all doses were ineffective after 48-h. In aged mice, 10 mg/kg progesterone enhanced object memory consolidation, relative to chance, after 24 h, whereas both 5 and 10 mg/kg progesterone enhanced memory after 48 h. Together, these results indicate that acute progesterone differentially enhances hippocampal-dependent memory in middle-aged and aged females.

Introduction

Interest in the effects of sex-steroid hormones, including estrogen and progesterone, on cognitive function has increased substantially in recent years due, in part, to clinical findings suggesting that the reduction in hormone levels that occurs during menopause may contribute to age-related memory decline (Sherwin, 1999; Yaffe, 1998). Thus, a thorough understanding of the roles these hormones play in modulating memory formation could lead to improved treatment for age-related memory dysfunction. The effects of estrogen on regions of the brain involved in memory, including the hippocampus, have been well studied. One of the most potent forms of estrogen, 17β-estradiol, has numerous effects on hippocampal physiology including increased CA1 dendritic spine density (Woolley and McEwen, 1992; Woolley and McEwen, 1993) and synaptic proteins (Choi et al., 2003; Stone et al., 1998), reduced GABAergic inhibition of CA1 pyramidal neurons (Murphy et al., 1998), and enhanced long-term potentiation (Foy et al., 1999; Warren et al., 1995) and neurogenesis (Tanapat et al., 1999). Estradiol also phosphorylates hippocampal signal transduction proteins such as extracellular signal-regulated kinase (ERK) (Carlstrom et al., 2001; Kuroki et al., 2000) and cyclic AMP response element binding protein (CREB) (Murphy and Segal, 2000). Most studies that have examined the effects of estradiol administration on hippocampal-dependent memory (e.g., spatial memory, object recognition) typically report improvements in young ovariectomized female rodents (Bimonte and Denenberg, 1999; Daniel et al., 1997; Fader et al., 1998; Farr et al., 1995; Gibbs, 1999; Gresack and Frick, 2004; Heikkinen et al., 2002; Luine and Rodriguez, 1994; O'Neal et al., 1996; Rissanen et al., 1999; Sandstrom and Williams, 2001).

In contrast, much less is known about the effects of progesterone on hippocampal physiology and memory. Similar to estradiol, progesterone increases levels of the presynaptic protein synaptophysin in the female hippocampus (Choi et al., 2003), and increases ERK activation in hippocampal cultures (Nilsen and Brinton, 2002; Nilsen and Brinton, 2003). However, progesterone does not appear to be as beneficial for hippocampal function as estradiol. For example, progesterone blocks estradiol-induced increases in CREB phosphorylation in hippocampal cultures (Murphy and Segal, 2000). Further, estradiol alone, but not progesterone alone, modulates hippocampal NMDA receptor expression after ovariectomy (Cyr et al., 2001; El-Bakri et al., 2002). Progesterone administration 48 h after two estradiol injections (spaced 24 h apart) initially increases CA1 dendritic spine

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density for 2–6 h after treatment, but then sharply decreases spine density much more than is observed with estradiol alone (Woolley and McEwen, 1993). Interestingly, this same estradiol and progesterone treatment improves spatial memory if rats are tested 90 min, but not 24 h, after progesterone injection (Sandstrom and Williams, 2001). A similar estradiol and progesterone treatment impaired spatial reference memory in the Morris water maze 4 h after progesterone injection, whereas estradiol alone or progesterone alone had no effect (Chesler and Juraska, 2000). Other studies have reported that chronic administration of progesterone or its metabolite, allopregnanolone, alone impairs spatial memory and footshock avoidance in young female rodents (Farr et al., 1995; Johansson et al., 2002). On the other hand, acute post-training systemic injections of estradiol alone, progesterone alone, or both hormones together improve inhibitory avoidance and novel object recognition (Frye and Lacey, 2000; Walf et al., 2006). Further, treatment with estradiol plus progesterone reduces spatial memory impairments induced by the cholinergic agonist scopolamine (Tanabe et al., 2004) and protects against memory impairments induced by intra-hippocampal administration of the neurotoxin colchicine (Vongher and Frye, 1999). As such, the effects of progesterone in young females may be highly dependent on factors such as the timing of testing relative to treatment and whether it is given alone or in combination with estradiol.

Of particular relevance to the study of hormones and cognition are the effects of sex-steroid hormone loss during aging in females. Although hippocampal-dependent memory deteriorates with age in both male and female rodents, age-related deficits in spatial memory tested in the Morris water maze occur earlier in females than males, and this deficit is associated with declining estrous cyclicity (Frick et al., 2000; Markowska, 1999). Because estrogen functions as a trophic factor in the adult hippocampus (Brinton, 2001), hormone deficiency during aging may render the hippocampus more vulnerable to deterioration and exacerbate emerging age-related memory deficits. Numerous studies have shown that estradiol treatment can reduce age-related memory decline in middle-aged (Daniel et al., 2006; Fernandez and Frick, 2004; Foster et al., 2003; Markham et al., 2002) and aged (Frick et al., 2002; Gibbs, 2000; Heikkinen et al., 2002; Markowska and Savonenko, 2002; Vaucher et al., 2002) female rodents. However, very few studies have examined the effects of progesterone on memory decline in aging females. The handful of studies that have tested the effects of estradiol plus progesterone on memory have yielded inconsistent results: although chronic treatment with estradiol plus progesterone improved spatial reference and working memory in aged females (Gibbs, 2000; Markham et al., 2002), progesterone reversed the beneficial effects of estradiol on spatial reference memory in middle-aged (Bimonte-Nelson et al., 2006) and aged (Harburger et al., 2007) females. No study to date has examined the effects of progesterone alone on memory in aging females, and therefore, it is unclear whether progesterone itself can ameliorate age-related cognitive deficits.

