Ovarian Hormones and Cognition in the Aged Female Rat: I. Long-Term, but Not Short-Term, Ovariectomy Enhances Spatial Performance

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Although research suggests that ovariectomy (ovx) is detrimental to spatial cognition in young rats, little work has evaluated the cognitive effects of ovx in aged rats. The authors investigated the effects of ovx in aged rats using the water radial-arm maze. In Study 1, young rats and aged rats receiving ovx 1.5 months before testing outperformed aged rats receiving sham surgery or ovx 21 days before testing. In Study 2, young rats and aged rats receiving ovx 2.0 or 6.0 months before testing outperformed aged sham rats. Aged rats exhibited estradiol and elevated progesterone levels comparable to those of young rats. The findings suggest that 1.5–6.0 months, but not 21 days, of ovx improves spatial memory in aged rats. The hypothesis that long-term ovarian hormone loss is detrimental to spatial memory in aged rats was not supported. The authors hypothesize that removal of elevated progesterone levels is related to the ovx-induced cognitive enhancement.

It is well documented that gonadal hormones influence the brain and behavior during early development, as well as later in life. Both cognitive performance and brain substrates that mediate learning and memory respond to activational, or nonpermanent, effects of ovarian hormones in adulthood. For example, research in women has shown that during menstruation, when estrogen and progesterone levels are lowest, spatial skills are strongest, whereas verbal skills are poorest (Hampson, 1990a). In contrast, during the midluteal cycle phase, when estrogen and progesterone levels are high, verbal fluency and fine motor skills are strongest, whereas spatial skills are poorest (Hampson, 1990a, 1990b; see also Phillips & Sherwin, 1992b).

Menopause is defined as the permanent cessation of menses in women as a result of natural or surgical loss of ovarian hormones (Timiras, Quay, & Vernadakis, 1995). In women, the ovary is an endocrine gland that gradually ceases hormonal function as aging ensues (Timaras et al., 1995). Hence, by means of either natural ovarian cessation or surgical removal of the ovaries, ovarian hormone output eventually terminates in most women, resulting in very low circulating levels of gonadal hormones, including estrogen and progesterone, relative to the hormonal state seen in younger women.

The issue of hormone replacement therapy in naturally or surgically menopausal women has yielded much debate. Some studies suggest that one risk factor in developing Alzheimer's disease (AD) is female gender, and in general, women comprise the majority of the AD group in cohort studies (Corey-Bloom, Galasko, Hofstetter, Jackson, & Thal, 1993; Katzman et al., 1989). The loss of ovarian hormones at menopause has been argued to play a role in the increased prevalence of AD in women by exacerbating neurodegeneration and subsequent cognitive decline (Birge, 1996; Simpkins, Singh, & Bishop, 1994). Supporting this hypothesis, studies have shown that women exhibited memory decline after oophorectomy (Sherwin, 1988), that surgically menopausal women had lower memory scores than naturally menopausal women (possibly due to a more drastic hormone loss; Nappi et al., 1999), and that age at oophorectomy and years since surgery correlated with memory (Nappi et al., 1999). The fact that hormone-related variables alter AD risk points to a role for ovarian hormones, or lack thereof, in developing AD. Several studies in humans have suggested that hormone replacement therapy in menopausal women may reduce the risk or delay the onset of developing AD (Henderson, Paganini-Hill, Emanuel, Dunn, & Buckwalter, 1994; Tang et al., 1996). In addition, there are a number of well-controlled studies suggesting that removal of the ovaries results in an eventual decline on specific tests of memory function in women without AD, and that estrogen treatment prevents this postoopherectomy cognitive decline (Phillips & Sherwin, 1992a; Sherwin & Phillips, 1990). However, some recent reports have not found positive effects of hormone replacement therapy and have emphasized the need for more placebo-controlled, randomized studies (Mulnard et al., 2000; Seshadri et al., 2001; Yaffe, Sawaya, Lieberburg, & Grady, 1998). Recently, a large, randomized, placebo-controlled evaluation (The Heart and Estrogen/Progestin Replacement Study) using an estrogen-
pseudopregnant–persistent diestrus state characterized by high progesterone levels caused by an increase in ovulation and subsequent corpora lutea development (Lu, Hopper, Vargo, & Yen, 1979; Meites et al., 1994). Most, but not all, middle-aged rats in persistent estrous eventually progress to the pseudopregnant or persistent diestrus state. One study suggests that the onset of female rats’ spatial reference memory decline at 12 months may be related to the reproductive senescence that occurs at about this same age (Markowska, 1999). Furthermore, in aged female rats, the particular stage of estropause has been associated with spatial reference memory performance as evaluated in the Morris water maze, with the pseudopregnant state related to poorer test scores (Warren & Juraska, 2000). Correspondingly, one study has shown that in young, cycling rats, spatial performance on the Morris water maze was worse during proestrus, when estrogen and progesterone levels are highest, and best during estrus, when estrogen and progesterone are lowest (Warren & Juraska, 1997). In addition, Frye (1995) found that female rats tested on the evening of proestrus (behavioral estrus) exhibited poorer Morris maze performance on Trial 3 of 6 total trials as compared with females in diestrus. Still others did not find cognitive changes across the estrous cycle, as evaluated in pretrained young rats on the Morris water maze (Berry, McMahan, & Gallagher, 1997) or in rats tested on the appetitively motivated version of the radial-arm maze (Stackman, Blasberg, Langan, & Clark, 1997).

