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Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats

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Abstract

Learning and memory processes may be influenced by fluctuations in steroid hormones, such as estrogens and progestins. In this study, we have used an animal model to investigate the effects of endogenous fluctuations in ovarian steroids in intact female rats and effects of administration of ovarian steroids to ovariectomized rats for non-spatial, working memory using the object recognition task. Performance in this task relies on cortical and hippocampal function. As such, serum, cortical, and hippocampal concentrations of estradiol (E_2), progesterone (P_4), and P_4 's metabolite, 5 α -pregnan-3 α -ol-20-one (3 α , 5 α -THP), were measured by radioimmunoassay. Experiment 1: Rats in behavioral estrus, compared to those in diestrus or estrus, spent a greater percentage of time exploring a novel object concomitant with increases in serum E_2 , P_4 , and 3 α , 5 α -THP levels. Regression analyses revealed that there was a significant positive relationship between E_2 levels in the hippocampus and 3 α , 5 α -THP levels in the hippocampus and cortex and performance in this task. Experiment 2: Administration of E_2 and/or P_4 immediately post-training increased the percentage of time spent exploring the novel object and produced levels of E_2 , P_4 , and 3 α , 5 α -THP akin to that of rats in behavioral estrus. Experiment 3: Post-training administration of selective estrogen receptor modulators, including 17 β - E_2 , propyl pyrazole triol, and diarylpropionitrile increased the percentage of time spent exploring the novel object compared to vehicle-administration. Experiment 4: Post-training P_4 or 3 α , 5 α -THP administration, compared to vehicle, increased the percentage of time spent exploring the novel object and produced P_4 and/or 3 α , 5 α -THP levels within the physiological range typically observed for rats in behavioral estrus. Experiment 5: If post-training administration of E_2 and/or P_4 was delayed one hour, no enhancement in object recognition was observed. Together, these results suggest that E_2 and progestins can have mnemonic effects through actions in the cortex and/or hippocampus.

Keywords

Estrogen; Progesterone; Allopregnanolone; Selective estrogen receptor modulators; Prefrontal cortex; Hippocampus; Learning; Memory

1. Introduction

Fluctuations in ovarian steroids, such as estrogens and progestins, across the estrous cycle alter several behavioral processes among rodents, including cognitive performance.

Circulating estradiol (E_2) levels are low early in diestrus, increase later in diestrus, peak during behavioral estrus, and decline during estrus. Progesterone (P_4) and its metabolite, 5 α -pregnan-3 α -ol-20-one (3 α , 5 α -THP), are low during diestrus and increase following the peak in E_2 levels during behavioral estrus (Frye & Bayon, 1999). The extent to which these endogenous fluctuations in estrogens and progestins influence cognitive performance is not entirely clear. Acute elevations to physiological levels of E_2 and progestins in young, intact female rats are associated with enhanced cognitive performance in some tasks. For example, in the classical eye-blink conditioning task and the inhibitory avoidance task, performance is better during behavioral estrus compared to other phases of the cycle (Rhodes & Frye, 2004a; Shors, Lewczyk, Pacynski, Mathew, & Pickett, 1998; Wood, Beylin, & Shors, 2001). No differences, or poorer performance, are associated with estrous cycle variations in E_2 that would not be expected to produce low physiological levels of E_2 during memory consolidation in tasks that are dependent upon the hippocampus (i.e., water and radial arm maze) or hippocampus and amygdala (i.e., active avoidance; Frye, 1995; Galea, Kavaliers, Ossenkopp, & Hampson, 1995; Mora, Dussaubat, & Diaz-Veliz, 1996; Stackman, Blasberg, Langan, & Clark, 1997; Warren & Juraska, 1997). Thus, these data from tasks that involve hippocampal function suggest that the hippocampus is a target of E_2 for its effects to enhance cognitive performance.