As such, the current experiment examined whether progesterone alone could improve hippocampal-dependent spatial and object memory in ovariectomized middle-aged and aged female mice using Morris water maze and object recognition tasks. In order to pinpoint effects of progesterone on memory consolidation specifically, a single injection of water-soluble progesterone was given immediately after training (post-training) in each task. Because progesterone was not in the circulation during training or testing, the post-training design allowed effects on memory consolidation to be observed in the absence of non-mnemonic confounds (e.g., effects on motivation, anxiety) that may influence performance (Gresack and Frick, 2006; Harburger et al., 2007). The results suggest that acute post-training progesterone administration differentially affects both spatial and non-spatial forms of hippocampal-dependent memory in middle-aged and aged female mice.

Methods

Subjects

Subjects were 16 and 22 month-old female C57BL/6 mice (N=8–11/group) 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 for at least 5 days 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.

Ovariectomy

All mice were ovariectomized to eliminate endogenous ovarian production of estrogen and progesterone prior to treatment. Surgeries were conducted 1 week prior to behavioral testing as previously described (Fernandez and Frick, 2004). Briefly, mice were anesthetized using 2% isoflurane 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, which were removed 1 week after surgery. Mice were given 1 week to recover from surgery, during which they were housed singly and received 30 mg/kg children's ibuprofen in the drinking water for analgesia. Mice were then re-housed with their original cage mates after wound clip removal.

Hormone administration

Water-soluble progesterone (Progesterone: 2-hydroxypropyl-β-cyclodextrin complex) and HBC vehicle (2-hydroxypropyl-β-cyclodextrin complex) were dissolved in physiological saline. Doses of 5, 10, or 20 mg/kg progesterone were administered and hormone solutions were prepared to ensure these doses reflected the progesterone content of the HBC complex. Drugs were administered intraperitoneally (i.p.) immediately upon completion of training in each behavioral task. All animals were run in each task (water maze and object recognition with 24 and 48 delays). Thus, to minimize any effects of previous behavioral testing and/or drug administration, a minimum of 2 weeks elapsed between all behavioral procedures, including between the 24 and 48-h versions of the object recognition task. Further, the order in which the mice were run in the various tasks was pseudo-randomly varied such that some groups ran in the water maze first, while others were initially run in the object recognition task. The HBC conjugate enhances the water solubility of progesterone without affecting the pharmakokinetics of the hormone (Pitha and Pitha, 1985). The advantage of HBC preparations is that they are readily metabolized within 24 h and, thus, are not present during testing (Pitha et al., 1986). This minimizes potential confounding effects of the drug on non-mnemonic aspects of task performance. Further, although plasma progesterone levels were not measured in this study, previous work has demonstrated that doses of progesterone between 0.1 and 1.0 mg produce physiological levels of progesterone (Akinci and Johnston, 1997). Thus, given the average weight of the middle-aged (26.5 g) and aged (27.8 g) mice, the doses of progesterone administered in the current study (5, 10, and 20 mg/kg) equate to (0.13, 0.26, and 0.52 mg) for middle-aged mice and (0.14, 0.28, and 0.56 mg) for aged mice, which are all in the physiological range.

Morris water maze

Morris water-maze testing was conducted as previously described (Gresack and Frick, 2006; Harburger et al., 2007). Briefly, 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 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 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 the experimenter led the mouse to it. No data were collected during shaping.

During testing, a transparent Lucite platform (10×10 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 into the tank at one of the four cardinal (N, S, E, or W) start positions, which varied for each trial. The mouse was given 60 s to find the platform in each trial. If the platform was not located, then the experimenter led the mouse to the platform where it remained for 10 s. The mouse 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 vehicle or progesterone (see Hormone administration). Twenty-four hours later, mice were re-tested in four consecutive spatial trials to examine spatial memory consolidation. As in previous studies (Gresack and Frick, 2006; Harburger et al., 2007; Packard and Teather, 1997), spatial memory consolidation can only be measured during the first trial of Day 2 because re-exposure to the platform in this trial serves to remind the mouse of the platform location. The remaining trials ascertain whether the mice can re-learn the platform location. Swim distance (m) was used to measure memory and swim speed (m/s) was used to measure general motor performance throughout testing.

Object recognition

This task assesses hippocampal-dependent, non-spatial object memory and consists of habituation, sample, and choice phases (Baker and Kim, 2002; Clark et al., 2000; Frick and Gresack, 2003; Mumby et al., 2005; O'Brien et al., 2006). Mice were first habituated to an empty white box by allowing them to freely explore for 5 min. Twenty-four hours later, mice were placed in the empty box for 1 min of additional habituation. Mice were removed while two identical objects were placed in the northeast and northwest corners of the box, approximately 5 cm from the walls. Mice were then placed back in the box facing the middle of the south wall and allowed to freely investigate the objects until they accumulated a total of 30 s exploring the objects, at which point the sample phase was terminated. Object exploration was scored only when the mouse's nose was within approximately 1 cm of the object or if the front paws were in contact with the object. Immediately after the completion of the sample phase, mice received vehicle or progesterone injections. The use of 30 s of total exploration time rather than a fixed trial duration minimizes the effects of group differences in activity (Frick and Gresack, 2003). Twenty-four and 48 h later, mice were tested in the choice phase. This phase was identical to the sample phase, except that a novel object was substituted for one of the objects used in the sample phase and different objects were used for each delay. Time spent with the objects during the choice phase was recorded and compared to chance performance of 15 s (see Data analysis). Total time to complete the choice phase was also recorded. The location of the novel object (northeast or northwest corner) was counterbalanced across mice and different objects were used for each of the delays. For both phases, objects and the box were cleaned with 70% EtOH between mice.