To more directly evaluate ovarian hormone contributions to learning and memory during both young adulthood and aging, experimental hormone manipulation procedures have been performed in animal models. Using young female rats, some research has shown that ovariectomy (ovx) disrupts spatial working memory performance on the water escape radial-arm maze and the land radial-arm maze, but enhances performance on the spatial reference memory Morris water maze (Bimonte & Denenberg, 1999; Daniel, Roberts, & Dohanich, 1999). Effects of hormone replacement have also been assessed. Indeed, estradiol treatment enhanced the cognitive performance of young ovx and aged ovx rats, although only one of the studies in aged female rats used an intact control group (Bimonte & Denenberg, 1999; Gibbs, 2000; Luine & Rodriguez, 1994; Markowska & Savonenko, 2002). However, not all rodent studies show positive mnemonic effects of female gonadal steroid treatment. Young ovx rats acutely treated with estrogen plus progesterone showed poorer performance on the Morris water maze than young ovx rats given oil, estrogen alone, or progesterone alone (Chesler & Juraska, 2000). Accordingly, Frye (1995) demonstrated that young ovx rats administered a hormone regimen designed to mimic estrus had poorer scores during acquisition of the Morris water maze than young ovx rats administered vehicle. Others have shown that ovx improved the performance of young mice on a footshock-avoidance task compared with their intact counterparts, whereas treatment of ovx mice with progesterone or estrogen plus progesterone resulted in impaired performance (Farr et al., 1995).

The majority of animal studies investigating ovarian hormone effects on learning and memory have used young animals. Only a few studies have evaluated potential relationships between endogenous ovarian hormones and cognitive performance in aged rodent models (Gibbs, 2000; Markowska, 1999; Markowska & Savonenko, 2002; Warren & Juraska, 2000). Moreover, there has been only one study (Markowska & Savonenko, 2002) addressing whether removal of ovarian hormones in the aging female rat affects cognitive behavior. Markowska and Savonenko (2002) found that ovx had a negative impact in middle-aged female rats on a delayed nonmatching-to-position working memory task, but not on spontaneous alternation in a Y-maze, or on place discrimination or repeated acquisition of the Morris water maze. On the other hand, a recent study using aged monkeys showed that ovx had a positive impact on the spatial component of the delayed recognition test (Lacreuse, Herndon, & Moss, 2000). Thus, to date, the cognitive effects of ovx during aging have not been fully clarified.

We have recently shown that aged female rats perform profoundly worse than young female rats on a spatial water radial-arm maze task designed to evaluate both working and reference memory simultaneously (Bimonte, Granholm, Seo, & Isacson, 2002; Bimonte, Nelson, & Granholm, 2003). On this task, 8 of the 12 maze arms contained hidden escape platforms. Once a rat found a platform, the platform was removed for the remainder of that testing day. Hence, a rat had to avoid arms that never contained a platform (reference memory), as well as remember and avoid arms where a platform had already been located within that session (working memory). As a rat progressed through a daily session, the number of previously reinforced choices, and thus locations to be remembered, increased. Therefore, the ability to handle an increasing memory load was required to perform successfully. Our previous research has shown that the detrimental effects of aging are especially pronounced as working memory load increases (Bimonte, Granholm, et al., 2002; Bimonte et al., 2003; Bimonte-Nelson, Singleton, et al., 2003).

The first goal of the current experiments was to determine whether age-related performance decrements are observed in female rats on the water radial-arm maze with a lesser memory load: a maze containing only eight arms, with four arms designated as reference memory (containing no platforms) and the other four designated as working memory (containing platforms). The second goal of the current experiments was to assess the effects of ovarian hormone removal on spatial working and reference memory in aged female rats, methodically investigating the cognitive consequences of different pretest time lengths of ovx. Our initial hypothesis was that ovx would have a negative impact on spatial memory in aged female rats. This hypothesis was based on research showing that ovx is detrimental to cognitive performance in young rats, and that estrogen treatment improves such performance in young ovx and aged ovx rats. However, Study 1 revealed that ovx 1.5 months, but not 21 days, before test enhanced water radial-arm maze performance in aged rats. Because these results
were novel and unexpected, we performed a second, independent study to replicate and extend the investigation. Hence, Study 2 assessed the effects of 2 and 6 months of ovx in aged rats. Because hormone levels change with age, in both studies we also obtained serum samples following testing to determine circulating estradiol and progesterone levels.

Method

Subjects and Treatment Procedures

As described in Table 1, subjects in Study 1 and Study 2 were Fischer-344 female rats born and reared at the aging colony of the National Institute on Aging at Harlan Laboratories (Indianapolis, IN). In Study 1, subjects were 9 young (5 months old at behavioral testing) and 34 aged (22–23 months old at behavioral testing) rats. In Study 2, subjects were 9 young (2.5–3.0 months old at test) and 20 aged (20 months old at test) rats. All rats were naive to behavioral testing prior to testing on the water radial-arm maze. Prior to surgery, rats were acclimated for several weeks, pair-housed with a same-age and treatment assignment cage mate in a barrier environment in the Medical University of South Carolina animal facility. The rats had ad-lib exposure to food and water and were maintained on a 12-hr light–dark cycle at 80 °F (26.7 °C) according to National Institute on Aging guidelines for aged rats. All procedures were approved by the local International Animal Care and Use Committee and adhered to National Institutes of Health standards.