Although the data discussed above suggest that there may be concentration-dependent effects of endogenous E_2 across the estrous cycle to alter cognitive performance, other steroids, such as progestins, also vary with the estrous cycle. One way to investigate E_2 's effects is to administer E_2 to ovariectomized (OVX) rats. Administration of E_2 that likely produces low, physiological levels of E_2 during training and/or testing (similar to naturally receptive rats) improves performance in tasks that are mediated by the striatum, hippocampus, amygdala, and/or neocortex (Bimonte & Denenberg, 1999; Daniel, Fader, Spencer, & Dohanich, 1997; Davis, Jacobson, Aliakbari, & Mizumori, 2005; Diaz-Veliz, Urresta, Dussaubat, & Mora, 1991; Gibbs, 1999, 2000; Gibbs, Gabor, Cox, & Johnson, 2004; as reviewed in Korol, 2004; Korol & Kolo, 2002; Luine, Richards, Wu, & Beck, 1998; Marriott & Korol, 2003; O'Neal, Means, Poole, & Hamm, 1996; Sandstrom & Williams, 2001, 2004; Vazquez-Pereyra, Rivas-Arancibia, Loaeza-Del Castillo, & Schneider-Rivas, 1995). However, beneficial effects of E_2 are not observed in all studies using tasks that are dependent upon the hippocampus (Morris water maze, radial arm maze) or striatum (win-stay task, conditioned place preference) when E_2 is administered pre-training and/or pre-testing (Chesler & Juraska, 2000; Galea et al., 2001; Galea, Lee, Kostaras, Sidhu, & Barr, 2002; Holmes, Wide, & Galea, 2002). It may be that clearer effects of E_2 to enhance learning/memory are observed when E_2 is present during the post-training/consolidation period (Packard, 1998). Together, these findings suggest that effects of E_2 to enhance learning/memory may be dependent upon many factors, such as regimen utilized, task difficulty, type of task, strategy utilized, etc. Furthermore, it may be that some of these variable effects of E_2 for cognitive function are due to actions at different putative receptor substrates of E_2 .

In considering the effects of E_2 for cognitive performance, it is also of interest to examine putative mechanisms for these effects. For instance, studies utilizing mice with deletions of the intracellular estrogen receptor (ER) suggest that E_2 's effects at ERs may be critical for its effects on cognitive behavior (Fugger, Foster, Gustafsson, & Rissman, 2000). Furthermore, there is some suggestion from studies with knockout mice and administration of selective estrogen receptor modulator (SERMs) that have greater affinity for the α or β isoform of the ER that E_2 can have effects involving both of these isoforms (Fugger et al., 2000; Gibbs et al., 2004; Luine, Jacome, & Maclusky, 2003; Lund & Lephart, 2001; Rhodes & Frye, in press; Rissman, Heck, Leonard, Shupnik, & Gustafsson, 2002). Notably, both ER α and ER β have been localized in brain regions considered important for learning and

memory processes, such as the hippocampus and prefrontal cortex (PFC; Shughrue, Lane, & Merchenthaler, 1997, 1998).

The behavioral data discussed above regarding E_2 's effects for cognitive performance suggest that a likely target of E_2 for these effects is the hippocampus. However, the PFC may be another important target for steroid hormones' effects on cognitive performance. Two tasks that are typically utilized to assess PFC function are the T-maze and object recognition tasks (Ennaceur, Neave, & Aggleton, 1997; Granon, Vidal, Thinus-Blanc, Changeux, & Poucet, 1994). E_2 administration to young or aged mice and young, OVX rats enhances performance in both of these tasks (Fader, Hendricson, & Dohanich, 1998; Heikkinen, Puolivali, & Tanila, 2004; Luine et al., 2003; Miller et al., 1999; Vaucher et al., 2002). These data suggest that the PFC may also be a target for E_2 's effects on cognitive performance.