Data analysis

For the Morris water maze, a series of analyses were performed for swim distance. Acquisition of the spatial task was analyzed using both a two-way mixed design ANOVA with one between-factor (drug treatment) and one within-factor (training trials), as well as a trend analysis designed to examine the rate of acquisition across the training trials on Day 1. A one-way ANOVA without repeated-measures was then performed on the last training trial (trial 8) to examine whether the groups exhibited similar performance at the end of training. We next performed a repeated-measures ANOVA comparing performance in trial 8 of Day 1 and trial 1 of Day 2 as a measure of memory consolidation. In addition, a one-way ANOVA was performed on difference scores calculated between these two trials (Trial 8 of Day 1−Trial 1 of Day 2) to further examine changes in performance between days. We performed a two-way mixed ANOVA on all trials of Day 2 to examine whether the mice could re-learn the platform location after a reminder trial. Finally, a one-way repeated-measures ANOVA was performed on the last trial of day 2 to examine reacquisition among treatments groups. For swim speed, identical analyses were run as were run for swim distance to examine motor performance between groups in each phase of the task.

For object recognition, time spent with the novel object during the choice phase was analyzed using separate one-sample *t*-tests for each group to determine if the time spent with the novel object differed from the chance value of 15 s. This type of analysis was used because the time spent with each object in a total time of 30 s is not independent; time spent with one object necessarily reduces the time spent with the other object (Frick and Gresack, 2003; Baker and Kim, 2002). Because each group was analyzed individually relative to chance, between-group comparisons of exploration were made relative to chance and not directly between groups. Total time to complete the choice phase at each delay was analyzed with one-way ANOVAs including treatment as the independent variable.

Results

Morris water maze

Middle-aged mice

A repeated-measures ANOVA examining swim distances throughout training on Day 1 (Fig. 1A) revealed a significant main effect of