Ovx and Sham Surgeries

For Study 1 and Study 2, all young rats were given sham surgery. In addition to the young sham group, in Study 1 there were three groups of aged rats: intact sham (aged sham), ovx 21 days before testing (aged 21-day ovx), and ovx 1.5 months before testing (aged 1.5-month ovx). For Study 1, young rats were given sham surgery 21 days and 1.5 months before testing (young sham). One aged group received sham surgery 21 days and 1.5 months before testing (aged sham); the second aged group received sham surgery 1.5 months before testing followed by ovx 21 days before testing (aged 21-day ovx); the third aged group received ovx 1.5 months before testing, followed by sham surgery 21 days before testing (aged 1.5-month ovx). Behavioral testing began when aged rats were 22–23 months old and young rats were 5 months old.

For Study 2, in addition to the young sham group, there were also three groups of aged rats: intact sham (aged sham), ovx 2 months before testing (aged 2-month ovx), and ovx 6 months before testing (aged 6-month ovx). The aged sham group received sham surgery 2 months and 6 months before testing; the aged 2-month ovx group received sham surgery 6 months before testing followed by ovx 2 months before testing; and the aged 6-month ovx group received ovx 6 months before testing, followed by sham surgery 2 months before testing. Behavioral testing for Study 2 began when aged rats were 20 months old and young rats were 2.5–3.0 months old.

For surgeries, all rats were anesthetized with an intraperitoneal injection of Ketamine/Xylazine (7:2 ratio). For ovx, two dorsolateral incisions were made in the skin and peritoneum, and the ovaries and tips of the uterine horns were ligatured and removed. The muscle was then sutured and the skin stapled. Sham surgery consisted of the skin incision and staple procedures only. Heat lamps were used to maintain body temperature during and immediately after surgery. Vaginal smears were performed after surgery, before behavioral testing ensued, to confirm complete removal of ovarian tissue. As expected, all ovx rats exhibited leukocytic acyclic smears, confirming complete removal of ovarian tissue.

Radial-Arm Maze Testing

We tested subjects on the water escape radial-arm maze to evaluate the effects of aging and the effects of ovx in aged rats. The win–shift radial-arm maze used water escape onto hidden platforms as the reinforcer, thereby avoiding food deprivation (Bimonte & Denenberg, 1999, 2000;
Bimonte, Granholm et al., 2002; Bimonte, Hunter, Nelson, & Granholm, 2003; Bimonte, Hyde, Hoplight, & Denenberg, 2000, Hyde, Hoplight, & Denenberg, 1998; Hyde, Sherman, & Denenberg, 2000). The maze had 12 arms; 4 arms were consistently blocked off to make a symmetrical 8-arm maze (see Bimonte et al., 2003 for details of maze dimensions). The maze was constructed of galvanized steel, painted black, and filled with water (room temperature). It had hidden escape platforms, with wire mesh tops (hidden about 1 cm below the water surface) placed in the ends of 4 of the 8 accessible arms. Each subject had different platform locations that were semirandomly determined and that remained fixed throughout the experiment. There was never a platform in more than three adjacent arms nor in the arm from which the rat was released. The testing room had salient extramaze cues that remained constant throughout testing, including a door; cabinets; a row of warming lamps; a solid black panel on one wall; a black-and-white striped panel on the opposite wall; and the experimenter, who sat behind the start arm.

A subject was released from the start arm, facing the center, and had 3 min to locate a platform. If the allotted time expired, the subject was guided to the nearest available platform. Once a platform was found, the rat remained on it for 15 s, and was then returned to its heated home cage for 30 s until its next trial. During the interval, the just-chosen platform was removed from the maze. The rat was then placed again into the start alley and allowed to locate another platform. A daily session consisted of this sequence of events repeated until all four platforms were located. Consequently, for each rat, a daily session consisted of four trials, with the number of arms containing platforms reduced by one on each subsequent trial. Thus, four arms contained platforms on Trial 1, three arms contained platforms on Trial 2, et cetera. This pattern continued so that by Trial 4, only one arm contained a platform. Because one platform was removed after every trial, the rat needed to remember one more item of information after every trial. Hence, the working memory system was increasingly taxed as trials progressed. This version is similar to the land version of the radial-arm maze in that rats had to avoid arms that never contained a reinforcer (reference memory) and enter only once into arms that contained a reinforcer (working memory).

The following quantification and blocking procedures are based on previous studies using the water radial-arm maze (Bimonte & Denenberg, 1999, 2000; Bimonte et al., 2003; Bimonte, Granholm, et al., 2002; Bimonte, Hyde, et al., 2000; Bimonte, Hunter, et al., 2003; Hyde et al., 1998, 2000). Each subject was given one session a day for 12 consecutive days. Day 1 was considered a training session because the rat had no previous experience in the maze. Days 2–12 were testing sessions. The 11 testing days were blocked into two phases: the initial phase consisting of Days 2–6, and the latter phase consisting of Days 7–12. Behavioral testing took place between 0900 and 1400. An arm entry was counted when the tip of a rat’s snout reached a mark delineated on the outside of the arm that was not visible from the inside of the maze (11 cm into the arm). Errors were quantified for each daily session using the orthogonal measures of working and reference memory errors (Jarrard, Okaichi, Steward, & Goldschmidt, 1984), as done previously in studies using the water escape radial-arm maze (Bimonte et al., 2000; Bimonte, Granholm, et al., 2002; Bimonte, Hunter, et al., 2003; Hunter, Bimonte, & Granholm, 2003; Hyde et al., 2000). Working memory correct errors were the number of first and repeat entries into any arm from which a platform had been removed during that session. Reference memory errors were the number of first entries into any arm that never contained a platform. Working memory incorrect errors were the number of repeat entries into an arm that never contained a platform in the past (thus, repeat entries into a reference memory arm).