Although the data discussed above provide support for E_2 's effects to alter cognitive performance, the role of progestins for these effects has received less attention and is also of interest. First, in addition to circulating E_2 , progestin levels change over the estrous cycle, such that increased P_4 and $3\alpha, 5\alpha$ -THP levels may improve cognitive performance. Second, there is also evidence from extirpation and replacement studies that progestins may enhance cognitive performance of young rats. Young, OVX rats show consistent decrements in water maze and inhibitory avoidance that can be ameliorated with administration of P_4 or $3\alpha, 5\alpha$ -THP (Frye & Lacey, 2001; Frye & Sturgis, 1995). Co-administration of E_2 and P_4 enhances performance of young OVX rats, with or without neurodegeneration, in the hippocampally-mediated water or radial arm maze (Sandstrom & Williams, 2001; Sato et al., 2004; Vongher & Frye, 1999). Although there has been less investigation of the effects of progestins to modulate performance in cognitive tasks mediated by the PFC and hippocampus, in one study, administration of E_2 alone, or with P_4 , to OVX rats attenuated scopolamine-induced deficits in T-maze alternations (Dohanich, Fader, & Javorsky, 1994). Third, it may be that E_2 's effects to enhance cognitive performance are through modulating endogenous production and/or functional effects of progestins. For example, E_2 enhances central $3\alpha, 5\alpha$ -THP synthesis (Cheng & Karavolas, 1973; Malendowicz, 1976; Resko, Stadelman, & Handa, 1986; Vongher & Frye, 1999) and can enhance anti-nociceptive and/or neuroprotective effects of neuroactive progestins (Frye & Duncan, 1996; Vongher & Frye, 1999). Thus, it is intriguing to consider how E_2 and/or progestins may interact in the PFC and hippocampus to alter cognitive performance.

To investigate the role of ovarian hormones' actions in the PFC and hippocampus for cognitive performance, we examined effects of estrogens and progestins for performance in the object recognition task, a memory task that relies on the hippocampus and PFC. We hypothesized that if estrogens and/or progestins have beneficial effects to enhance cognitive performance that involves the PFC and hippocampus, then higher endogenous levels of estrogens and/or progestins in the PFC and hippocampus should occur with enhanced performance in the object recognition task. Further, administration of estrogens and/or progestins post-training to OVX rats should produce increased levels of these steroids in the PFC and hippocampus concomitant with enhanced performance in this task.

2. Methods

These methods were pre-approved by the Institutional Animal Care and Use Committee at S.U.N.Y.-Albany.

Adult, female Long-Evans rats ($N = 160$), approximately 55 days old, were obtained from the breeding colony at S.U.N.Y.-Albany (stock originally purchased from Taconic Farms, Germantown, NY). Rats were housed four per cage in polycarbonate cages ($45 \times 24 \times 21$

cm) in a temperature-controlled room (21 ± 1 °C) in the Laboratory Animal Care Facility in The Life Sciences Research Building. Rats were maintained on a 12/12 h reversed-light cycle (lights off at 0800) with continuous access to Purina Rat Chow and tap water.

In Experiment 1, effects of endogenous variations in E_2 and progestins for cognitive performance were examined. Intact rats were cycled to determine estrous cycle phase. As per previously published methods, estrous cycle phase was determined with examination of vaginal cytology (Frye & Bayon, 1999; Long & Evans, 1922). Upon examination of vaginal cytology, nucleated cells were used to identify rats in behavioral estrus, cornified cells were used to identify rats in estrus, and a heterogeneous vaginal epithelia were used to identify rats in diestrus. Immediately after screening (~0800–900), diestrous ($n = 6$), estrous ($n = 7$), and behavioral estrous ($n = 6$) rats were trained in the object recognition task. Data from one diestrous rat was excluded because central levels of E_2 and progestins could not be determined.