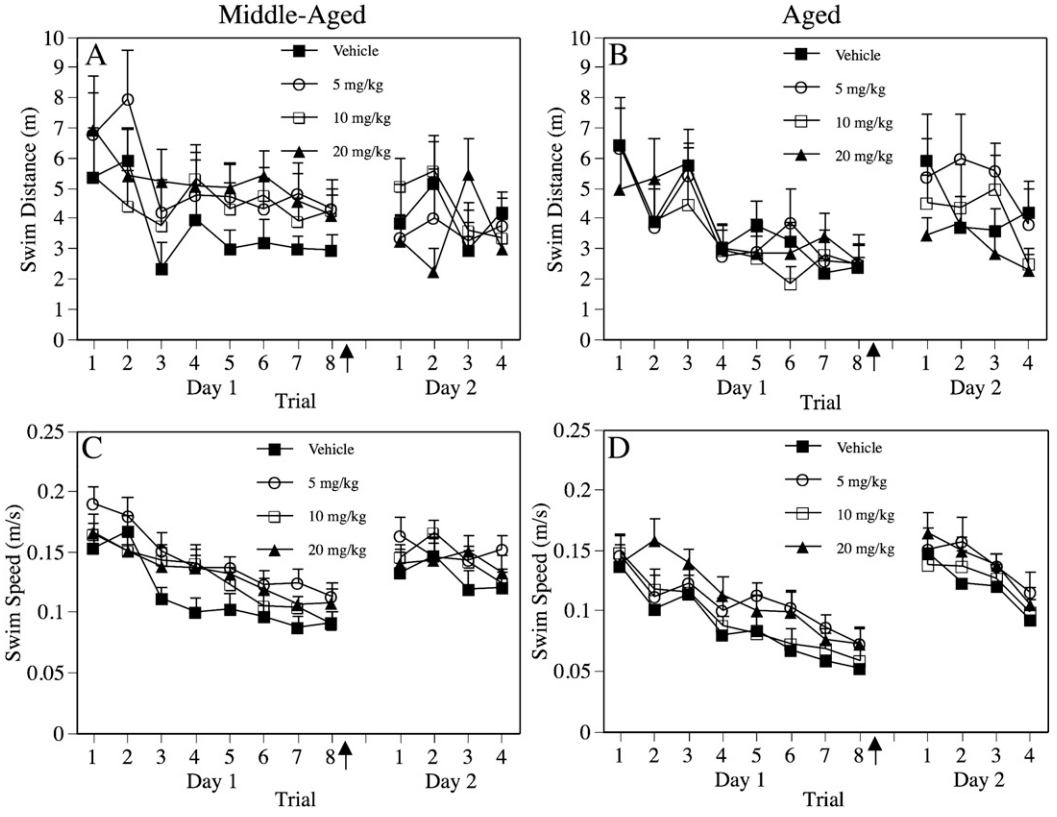

Fig. 1. Effects of post-training progesterone injection on water-maze performance in middle-aged (A and C) and aged (B and D) females. For all graphs, each point represents the group mean for each trial. Error bars represent ± standard error of the mean (SEM). The filled arrow represents the progesterone injection immediately after training trial 8. (A) Spatial memory consolidation in middle-aged mice. Swim distance in all middle-aged groups decreased over the eight training trials on Day 1. The groups did not differ on trial 8 of Day 1, suggesting that all groups performed similarly during the last trial before training. None of the groups demonstrated significant overnight forgetting after 24 h, as evidenced by no significant increase in swim distance between trial 8 of Day 1 and trial 1 of Day 2. There were no differences among groups across the 4 testing trials of Day 2, likely due to a lack of forgetting after 24 h. (B). Spatial memory consolidation in aged mice. Swim distance in all aged groups decreased over the eight training trials on Day 1. The groups did not differ in trial 8 of Day 1, suggesting similar performance during the last training trial. During the first trial of Day 2, swim distances in the vehicle, 5 mg/kg, and 10 mg/kg groups were significantly higher than during trial 8 of Day 1 (148%, 112%, and 84%, respectively). In contrast, the 35% increase in swim distance exhibited by the 20 mg/kg group was not significant, suggesting that this dose of progesterone can enhance spatial memory consolidation in aged female mice. Swim speeds were not affected by progesterone in either the middle-aged (C) or aged (D) mice. Within an age, swim speeds decreased similarly during Day 1 and were significantly faster on the 1st trial of Day 2 relative to the last trial of Day 1. Further, swim speeds decreased similarly in all groups over the four testing trials on Day 2, suggesting that alterations in swimming ability did not contribute to the differences observed in swim distance.

Trial (F (7,273)=3.30, $p<0.05$), a significant main effect of Treatment (F (3,39)=3.06, $p<0.05$), but no Treatment X Trial interaction (F (21, 273)=0.49, $p>0.05$). Further, a trend analysis examining the curve-fit to an inverse function across the 8 training trials of Day 1 revealed a significant effect of trial (F (1, 342)=16.43, $p<0.001$), suggesting that all groups decreased their path to the platform as a function of training trial. Despite the overall main effect of treatment, a one-way ANOVA performed on Trial 8 of Day 1 revealed no differences in swim distance among the groups (F (3,42)=0.87, $p>0.05$), demonstrating no significant differences among the groups in performance in the last training trial before hormone administration.

The effects of post-training progesterone administration are shown on the right side of Fig. 1A. A repeated-measures ANOVA examining swim distance during the last trial of Day 1 and the first trial of Day 2 revealed no significant main effect of Trial (F (1,39)=0.01, $p>0.05$), no significant main effect of Treatment (F (3,39)=0.84, $p>0.05$), and no Trial×Treatment interaction (F (3,39)=0.90, $p>0.05$), suggesting that none of the groups exhibited significant overnight forgetting. In addition, a one-way ANOVA examining difference scores revealed no differences among any of the groups (F (3,39)=0.90. $p>0.05$). As such, no beneficial effect of progesterone administration on spatial memory consolidation was observed. The similarities in performance between trial 8 of Day 1 and trial 1 of Day 2 is illustrated in Fig. 2A, which plots only these trials. A repeated-measures ANOVA on the four trials of Day 2 revealed no significant main effect of Trial (F (3,117)=0.18, $p>0.05$), no significant main effect of Treatment (F (3,39)=0.45, $p>0.05$) and no Treatment×Trial interaction (F (9,117)=1.28, $p>0.05$), which is likely attributable to the fact that no overnight forgetting was apparent and, thus, improved performance was not easily observable. Lastly, a one-way ANOVA examining performance on the last trial of Day 2 revealed no significant differences between groups (F (3,39)=0.50, $p>0.05$), demonstrating that all groups performed similarly at the end of Day 2.

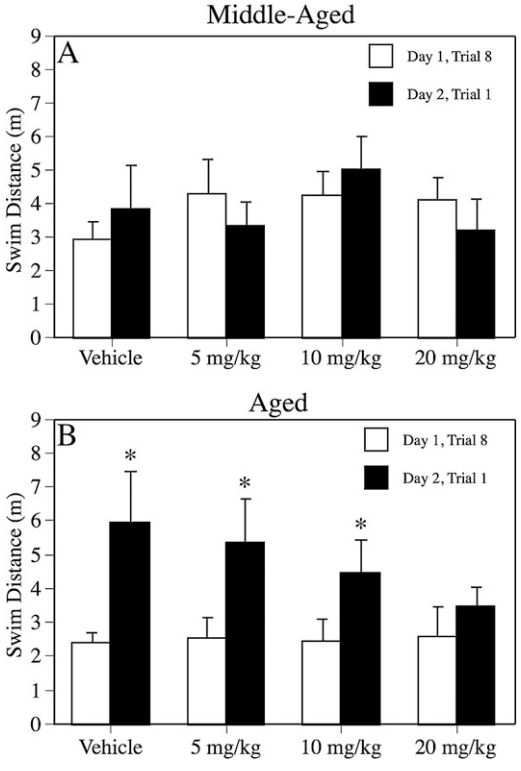

Fig. 2. Change in performance from trial 8 of Day 1 to trial 1 of Day 2 in middle-aged (A) and aged (B) females. (A) There were no significant differences in swim distance from Day 1 to Day 2 in any of the middle-aged groups. Among aged females, (B), vehicle-treated mice demonstrated significantly increased swim distances on the first trial of Day 2 relative to the last trial of Day 1. The 20 mg/kg dose of progesterone, but not 5 or 10 mg/kg progesterone, prevented this decline in performance. *= $p < 0.05$ relative to Day 1, Trial 8.