Statistical Analysis

Study 1 and Study 2 were analyzed separately. In each study, the experiments were designed so that we could evaluate two distinct scientific questions: effects of age, by comparing young sham to aged sham rats, and effects of different time lengths of ovx in aged rats, by comparing the three aged treatment groups (Study 1: aged sham, aged 21-day ovx, aged 1.5-month ovx; Study 2: aged sham, aged 2-month ovx, aged 6-month ovx).

Each type of error was analyzed separately. For working memory correct and working memory incorrect measures, to assess age effects, the data for each phase were analyzed with a 1 Between (age) × 2 Within (days and trials) repeated measures analysis of variance (ANOVA). To investigate ovx effects in the aged rats, the data for each phase were analyzed with a 1 Between (ovx) × 2 Within (days and trials) repeated measures ANOVA. Trial 1 was not included in the analysis for working memory correct because it is not possible to make a working memory correct error on the first trial. For reference memory, each phase was analyzed with a 1 Between (either age or ovx) × 1 Within (days) ANOVA. One-tailed tests were used for all young sham versus aged sham comparisons because the direction of the effect was anticipated on the basis of previous studies using the water radial-arm maze (Bimonte, Granholm, et al., 2002; Bimonte-Nelson, Singleton, et al., 2003). Two-tailed tests were used for all ovx analyses.

Assessment of Serum Hormone Levels

After behavioral testing, rats were anesthetized with Halothane inhalant anesthesia and decapitated. Blood from the trunk was immediately collected into a serum separator tube (Vacutainer 367986, Becton Dickinson and Company, Franklin Lakes, NJ). The blood was allowed to clot at 4 °C, and serum was collected after centrifugation (3,220 G, 20 min). The serum was stored at 20 °C until assays were performed to determine estradiol and progesterone concentrations.

Progesterone concentrations in rat serum were measured with commercially available enzyme immunoassay kits from Diagnostic Systems Laboratories (progesterone DSL-10-3900, Webster, TX) based on goat anti-rabbit IgG immobilized to the inside wall of each well in a 96-well plate. Progesterone standards (50 μl) or samples containing progesterone (25 μl) plus 25 μl of zero standard were added to each well along with rabbit anti-progesterone serum and progesterone–horseradish peroxidase conjugate. This was incubated for 1 hr at 22 °C, and then tetramethylbenzidine was added and allowed to react for 30 min at room temperature. The reaction was stopped by the addition of 0.2 M sulfuric acid. The absorbances were read with a microplate reader (Spectramax 340PC; Molecular Devices, Sunnyvale, CA) set to 450 nm, with wavelength correction set at 600 nm. The mean absorbances versus the standard concentrations were plotted by using a four parameter curve-fit, and the concentrations of the unknowns were calculated.

The Core Endocrinology Laboratory at Pennsylvania State University College of Medicine performed estradiol hormone assays using the Coat-a-Count estradiol kit (Product Number TKE21; Diagnostic Products Corporation, Los Angeles, CA). Estradiol was determined in serum by a solid-phase radioimmunoassay based on estradiol-specific antibodies that are immobilized to the wall of polypropylene tubes and 125I-labeled estradiol as the tracer, following extraction with diethyl ether. Serum (2.4 ml) was extracted, and the ether portion was collected and evaporated to dryness. The sample was reconstituted in assay buffer, and a competitive radioimmunoassay was performed using 125I estradiol with high specific activity and a high-affinity, highly specific antibody. Separation of bound from free was achieved with activated charcoal, and the data reduction was performed with the use of a 5-point standard curve and purified estradiol standards. The functional sensitivity of the assay was 5 pg/ml. The interassay precision at a concentration of 35 pg/ml was 8%.
Results

Study 1

Effects of aging. We have previously shown age-related performance deficiencies in the female rat in the water escape radial-arm maze with 8 of 12 arms containing platforms (Bimonte et al., 2003). The first aim of the current study was to evaluate whether age-related changes in performance are also observed on the water radial-arm maze with 4 of 8 arms containing platforms (i.e., with a lesser working memory demand). Although there were no age effects on Days 2–6, age-related performance deficiencies were noted on Days 7–12. Indeed, consistent with previous studies, aged shams made more working memory correct, $F(1, 17) = 4.42, p < .05$; reference memory, $F(1, 17) = 6.33, p < .025$; and working memory incorrect, $F(1, 17) = 2.99, p = .05$, errors than young shams during this latter portion of testing (see Figure 1). Age did not interact with days for any measure.

Effects of 21-day or 1.5-month ovx in aged rats. The second aim of the current studies was to evaluate the mnemonic effects of ovx in aged rats. There were no ovx effects for any measure on Days 2–6. On the contrary, there were ovx effects during the latter portion of testing, on Days 7–12. As graphically represented in Figure 1, the omnibus ANOVA showed an ovx effect for working memory correct, $F(2, 31) = 4.28, p < .05$. Fisher’s post hoc tests showed that the 1.5-month ovx group committed fewer errors than both the aged sham ($p < .05$) and the aged 21-day ovx ($p < .01$) groups. There was also a marginal ovx effect for working memory incorrect, $F(2, 31) = 2.68, p = .08$, and no ovx effect for reference memory. There were no significant Ovx × Days interactions for any memory measure within either testing phase. To further evaluate potential differences between aged sham and aged 21-day ovx rats, we analyzed these two groups alone (with treatment as the between-groups variable) in a repeated measures ANOVA (see the Method section). There were no significant main effects or interactions with treatment for any memory measure, suggesting that aged sham and aged 21-day ovx groups did not differ in maze performance.