Rats in Experiments 2–5 were ovariectomized under Rompum (12 mg/kg; Bayer Corp., Shawnee Mission, KS) and Ketaset (80 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) anesthesia at least 1 week before behavioral testing commenced. In Experiment 2, OVX rats received subcutaneous (SC) injections of sesame oil vehicle (0.2 cc; $n = 15$), 17β -estradiol (17β - E_2 ; Sigma Chemical Co.; 0.9 mg/kg; $n = 14$), P_4 (Sigma Chemical Co.; 4 mg/kg; $n = 16$), or 17β - E_2 (0.9 mg/kg) and P_4 (4 mg/kg; $n = 16$) immediately after training in the object recognition task. One week later, in Experiment 3, OVX rats ($n = 15$ /condition) were administered SC injections of sesame oil vehicle, 17β - E_2 (0.9 mg/kg), or SERMs that have greater affinity for estrogen receptor (ER) α (propyl pyrazole triol; PPT; 0.9 mg/kg; Stauffer et al., 2000) or ER β (diarylpropionitrile; DPN; 0.9 mg/kg; Meyers et al., 2001) immediately after training. The next week, in Experiment 4, OVX rats were administered SC sesame oil vehicle (0.2 cc; $n = 11$), P_4 (4 mg/kg; $n = 14$), or 3α , 5α -THP (4 mg/kg; $n = 14$) immediately after training. One week later in Experiment 5, OVX rats were administered sesame oil vehicle (0.2 cc; $n = 7$), 17β - E_2 (0.9 mg/kg; $n = 5$), or P_4 (4 mg/kg), or 17β - E_2 and P_4 ($n = 5$) 1 h following training to address whether steroid hormones would enhance performance if present outside the 1 h post-training consolidation period. The same OVX rats were retested once per week in different conditions that were randomized with no evidence of test-decay effects, as has been previously demonstrated (Luine et al., 2003).

The object recognition task is a working memory task that relies on cortical and hippocampal functioning (Ennaceur et al., 1997). This task was used as modified from previously published methods (Ennaceur & Delacour, 1988; Frye & Lacey, 2001; Luine et al., 2003). During training, rats are placed in a white open field (76×57 cm with 35 cm high walls) in the brightly lit testing room. During training, the open field has two identical objects in adjacent corners. The identical objects were colored spheres (“orange” or “lemon” plastic toys). During testing, one sphere was replaced by a cone-shaped object (blue or red “buoy” plastic toys) of a similar size. Four hours after training, rats are placed back in the open field with two objects, one that rats had previously been exposed to during training and one novel object. Rats were allowed to explore the objects for 3 min in both trials. Rats spent equal amounts of time exploring objects in this task (typically 4.7 ± 1.0 s) and only data of rats that spent time exploring both objects during training are considered in analyses. Although rats did not show a bias towards exploring objects on the right vs. left side of the open field, placement of the novel object was counterbalanced across treatment groups and testing sessions to eliminate any possible effects of side preference. An increased percentage of time spent exploring the novel object as a function of the total amount of time spent exploring both objects during testing (duration spent with novel object/(duration spent with novel object + duration spent with familiar object) $\times 100$) is considered an index of enhanced cognitive performance in this task.

Immediately after testing, intact female rats ($n = 18$) and, a group of OVX rats that were trained, administered vehicle ($n = 2$), E_2 ($n = 3$), E_2 and P_4 ($n = 3$), P_4 ($n = 3$), or 3α , 5α -THP ($n = 3$) post-training, and were tested 1 week following completion of Experiment 5, were rapidly decapitated. Blood and whole brains were collected for later hormonal measurement by radioimmunoassay. After decapitation, blood was collected in empty glass test tubes on ice and then blood was spun in a centrifuge $3000g$ for 10 min. After spinning, serum was decanted into empty plastic eppendorf tubes and stored at -80°C (~1 week). Whole brains were dissected out from the skull in under 30 s and were placed on weighboats on dry ice. Whole brains were stored at -80°C (~1 week) until immediately before radioimmunoassay when bilateral sections of the hippocampus and cortex were dissected out on ice. These dissected out sections were then homogenized with a glass/glass homogenizer and steroids were extracted from serum and CNS tissue on the same day as radioimmunoassay was performed. Radioimmunoassay was utilized to determine E_2 , P_4 , and 3α , 5α -THP levels in these tissues, as per previously published methods (Frye & Bayon, 1999).