A repeated-measures ANOVA examining swim speed during training on Day 1 (Fig. 1C) revealed a significant main effect of Trial (F (7,273)=28.63, $p<0.001$), a significant main effect of Treatment (F(3,39)=3.85, $p<0.05$), but no Trial×Treatment interaction (F(21,273)= 0.92, $p>0.05$). There were no differences in swim speed on trial 8 of Day 1 (F (3,42)=1.39, $p>0.05$), suggesting that, while some differences in swim speed existed between groups during training on trials 1–7, all mice had decreased in swim speed to a similar extent by trial 8. A repeated-measures ANOVA examining swim speed on Trial 8 of Day 1 to Trial 1 of Day 2 revealed a significant main effect of Trial (F(1,39)=27.00, $p<0.05$), no significant main effect of Treatment (F(3,39)=1.87, $p>0.05$), and no Trial×Treatment interaction (F(3,39)=0.32, $p>0.05$), suggesting that all mice swam faster on the first trial of Day 2. A repeated-measures ANOVA examining the four trials of Day 2 revealed no significant main effect of Trial (F (3,117)=1.71, $p>0.05$), no significant main effect of Treatment (F (3,39)=1.43, $p>0.05$) and no Treatment×Trial interaction (F (9,117)=0.71, $p>0.05$), indicating that swim speed did not decrease significantly across trials. Finally, a one-way ANOVA examining swim speed on the last trial of Day 2 revealed no significant differences among groups (F (3,42)=1.20, $p>0.05$) suggesting that all mice swam similarly at the end of Day 2.

Aged mice

A repeated-measures ANOVA examining swim distances during training on Day 1 (Fig. 1B) revealed a significant main effect of Trial (F (7,245)=7.15, $p<0.001$), no significant main effect of Treatment (F(3,36)=0.76, $p>0.05$) and no Treatment×Trial interaction (F(21, 245)= 0.51 $p>0.50$). Further, a trend analysis examining the curve-fit to an inverse function across the 8 training trials of Day 1 revealed a significant effect of trial (F (1, 318)=38.18, $p<0.001$), suggesting that all groups decreased their path to the platform as a function of training trial. A one-way ANOVA performed on trial 8 revealed no differences in swim distance among the groups (F (3,36)=0.62, $p>0.05$), suggesting that all groups performed similarly in the last training trial prior to hormone administration.

The effects of post-training progesterone administration are illustrated on the right side of Fig. 1B. A repeated-measures ANOVA examining swim distance during the last trial of Day 1 and the first trial of Day 2 revealed a significant main effect of Trial (F(1, 36)=14.96, $p<0.001$) a trend towards a significant main effect of Treatment (F (3,36)=2.37, $p=0.08$) and a trend for a Trial×Treatment interaction (F (3, 36)=2.63, $p=0.06$). In addition, a one-way ANOVA examining difference scores revealed a trend toward significance (F (3,36)=2.63, $p=0.06$). To further examine treatment effects from the last trial of Day 1 to the first trial of Day 2, paired-samples t-tests were conducted for each group (Fig. 2B). The paired-samples t-tests revealed a significant 148% increase in swim distance from Day 1 to Day 2 in the vehicle treated group (t (9)=3.47, $p<0.05$), indicating that the mice did not remember the platform location after 24 h. Further, swim distances for both the 5 mg/kg (t (9)=2.01, $p<0.05$) and 10 mg/kg (t (9)=2.11, $p<0.05$) progesterone groups were also significantly higher on Day 2 (112% and 82% for the 5 and 10 mg/kg groups, respectively), suggesting that these doses of progesterone did not enhance memory for the platform location. Importantly, swim distances for the 20 mg/kg progesterone group did not significantly increase between Day 1 and Day 2 (t (9)=0.13, $p>0.05$; swim distance increased by only 35%), which suggests that this dose of progesterone prevented the memory decline observed in the other groups, thereby enhancing spatial memory consolidation. A repeated-measures ANOVA on the four trials of Day 2 revealed a trend toward a significant main effect of Trial (F (3, 108)=2.45, $p=0.06$), a significant main effect of Treatment (F(3,36)=6.57, $p<0.05$), but no Treatment×Trial interaction (F(9,108)= 0.85, $p>0.05$), suggesting re-learning of the platform location on Day 2. To further examine whether mice in each group were able to re-learn