As shown in Figure 2, the aged treatment groups differed in their ability to handle an increasing memory load, with the aged 1.5-month ovx group exhibiting the best performance of the aged groups as trials progressed and working memory load increased: Ovx × Trials interaction for working memory correct, $F(4, 62) = 3.39, p < .05$. In fact, separate repeated measures ANOVAs for working memory correct and working memory incorrect showed that the aged 1.5-month ovx and young groups did not differ in any main effect or interaction, indicating that the performance of the two groups was comparable on the working memory measures. These data suggest that, as seen in Figure 2, ovx given 1.5 months before testing in the aged rat improves working memory performance to that of young intact rats. Further analyses indicated that the working memory benefits of ovx in aged female rats are temporally specific. Indeed, the aged group that was ovariectomized 1.5 months before testing was also better able to handle a higher working memory load than the group that was ovariectomized only 21 days before testing. This was verified by $t$ tests showing that the aged 1.5-month ovx group made fewer errors than the aged 21-day ovx group on the trials with the highest memory load (collapsed across Trials 3 and 4 for Days 7–12) for both working memory correct, $t(22) = 3.07, p < .01$, and working memory incorrect, $t(22) = 2.30, p < .05$.

Estradiol and progesterone levels. Blood was collected following behavioral testing to determine circulating estradiol and progesterone levels. Figure 3 shows the mean (± SEM) estradiol and progesterone levels for each treatment group. The omnibus ANOVA including all groups was significant for progesterone levels, $F(3, 39) = 24.20, p < .0001$. Fisher’s post hoc tests revealed that both 21-day ($p < .0001$) and 1.5-month ($p < .0001$) aged ovx groups had lower progesterone levels than aged shams. Aged sham rats had markedly higher progesterone levels than young shams ($p < .0001$).

Serum estradiol levels differed between groups, $F(3, 39) = 7.66, p < .0005$. Fisher’s post hoc tests showed that, as expected, ovx 21 days ($p < .005$) and 1.5 months ($p < .0005$) before testing decreased estradiol levels relative to that of aged shams. Estradiol

![Graphs showing working memory correct, reference memory, and working memory incorrect errors](image-url)

*Figure 1. Mean (± SEM) number of working memory correct, reference memory, and working memory incorrect errors committed on the water radial-arm maze averaged across the latter testing phase (Days 7–12) for each treatment group in Study 1. * $p < .05$. ** $p < .01$. d = day; mo = month; Ovx = ovariectomy.*
levels did not differ between aged shams and young shams (*p* = .50).

**Study 2**

Study 1 showed that whereas short-term (21 days) ovx yielded no significant effects on maze performance, ovx 1.5 months before testing resulted in an enhancement in performance. To further investigate this finding, we wished to evaluate the effects of ovx at a longer duration. Hence, Study 2 compared the performance of 20-month-old sham, intact female rats to 20-month-old female rats receiving ovx either 2 months before testing (at 18 months) or 6 months before testing (at 14 months; see Table 1).

**Effects of aging.** As shown in Study 1 and in previous studies from our laboratory, aged sham rats made more working memory correct, *F*(1, 12) = 7.02, *p* < .025; reference memory, *F*(1, 12) = 9.96, *p* < .005; and working memory incorrect, *F*(1, 12) = 4.20, *p* < .05, errors than young shams on Days 7–12 on the water radial-arm maze in Study 2 (see Figure 4). Age did not interact with days for any measure within either testing phase.

**Effects of 2- or 6-month ovx in aged rats.** Similar to the findings in Study 1, there were no significant treatment effects for Days 2–6. However, ovx had profound effects on aged female performance during the last 6 days of testing (Days 7–12; Figure 4). The omnibus ANOVA for Days 7–12 revealed an ovx effect for working memory correct, *F*(2, 17) = 4.73, *p* < .05; reference memory, *F*(2, 17) = 4.84, *p* < .05; and working memory incorrect, *F*(2, 17) = 4.55, *p* < .05. Aged female rats that received ovx either 2 or 6 months before testing made fewer errors than aged shams on all three measures on Days 7–12 (Fisher’s post hoc tests—aged 2-month ovx group vs. aged sham group: working memory correct *p* < .05, reference memory *p* < .01, working memory incorrect *p* < .01; aged 6-month ovx group vs. aged sham group: working memory correct *p* < .05, reference memory *p* < .05, working memory incorrect *p* < .05).
memory correct \( p < .01 \), reference memory \( p < .05 \), working memory incorrect \( p < .05 \). The aged 2-month ovx and 6-month ovx groups did not differ from each other on any memory measure. There were no Ovx \( \times \) Days interactions for any memory measure within either testing phase.

Study 1 showed that aged female rats receiving ovx 1.5 months before testing made fewer errors on the trials with the highest working memory demand compared with the other aged groups, indicating that the 1.5-month ovx rats were better able to handle this increased memory load relative to aged rats that received sham surgery or ovx 21 days before test. In Study 2, we found similar results, with aged 2- and 6-month ovx groups exhibiting a better ability than aged shams to handle a higher working memory load (see Figure 5). Specifically, compared with aged sham rats, aged rats that received ovx made fewer working memory correct and working memory incorrect errors on the last two trials, when working memory demand was highest: combined 2- and 6-month ovx groups versus aged sham group, collapsed across Trials 3 and 4 for Days 7–12 for working memory correct, \( t(18) = 2.68, p < .05 \), and working memory incorrect, \( t(18) = 2.84, p < .05 \).