Data from rats were analyzed using analyses of variance (ANOVAs) to determine effects of hormone condition on performance in the object recognition task. In Experiment 1, simple regression analyses were carried out to determine the contribution of steroid hormone levels in intact, cycling rats to performance in the object recognition task. An α level of $p < .05$ was used to determine statistical significance. When appropriate, significant overall analyses were further analyzed with Fisher PLSD comparisons to determine group differences.

3. Results

3.1. Experiment 1—Endogenous variations in object recognition

Endogenous variations in E_2 and progesterone levels influence performance in the object recognition task. There was a main effect for endogenous hormone condition to alter percentage of time spent with the novel object $F(2, 15) = 10.23$, $p < .01$ (Fig. 1). Post hoc analyses revealed that rats in behavioral estrus (4.7 ± 0.6 s with novel object vs. 1.3 ± 0.4 s with familiar object) spent a greater percentage of time with the novel object compared to estrous (4.1 ± 0.6 s with novel object vs. 2.0 ± 0.3 s with familiar object) or diestrous (2.6 ± 0.7 s with novel object vs. 3.0 ± 0.8 s with familiar object) rats. Estrous rats spent a significantly greater percentage of time with the novel object than did diestrous rats.

3.1.1. Experiment 1—Endogenous variations in E_2 and progesterone levels—There were endogenous variations in E_2 and progesterone levels in serum, cortex and hippocampus (Table 1). Serum E_2 $F(2, 16) = 30.77$, $p < 0.01$, P_4 $F(2, 16) = 23.73$, $p < 0.01$, and 3α , 5α -THP $F(2, 16) = 4.48$, $p = 0.03$ concentrations were increased in rats in behavioral estrus compared to diestrus. Cortical P_4 $F(2, 16) = 4.21$, $p = 0.03$, and 3α , 5α -THP $F(2, 16) = 5.49$, $p = 0.02$ concentrations were significantly increased in rats in behavioral estrus compared to diestrus. Hippocampal E_2 $F(2, 16) = 4.04$, $p < 0.03$, and 3α , 5α -THP $F(2, 16) = 50.04$, $p < 0.01$ concentrations were increased in rats in behavioral estrus compared to diestrus.

3.1.2. Experiment 1—Regression analyses of endogenous variations in steroids and object recognition—Regression analyses revealed that physiological steroid hormone concentrations, as measured in serum, cortex, and hippocampus, contributed to performance in the object recognition task (Fig. 2). There were positive relationships between serum levels of E_2 ($r = 0.67$; $r^2 = 0.45$; $p = 0.01$, P_4 ($r = 0.67$; $r^2 = 0.44$; $p = 0.01$), and 3α , 5α -THP ($r = 0.57$; $r^2 = 0.32$; $p = 0.01$) and the percentage of time spent exploring the novel object among intact females. In analyzing the contribution of E_2 or progesterone concentrations in the cortex for object recognition, a positive relationship between

cortical levels of 3α , 5α -THP ($r = 0.48$; $r^2 = 0.23$; $p = 0.04$), but not E_2 or P_4 , and total percentage of time spent exploring the novel object was found. Positive relationships between hippocampal levels of E_2 ($r = 0.60$; $r^2 = 0.36$; $p = 0.01$), and 3α , 5α -THP ($r = 0.67$; $r^2 = 0.46$; $p = 0.01$), and the percentage of time spent exploring the novel object among intact females were also found.

3.2. Experiment 2—Effects of 17β - E_2 and/or P_4 to OVX rats for object recognition

A similar pattern of results were observed in experiments investigating effects of administration of ovarian steroids to OVX rats. There was a main effect of 17β - E_2 and/or P_4 treatment $F(3, 57) = 3.44$, $p = 0.02$. Rats administered 17β - E_2 (2.7 ± 0.4 s with novel object vs. 1.2 ± 0.3 s with familiar object), P_4 (3.8 ± 0.6 s with novel object vs. 1.8 ± 0.4 s with familiar object), or 17β - E_2 and P_4 (2.7 ± 0.3 s with novel object vs. 0.9 ± 0.2 s with familiar object) spent a significantly greater percentage of time exploring the novel object compared to rats administered vehicle (2.3 ± 0.7 s with novel object vs. 0.8 ± 0.2 s with familiar object; Fig. 3).