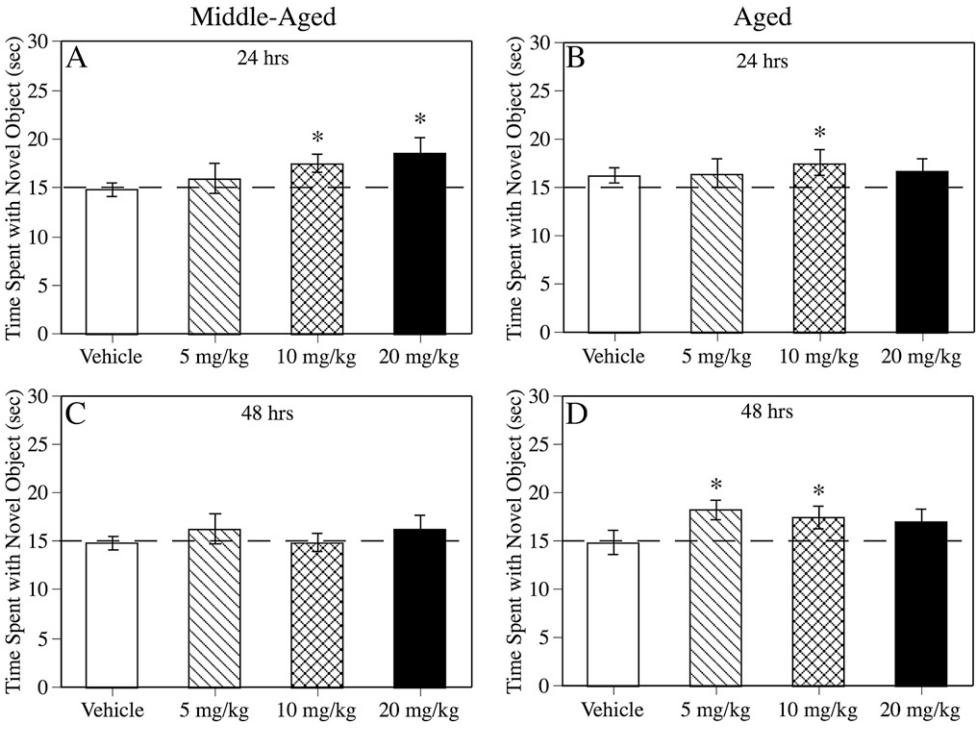

Fig. 3. Effects of post-training progesterone injection on novel object memory consolidation in middle-aged (A and C) and aged (B and D) females. For all graphs, error bars represent ± SEM and * = $p<0.05$ relative to chance (dotted line at 15 s). (A) In middle-aged females both 10 mg/kg and 20 mg/kg progesterone increased the time spent with the novel object relative to chance at the 24 h delay. In contrast, progesterone in middle-aged females (C) did not affect time spent with the novel object at the 48 h delay, relative to chance. In aged females, 10 mg/kg significantly increased the time spent with the novel object relative to chance after 24 h (B). After the 48 h delay (D), both 5 and 10 mg/kg doses increased the time that aged females spent with the novel object, relative to chance.

the platform position, two analyses were performed. A paired-samples *t*-test including all groups examining swim distance between trials 1 and 4 on Day 2 revealed a significant decrease across trials ($t(39)=2.96$, $p<0.05$), suggesting that overall swim distances were significantly lower by the end of Day 2. Further, a one-way ANOVA conducted on swim distance during the last trial of Day 2 revealed no significant main effect of Treatment ($F(3,79)=2.13$, $p>0.05$), suggesting that the groups performed similarly by the end of Day 2. These findings support the conclusion that all groups could re-learn the platform position after initial reminder trials.

A repeated-measures ANOVA examining swim speed during training on Day 1 (Fig. 1D) revealed a significant main effect of Trial ($F(7, 245)=28.61$, $p<0.001$), a significant main effect of Treatment ($F(3,36)=3.50$, $p<0.05$), but no Treatment×Trial interaction ($F(21, 245)=0.98$ $p>0.50$). A one-way ANOVA performed on trial 8 revealed no differences in swim speed among groups ($F(3,39)=0.91$, $p>0.05$), demonstrating that, while some groups differed in swim speed during training trials 1–7, all groups decreased to a similar degree by trial 8. A repeated-measures ANOVA examining swim speed during the last trial of Day 1 and the first trial of Day 2 revealed a significant main effect of Trial ($F(1, 36)=146.86$, $p<0.001$), no significant main effect of Treatment ($F(3,36)=1.22$, $p>0.05$), and no Treatment×Trial interaction ($F(3, 36)=.40$, $p>0.05$), suggesting that all groups swam faster on the first trial of day 2 relative to the last trial of Day 1. A repeated-measures ANOVA on the four trials of Day 2 revealed a significant main effect of Trial ($F(3, 108)=17.83$, $p<0.05$), no significant main effect of Treatment ($F(3,36)=1.50$, $p>0.05$), and no Trial×Treatment interaction ($F(9, 108)=.47$, $p>0.05$) suggesting that all mice swam similarly as testing trials progressed. Lastly, a one-way ANOVA conducted on swim speed during the last trial of Day 2 revealed no significant effect of Treatment ($F(3,39)=2.13$, $p>0.05$). Together, these findings suggest that decreased swim speed resulting from repeated swim trials were similar among all groups, and suggests that the effects of post-training progesterone treatment were not due to alterations in swimming ability.

Novel object recognition

Middle-aged mice

Time spent with the novel object during the choice phases of the object recognition task is presented in Fig. 3. At the 24-h delay (Fig. 3A), both the 10 mg/kg ($t(8)=2.75$, $p<0.05$) and 20 mg/kg ($t(9)=2.56$, $p<0.05$) progesterone groups spent significantly more time with the novel object than chance. Neither the vehicle nor the 5 mg/kg groups showed a preference for the novel object, suggesting that only 10 and 20 mg/kg progesterone can enhance object memory after 24 h in middle-aged mice. At the 48 h delay (Fig. 3C), none of the groups demonstrated a significant preference for the novel object, suggesting that progesterone has no beneficial effect on object memory consolidation after 48 h in middle-aged mice. There were no differences in total time to complete the choice phase during the 24-($F(3,40)=0.55$, $p>0.05$) or 48-h ($F(3,41)=1.87$, $p>0.05$) delays, indicating no effect of progesterone on the total time taken to accumulate 30 s of exploration during the choice phase (Table 1).

Aged mice

Time spent with the novel object in the choice phases of the object recognition task is presented in Fig. 3. At the 24-h delay (Fig. 3B), the 10 mg/kg progesterone group spent significantly more time with the

Table 1
Total time to accumulate 30 s of object exploration in middle-aged mice

Treatment	Delay	
	24 h	48 h
Vehicle ($N=10$)	457.1 ± 114.3	437.8 ± 123.9
5 mg/kg ($N=9$)	532.2 ± 110.7	568.4 ± 119.8
10 mg/kg ($N=10$)	589.5 ± 124.6	704.1 ± 136.6
20 mg/kg ($N=10$)	472.2 ± 121.2	464.4 ± 133.0

Values represent the mean ± SEM.