Estradiol and progesterone levels. As in Study 1, progesterone levels differed between groups, \( F(3, 25) = 16.22, p < .0001 \) (see Figure 6). Both 2- \( (p < .0001) \) and 6-month \( (p < .0001) \) ovx groups had lower progesterone levels than aged shams. Again, as seen in Study 1, aged shams had higher progesterone levels than young shams \( (p < .01) \).

Similar to the findings in Study 1, there were group differences in estradiol levels in Study 2, \( F(3, 25) = 10.60, p < .0001 \) (see Figure 6). In aged females, ovx given 2 \( (p < .01) \) and 6 \( (p < .005) \) months before test resulted in lower estradiol levels as compared with shams. Estradiol levels between aged shams and young shams did not differ \( (p = .39) \).

For progesterone and estradiol levels in both Studies 1 and 2, there was greater variability in the young sham and aged sham groups as compared with the ovx groups. This is likely due to the fact that these groups had their ovaries, which were excreting hormone, whereas the ovx groups were devoid of ovarian hormone excretion. The variability in the values in the young rats is most likely due to the normal hormonal fluctuations across the estrous cycle. The variability in hormone levels in aged sham rats is probably related to individual variations in ovarian output from abnormal corpora lutea development and, thus, hormonal status (e.g., estropause).

Discussion

We have previously shown that aged female rats made more working and reference memory errors on the water radial-arm maze with 8 of the 12 arms containing platforms. Because a platform is removed after it has been found, a subject must remember the just-located platform location and avoid reentries into that arm for successful working memory performance. Eight arms contained platforms; thus, seven items of information (platform locations) had to be remembered by the last trial. One goal of the current experiments was to evaluate age-related memory changes in female rats on the water radial-arm maze task with a lesser memory demand (e.g., a working memory load of three items of information). Hence, we tested young intact (sham) and aged intact (sham) rats on the water radial-arm maze with 4 of 8 arms containing platforms. Similar to our findings on the more difficult version of the maze, in the current experiments, on a maze with a lesser working memory load aged intact rats made more errors than young intact rats on all three memory measures: working memory correct, reference memory, and working memory incorrect. These findings suggest that aged female rats are impaired in both working and reference memory. Our findings agree with previous reports using the working and/or reference memory versions of the radial-arm maze, which found that aged male rats had more total errors or trials to criterion, less correct choices, or poorer performance on various other measures (Arendash, Sanberg, & Sengstock, 1995; Barnes, Nadel, & Honig, 1980; Beatty, Bierley, & Boyd, 1985; Bond, Everitt, & Walton, 1989; Chrobak, Hanin, Lorenz, & Napier, 1995; Kadar, Arbel, Silbermann, & Levy, 1994; Noda, Yamada, & Nabeshima, 1997; Pitsikas & Algeri, 1992; Stewart, Mitchell, & Kalant, 1989; Wallace, Krauter, & Campbell, 1980; Wellman & Pellemounter, 1999). In addition,
our findings add to accumulating data from aged female rats showing spatial working memory deficiency on the radial-arm maze (Luine & Hears, 1990) and spatial reference memory decline on the Morris maze (Fischer, Bjorklund, Chen, & Gage, 1991; Markowska et al., 1989).

The second goal of the current experiments was to evaluate the spatial working and reference memory effects of ovarian hormone removal in aged female rats. Our initial hypothesis that ovarian hormone removal in aged female rats would be detrimental to maze performance was not supported. In fact, our data suggest the contrary. The most important findings in this series of experiments are that ovx given 1.5, 2, or 6 months before testing improved performance of aged female rats on the water radial-arm maze. This effect of ovx was striking, improving the performance of aged female rats to that of young rats during the latter portion of testing. Further, the cognitive-enhancing effects of ovx were replicated in independent experiments and appeared to depend on the temporal parameters of ovx. Ovx 21 days before test did not yield an improvement in maze performance, whereas 1.5-, 2-, or 6-month ovx resulted in marked improvements in maze performance. Overall, the 2- and 6-month ovx effects were particularly salient, enhancing performance to that of young sham rats on all three orthogonal working and reference memory measures. The observed ovx-induced improvement in spatial reference memory scores is consistent with previous work evaluating ovx in young female rats on the Morris water maze (Daniel et al., 1999). Accordingly, on the Morris water maze, ovx rats outperformed ovx rats treated with ovarian hormones (Frye, 1995). Yet, others have shown that ovx had no effect on Morris water maze performance in aged rats (Markowska & Savonenko, 2002).

When we separated the working memory correct and working memory incorrect errors into trials, it was clear that rats that received ovx 1.5, 2, or 6 months before testing were better able to handle performance as trials, or working memory load, increased.

Figure 5. Mean (± SEM) number of working memory correct and working memory incorrect errors committed on the water radial-arm maze for each trial averaged across the latter testing phase (Days 7–12) for each treatment group in Study 2. Young sham rats and aged (A) rats that received ovariectomy (Ovx) 2 or 6 months (mo) before testing performed better as trials, and working memory load, increased compared with aged shams.