3.2.1. E_2 and progestin levels produced in OVX rats by E_2 and/or P_4 administration—

Results of radioimmunoassay demonstrate that serum, cortex, and hippocampal levels of E_2 and progestins were increased to a physiological range 4 h after training and post-training E_2 and/or P_4 injections, when rats were tested and tissues were collected (Table 2). E_2 levels were significantly increased in serum $F(4, 9) = 5.74$, $p = 0.01$, hippocampus $F(4, 23) = 22.31$, $p = 0.01$, and cortex $F(4, 23) = 2.65$, $p = 0.05$ of rats administered E_2 or $E_2 + P_4$ compared to rats administered vehicle or progestins. Cortical P_4 $F(4, 23) = 3.28$, $p = 0.03$ and 3α , 5α -THP $F(4, 23) = 28.12$, $p = 0.01$ and hippocampal P_4 $F(4, 23) = 4.33$, $p = 0.01$, and 3α , 5α -THP $F(4, 23) = 16.12$, $p = 0.01$ levels were significantly increased by $E_2 + P_4$ or P_4 , compared to E_2 or vehicle, administration to OVX rats. There was a tendency for serum levels of P_4 to be different among groups $F(4, 9) = 3.16$, $p = 0.07$, such that rats administered $E_2 + P_4$ or P_4 had higher serum P_4 levels compared to all other groups. Although not statistically different, rats administered E_2 , $E_2 + P_4$, or P_4 , had higher serum 3α , 5α -THP levels than did rats administered vehicle.

3.3. Experiment 3—Effects of 17β - E_2 or SERMs to OVX rats for object recognition

There was a main effect of estrogen treatment $F(3, 56) = 4.01$, $p = 0.01$. Rats administered 17β - E_2 (3.8 ± 0.9 s with novel object vs. 2.0 ± 0.5 s with familiar object), PPT (4.3 ± 0.8 s with novel object vs. 2.7 ± 0.7 s with familiar object), or DPN (5.5 ± 1.2 s with novel object vs. 2.7 ± 0.7 s with familiar object) spent a significantly longer percentage of time with the novel object compared to rats administered vehicle (2.7 ± 0.7 s with novel object vs. 3.1 ± 0.5 s with familiar object; Fig. 4).

3.4. Experiment 4—Effects of Progestins to OVX rats for object recognition

There was a main effect of progestin treatment $F(2, 36) = 4.77$, $p = 0.02$ for object recognition task performance. Administration of P_4 (2.4 ± 0.5 s with novel object vs. 1.2 ± 0.2 s with familiar object) or 3α , 5α -THP (2.4 ± 0.5 s with novel object vs. 1.7 ± 0.6 s with familiar object) significantly increased the percentage of time spent with the novel object compared to vehicle-administration (1.5 ± 0.5 s with novel object vs. 1.5 ± 0.4 s with familiar object; Fig. 5).

3.4.1. Progestin levels in OVX rats administered P_4 or 3α , 5α -THP—Results of radioimmunoassay demonstrate that serum, cortex, and hippocampal levels of progestins were increased to a physiological range 4 h after training and post-training P_4 (as described above in Section 3.2.1) and 3α , 5α -THP injections, when rats were tested and tissues were

collected (Table 2). Administration of 3 α , 5 α -THP increased cortical $F(4, 23) = 28.12$, $p < 0.01$ and hippocampal $F(4, 23) = 16.12$, $p < 0.01$, 3 α , 5 α -THP levels compared to vehicle or E_2 administration. Rats administered 3 α , 5 α -THP had the highest cortical concentration of 3 α , 5 α -THP compared to all other groups. Although not statistically different, rats administered 3 α , 5 α -THP had higher serum 3 α , 5 α -THP levels than did rats administered vehicle.