Table 2
Total time to accumulate 30 s of object exploration in aged mice

Treatment	Delay	
	24 h	48 h
Vehicle (N=9)	476.5±89.1	511.5±120.5
5 mg/kg (N=10)	374.2±92.1	598.0±111.0
10 mg/kg (N=11)	436.0±84.7	369.1±116.8
20 mg/kg (N=10)	382.6±96.2	452.5±91.3

Values represent the mean±SEM.

novel object than chance (t (10)=3.74, $p<0.05$). Neither the vehicle, 5 mg/kg progesterone, nor 20 mg/kg progesterone groups showed a significant preference for the novel object, suggesting that only 10 mg/kg progesterone enhanced object memory after 24 h. At the 48-h delay (Fig. 3D), the vehicle and 20 mg/kg groups exhibited chance performance, whereas the 5 mg/kg (t (7)=3.31, $p<0.05$) and 10 mg/kg (t (10)=2.4, $p<0.05$) progesterone groups spent significantly more time with the novel object. This finding suggests that both 5 and 10 mg/kg progesterone can enhance object memory consolidation in middle-aged mice after 48 h. There were no differences in total time to complete the choice phase during the 24- (F (3,44)=0.59, $p>0.05$) or 48-h (F (3,44)=1.22, $p>0.05$) delays, indicating no effect of progesterone on the total time taken to accumulate 30 s of exploration during the choice phase (Table 2).

Discussion

The present results demonstrate that acute post-training i.p. progesterone administration differentially affects spatial memory consolidation in the Morris water maze and hippocampal-dependent object memory consolidation in middle-aged and aged female mice. The 10 and 20 mg/kg doses of progesterone enhanced object memory consolidation, relative to chance, in middle-aged females when tested after 24, but not 48 h, and no dose affected spatial memory consolidation in the water maze. The lack of effect in the Morris water maze was likely due to generally poor learning and a lack of overnight forgetting in vehicle-treated mice, which has been observed previously in middle-aged mice (Benice et al., 2006). Thus, the possibility remains that acute, post-training progesterone administration may have had beneficial effects on memory consolidation in middle-aged mice if task acquisition had been sufficient to observe significant overnight forgetting. Interestingly, several studies have observed memory deficits in middle-aged rats and mice (e.g. Harburger et al., 2007; Francia et al., 2006; mild impairment observed in Verbitsky et al., 2004). Several factors could account for the discrepancy between the present and previous studies, including the number of days of training (1 vs. 5 or 8 days), the specific age at testing ("middle-aged" is generally defined as 12–16 months of age), and differences in endogenous steroid production prior to ovariectomy. It has been well established that middle-aged rodents demonstrate differences in the transition from normal estrous cycling to persistent estrous, to acyclicity (Frick et al., 2000). Thus, middle-aged mice may demonstrate differences in memory and/or responses to ovarian hormones based on the length of time since the transition from normal cycling, which was not directly examined in the present study. This possibility may also underlie the high variability and generally poor performance of middle-aged females in the current study. The high variability is highlighted by the significant main effect of Treatment observed prior to hormone administration, such that the group assigned to receive vehicle demonstrated a steeper learning curve than any of the groups assigned to receive progesterone. If middle-aged females are transitioning from regular cycling to acyclicity, then the middle-aged females might be expected to demonstrate more variable behavior relative to aged females, who have been persistently acyclic for a period of time (Markowska, 1999).

Our object recognition findings seem to suggest that middle-aged females are less sensitive to the memory-enhancing effects of progesterone than aged females. Only 10 and 20 mg/kg progesterone were effective in middle-aged mice at the 24 h delay (aged mice responded to 5 mg/kg), whereas no effect was observed after 48 h (aged mice respond to 5 and 10 mg/kg). The reasons for this apparent dose-response shift in the effects of progesterone at the two ages are unclear. One possible mechanism could be an increase in the number of progesterone receptors in response to decreased levels of circulating progesterone, thus increasing the sensitivity to progesterone in aged females. While we are unaware of any studies specifically examining age-related changes in progesterone receptor expression in learning-related brain regions, previous studies have demonstrated increased glutamate receptor expression in the hippocampus as a function of age. For example, Adams et al. (2001) demonstrated increased NR1 and NR2B expression in the ventral hippocampus of aged rats, relative to both young and middle-aged female rats. Further, the distribution of NR2B receptors in the hippocampus was altered by estrogen administration (Adams et al., 2004). Thus, changes in the number or distribution of progesterone receptors in the hippocampus of middle-aged or aged females could underlie the dose-response shift observed in the present study, but this has yet to be tested directly.

In aged mice, we observed enhanced object memory consolidation, relative to chance, after acute progesterone administration. These results are in accord with previous research in young ovariectomized female rats demonstrating that post-training i.p. injections of 4 or 8 mg/kg non water-soluble progesterone can enhance 24-h object recognition, working memory in a Y-maze, and inhibitory avoidance (Frye and Lacey, 2000; Walf et al., 2006). Our findings extend these results to demonstrate that, in aged female mice, post-training injections of 5 mg/kg water-soluble progesterone can enhance object recognition, relative to chance, after 48 h, whereas 10 mg/kg progesterone can enhance OR after both 24 and 48 h. The highest dose of progesterone, 20 mg/kg, had no effect on object recognition, but significantly improved spatial memory consolidation. Interestingly, another recent study in aged female mice found that the 10 mg/kg dose had no effect on the ability of 0.2 mg/kg estradiol to enhance spatial memory in the water maze, whereas 20 mg/kg progesterone, completely blocked the memory-enhancing effects of estradiol (Harburger et al., 2007). Together, these data suggest that post-training progesterone is beneficial for memory consolidation in aged females when given alone, but detrimental when given in combination with a beneficial dose of estradiol.