Figure 6. Mean (± SEM) estradiol and progesterone levels for each treatment group in Study 2. * p < .01, ** p < .005, *** p < .0005. Ovx = ovariectomy; mo = month.
In fact, on latter trials, these groups exhibited performance that was comparable to that of young rats. Our working memory findings may appear counterintuitive, as several studies using young rats suggest that ovarian hormone deprivation is associated with working memory decline. Daniel et al. (1999) showed that, compared with young intact female rats, young rats that were ovariectomized 1 month before testing exhibited worse working memory scores on the land radial-arm maze. We have also shown on the water radial-arm maze that 30-day ovx was detrimental to working memory performance in young rats, especially on trials with a higher memory load (Bimonte & Denenberg, 1999). In contrast, the current studies indicate that 21-day ovx has no effect on spatial working memory in the aged rat, and that 1.5-, 2-, or 6-month ovx has a significant positive impact on spatial working memory in the aged rat.

Why might ovx in young rats have detrimental effects on spatial working memory performance, whereas ovx in aged rats have enhancing effects on spatial working memory performance? Inspection of the gonadal hormone profile of intact aged female shams in the current studies may yield insight into the observed effects. Compared to young sham rats, aged sham rats exhibited comparable estrogen levels and elevated progesterone levels. These data indicate that, in the present study, estrogen levels did not change with age in female rats, whereas progesterone levels increased dramatically. Many endocrine studies have shown that aged female rats enter a pseudopregnant estropause state, in which progesterone values become significantly elevated but estradiol levels remain relatively unchanged (Huang, Steger, Bruni, & Meites, 1978; Wise & Ratner, 1980). This pseudopregnant state in aged female rats has in fact been associated with poorer spatial cognitive performance as assessed on the Morris water maze (Warren & Juraska, 2000). Accordingly, one study has shown that in young, cycling rats, spatial maze performance was worse during proestrus, when estrogen and progesterone levels are at their highest, and best during the estrous phase, when estrogen and progesterone are at their lowest (Warren & Juraska, 1997). It is noteworthy that in both intact cycling young rats and aged estropause rats, the rats in the phases with the highest progesterone levels performed the poorest relative to their age group, a pattern noted and discussed previously (Warren & Juraska, 2000). Hence, a reasonable explanation for our findings in aged female rats is that ovx removes the negative impact of elevated progesterone levels, thereby enhancing learning and memory.

One caveat to this interpretation is that ovx given 21 days before test appeared to be sufficient to reduce progesterone levels in aged rats (see Figure 3), but this time length of ovx did not enhance cognition as did 1.5-, 2-, or 6-month ovx. A feasible explanation for this is that 21 days may have been enough time to effectively reduce circulating progesterone levels, but not to effectively alter the neural parameters underlying our effects. In addition, stress can induce hormones such as progesterone to be released from the adrenals, and higher progesterone levels have been shown to correspond with increases in anxiolytic-like behavior in female rats (Frye, Petralia, & Rhodes, 2000). Thus, our maze performance effects of ovx and the concomitant decrease in circulating progesterone levels may have interacted with anxiety levels caused by test-related stress. This explanation, however, seems improbable because untested Fisher-344 female rats also show the same age-related pattern of estradiol and progesterone change (Bimonte-Nelson, Nelson, Moore, & Granholm, 2003), and ovx-related performance effects for working memory were seen only on the trials with the highest memory load. Indeed, when memory load was lowest but the noncognitive demands of the task were the same, there were no effects of ovx (see Figures 2 and 5).

Studies have been done to more directly evaluate the cognitive effects of progesterone and its metabolites, although these findings have shown mixed results. Young adult ovx female rats that received infusion of the progesterone metabolite allopregnanolone into the lateral ventricle exhibited poorer spatial performance on the Morris water maze as well as a delayed nonmatching-to-sample working memory Y maze task compared with vehicle-treated rats, whereas when the route of administration was via subcutaneous injection, allopregnanolone enhanced working and reference memory performance (Frye & Sturgis, 1995). Others have shown that intravenous administration of allopregnanolone impaired performance on the Morris water maze in young adult male rats (Johansson, Birzniece, Lindblad, Olsson, & Backstrom, 2002). Further, progesterone injections several hours before conditioned avoidance testing enhanced performance in the rat at the estrous, but not the diestrous, phase of the estrous cycle (Diaz-Veliz, Urresta, Dassaubat, & Mora, 1994). Although this and other evidence suggests that estradiol and progesterone may act in concert to affect cognitive performance, the magnitude and even the direction of the effects are unclear and may depend on the type of task (e.g., Chesler & Juraska, 2000; Diaz-Veliz et al., 1994; Gibbs, 2000). Indeed, progesterone treatment enhanced estradiol’s effects on a delayed matching-to-position spatial T maze (Gibbs, 2000), whereas progesterone plus estradiol injections impaired performance on the spatial, but not nonspatial, reference memory Morris water maze; estradiol or progesterone treatment alone had no effects (Chesler & Juraska, 2000).

There has been limited work evaluating the effects of ovx in aged nonhuman mammals. As far as we are aware, the current report is only one of two studies investigating the effects of ovx in the aged female rat. The other study (Markowska & Savonenko, 2002) was a longitudinal investigation which reported that ovx 4 months before testing in 17-month-old Fisher rats was detrimental to delayed nonmatching-to-sample performance when delays of 1–30 min were introduced, although ovx had no effect on spatial reference memory as evaluated in the Morris water maze (Markowska & Savonenko, 2002).

In accordance with our findings, a recent study reported that aged ovx monkeys outperformed aged intact monkeys on the spatial condition of the delayed recognition span test (Lacrey et al., 2000). Because the monkeys in that study were ovariectomized early in life, the authors suggested that long-term removal of ovarian hormones may protect against age-related spatial memory deficits (Lacrey et al., 2000). Although the findings in the present studies concur with this suggestion, it is as yet unclear how these findings relate to the cognitive effects of surgically or naturally occurring menopause in women. Indeed, studies in women receiving ovx for benign disease showed a decrease in verbal memory scores after ovx, whereas estrogen replacement attenuated this cognitive decline (for review, see Sherwin, 1998). Further, Nappi et al. (1999) found that time length of ovx correlated with verbal memory scores, with a longer ovarian hormone loss associated with worse performance. It may be of significance that most human studies evaluating the cognitive effects of the hormone loss...
at menopause and subsequent hormone replacement show enhancements specifically for verbal memory and the ability to learn or retain new information, with few or no effects on spatial tasks (for discussion, see Sherwin, 1998).

Clearly there is a need for further research evaluating the specific consequences of ovarian hormone loss. It may be of interest to further investigate the effects of ovx in aged animal models using a battery of spatial and non-spatial tasks that tap into various aspects of cognitive function, thereby potentially yielding implications to selective biological substrates (i.e., hippocampus-dependent vs. non-hippocampus-dependent tasks). This may be especially important given that the specific neural basis of the cognitive effects of ovarian hormone actions is, as yet, unclear. There is evidence suggesting several possible neurobiological mechanisms whereby ovarian hormones exert cognitive effects. Accumulating data indicate that the hippocampal formation is a likely candidate for ovarian hormone actions. In young female rats, hippocampal spine density is decreased by ovx and increased to control levels by estradiol treatment (Gould, Woolley, Frankfurt, & McEwen, 1990; Woolley & McEwen, 1992). It is interesting that estradiol plus progesterone treatment did not yield an increase in dendritic spine density, as did estradiol alone (Gould et al., 1990; Woolley & McEwen, 1992). Thus, progesterone decreased the efficacy of estradiol’s effects on this aspect of the physiology of the hippocampus, a region known to be intimately linked to spatial learning and memory. Choline acetyltransferase (ChAT) activity in the hippocampus and the number of ChAT-immunoreactive neurons in specific basal forebrain regions have also been shown to be altered with experimental ovarian hormone manipulation in young rodents (Gibbs, 1997; Heikkinen, Puolivali, Liu, Rissanen, & Tamila, 2002; Luine, 1985; Miller et al., 1999). Specifically, estradiol administration increased ChAT activity in some forebrain nuclei, the frontal cortex, and specific regions of the hippocampus (Luine, 1985), as well as increased the number of ChAT-like immunoreactive cells in basal forebrain (Gibbs, 1997). Further, estradiol-treated ovariecetomized rats showed elevated levels of high-affinity choline uptake relative to ovx rats given no hormone treatment (Singh, Meyer, Millard, & Simpkins, 1994). Growth factor protein levels are also affected by hormone status in aged rats in brain regions known to affect learning and memory, such as the hippocampus and basal forebrain, and growth factor receptor mRNA expression in the basal forebrain is altered by aging and ovx in female rats (Bimonte-Nelson, Singleton, et al., 2003; Gibbs, 1998). This is not surprising, given research showing intimate links between an estrogen response element and the gene for at least one growth factor (Sohrabji, Miranda, & Toran-Allerand, 1995). Because most research evaluating hormone effects on cognitive brain regions has focused on estrogenic actions, as yet there is limited information on whether progesterone also has effects on these systems.

Endocrine research has shown that there are both similarities and differences in age-related alterations in the reproductive system of female rats and women. Although cyclicity and hormone levels change with age in both rats and women, the relative differences between the species are both quantitative and qualitative in nature. In contrast to the herein observed and previously reported stable estradiol levels and increased progesterone levels in aged female rats, in women, both estrogen and progesterone levels decrease with age as menopause ensues, presumably because of a decrease in follicular reserves (Timmers et al., 1995). In fact, as women age, estradiol and progesterone levels progressively decline, eventually resulting in dramatically decreased levels in comparison to the hormonal profile of young women (Timmers et al., 1995). Thus, in light of such discrepancies between age-associated changes in the ovarian hormone profile of the rat versus the woman, it may be important to limit extrapolation to relationships between specific hormones and cognitive scores rather than relationships between the cognitive status of the older estrogenal rat and the older menopausal woman.

In conclusion, the findings in the current experiments suggest that ovx given 1.5 to 6 months, but not 21 days, before testing improves spatial memory in aged rats. The ovarian hormone profile of stable estradiol levels and elevated progesterone levels in the aged female rat may relate to the observed cognitive effects of ovx. It must also be taken into account that, because hormones other than estradiol and progesterone are reduced after ovx and individual hormones were not manipulated in the present studies, we are unable to determine which particular effect of ovx enhanced performance. Future studies will investigate the specific mnemonic contributions of estradiol versus progesterone. Finally, there is only one other study evaluating aging, ovx, and/or hormone replacement therapies that has included an intact comparison group. Specifically, most experiments compare an ovx group with an ovx-plus-hormone group, without an intact comparison group. This has consequently resulted in a limited amount of information regarding relative group rankings of intact aged, ovx aged, and ovx hormone-treated aged groups. Our findings therefore also underscore the importance of including an intact control group in evaluating ovarian hormone replacement therapies and cognitive performance.

References


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