Two potential issues of concern relating to the aged water-maze data are worth addressing. First, the Trial×Treatment interaction in the analysis from trial 8 of Day 1 to trial 1 of Day 2 only approached significance (p=0.06), which might indicate no effect of any progesterone dose. However, the conclusion that 20 mg/kg progesterone can enhance spatial memory consolidation is supported by t-tests showing that all groups except the 20 mg/kg group swam significantly longer distances on trial 1 of Day 2 than on trial 8 of Day 2. The fact that three of the four groups exhibited similar performance during these two trials likely overshadowed the positive effect of the 20 mg/kg dose in the omnibus test. The second issue is that although re-learning of the platform location did occur during the four trials on Day 2, the Trial main effect for this analysis only approached significance (p=0.06). This lack of statistical significance is likely due to high variability during Day 2, particularly in trials 1–3 (Fig. 1B). It is interesting to note that both vehicle and the 20 mg/kg progesterone groups appear to have reacquired the task earlier during testing than the 5 and 10 mg/kg groups, although the reason for this difference is unclear. Nevertheless, a t-test indicated that swim distance decreased significantly on Day 2 between trials 1 and 4 which, combined with the non-significant Treatment main effect on the final trial of Day 2, suggests that all groups re-learned the platform position to a similar extent after initial reminder trials.

Interestingly, the data suggest a shift in the aged dose-response curve for progesterone was observed in the Morris water maze relative to the novel object recognition task, with the former requiring a higher dose to produce an improvement. Although it is unclear what might cause such a shift, one possibility may involve differences in hippocampal involvement in each task. Spatial memory in the Morris water maze can be severely impaired by lesions of the dorsal hippocampus encompassing 30–50% of total hippocampal volume, whereas ventral hippocampal lesions have little effect on water-maze performance (reviewed in Moser and Moser, 1998). Novel object recognition memory, on the other hand, is only disrupted after lesions that damage 75–100% of the hippocampus (Broadbent et al., 2004). Thus, whereas spatial memory in the Morris water maze is critically dependent on the dorsal hippocampus, various regions of the hippocampus can support novel object memory (reviewed in Mumby, 2001). This differential regional involvement may contribute to the differential sensitivity of the tasks to progesterone, as progesterone receptors are expressed in all sub regions of the hippocampus (Guerra-Araiza et al., 2000; Kato et al., 1994). It is unknown how this expression changes with age, but it is likely that receptor levels in the hippocampus decrease somewhat with age, as progesterone receptor expression decreases with age in the hypothalamus (Chakraborty and Gore, 2004) and progestin levels are reduced in aged female mice relative to young female mice (Nelson et al., 1994). Nevertheless, the fact that a smaller region of the hippocampus is necessary for spatial memory in the water maze than for object recognition may render the water maze less sensitive to progesterone and, thus, necessitate a higher dose to observe a beneficial effect. The more widespread involvement of the hippocampus in object recognition could lead to the increased sensitivity of this task to progesterone if progesterone modulates performance by binding to more receptors throughout the hippocampus. This hypothesis, however, would need to be further supported by future studies mapping the distribution of progesterone receptors in the hippocampus to the sub regions critical for each task.

It should be noted that some previous studies have failed to demonstrate that progesterone alone improves memory in females. For example, a single progesterone injection given 4 h prior to training in the Morris water maze had no significant effect on spatial memory in young female rats, although neither did a single injection of estradiol alone (Chesler and Juraska, 2000). In contrast, injection of both estradiol and progesterone significantly improved performance (Chesler and Juraska, 2000). In addition, young female mice chronically implanted with pellets releasing either progesterone alone or estrogen plus progesterone were impaired in learning a footshock avoidance task relative to mice receiving placebo or estrogen pellets (Farr et al., 1995). The most likely explanation for the discrepancy between these two studies and the improvements observed in the present and previous studies (Frye and Lacey, 2000; Walf et al., 2006) is the timing of progesterone administration. The studies reporting no effect or an impairing effect of progesterone administered the hormone prior to training, whereas the three studies reporting improvements administered progesterone post-training. This timing is important given that progesterone and its metabolites have anxiolytic and analgesic effects (Bitran et al., 1991; Frye and Duncan, 1994), which could interfere with mnemonic function if given prior to training. Post-training paradigms, such as the one used in the present study, eliminate this confound because the hormone is not present in the circulation during training or testing; HBC-conjugated progesterone is metabolized within 24 h (Pitha and Pitha, 1985). Thus, although the present study cannot address the more clinically relevant issue of how chronic progesterone treatment influences memory function, it makes an important contribution towards understanding how progesterone specifically affects memory consolidation in the absence of the confounding factors that influence task performance.

In summary, the present study demonstrates that acute post-training progesterone injections can differentially affect spatial memory consolidation in the Morris water maze and hippocampal-dependent object memory consolidation in middle-aged and aged female mice. These findings provide an important initial step in clarifying the role of progesterone in memory consolidation.

Disclosure statement

The author's declare that there are no actual or potential conflicts of interest.

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