3.5. Experiment 5—Effects of 1 h delay in post-training E_2 or P_4 administration to OVX rats for object recognition

There was no main effect of hormone treatment, when rats were administered the drug 1 h after training was completed ($p = 0.90$). The percentage of time spent with the novel object was similar in rats administered vehicle (3.0 ± 0.9 s with novel object vs. 1.7 ± 0.5 s with familiar object), E_2 (4.0 ± 0.8 s with novel object vs. 2.8 ± 0.8 s with familiar object), P_4 (3.5 ± 0.6 s with novel object vs. 1.5 ± 0.5 s with familiar object), or $E_2 + P_4$ (4.6 ± 0.6 s with novel object vs. 2.4 ± 0.7 s with familiar object) 1 h post-training (Fig. 6).

4. Discussion

These results supported our hypothesis that estrogens and/or progestins from endogenous sources can have beneficial effects to enhance object recognition performance coincident with increasing endogenous steroid levels in serum, cortex, and hippocampus. Regression analyses suggest that E_2 levels in the hippocampus and/or 3 α , 5 α -THP levels in the cortex or hippocampus may be particularly important for the observed effects. Administration of these steroids immediately post-training in concentrations that produced similar physiological levels as were observed in behavioral estrous rats enhanced object recognition performance. ER ligands with selective affinity for ER α , ER β , or both increased performance in the object recognition task compared to vehicle-administration. Furthermore, administration of E_2 and/or P_4 1 h after training did not alter performance of OVX rats, suggesting that there is a critical time frame in which steroids must be present in order for observed beneficial effects to occur. Thus, these data support a role of E_2 and progestins having effects in the cortex and hippocampus to enhance object recognition.

The present results that behavioral estrous rats and OVX rats administered $E_2 + P_4$, both of which had physiological E_2 and P_4 levels, had better performance in the object recognition task confirm previous reports of the beneficial effects of physiological E_2 and/or P_4 levels in the hippocampus for cognitive performance. Administration of SC E_2 regimen that typically produces physiological, proestrous-like serum levels of E_2 in OVX mice (Edwards, 1970; Morgan, Schulkin, & Pfaff, 2004) enhances performance in the water maze of aged mice and increases hippocampal E_2 , synaptophysin, and BDNF levels compared to aged mice administered vehicle, which had hippocampal E_2 levels at nadir (Frick, Fernandez, & Bulinski, 2002; Frye, Rhodes, & Dudek, 2005). In the inhibitory avoidance task, a task that is considered to be mediated by the hippocampus and amygdala, naturally receptive rats perform better than do non-receptive rats when rats are tested 4 h later (Rhodes & Frye, 2004a). Notably, the present study, investigating effects of estrous cycle differences and effects of exogenous E_2 or progestin administration (that produces similar circulating and central E_2 , P_4 , and 3 α , 5 α -THP levels), suggests that ovarian steroids may enhance performance in the object recognition task, a task that relies on the functioning of the cortex and the hippocampus. Furthermore, these data suggest that there may be a relationship between levels of E_2 in the hippocampus, but not the cortex, and 3 α , 5 α -THP in the hippocampus and cortex and performance of rats in the object recognition task. Higher cortical levels of E_2 appear to be disruptive to performance among cycling females in this task, which may be related to regimen-dependent effects of E_2 , as already discussed. Although it is not evident from these data how E_2 and 3 α , 5 α -THP may be interacting to

produce these functional effects, E_2 can increase production of 3α , 5α -THP by altering activity of enzymes necessary for the metabolism of P_4 to 3α , 5α -THP and this may underlie the observed effects (Cheng & Karavolas, 1973; Malendowicz, 1976; Resko et al., 1986). Administration of a lower dose of E_2 (2 μ g) than was used in this study, increases 3α , 5α -THP levels in the hippocampus of OVX rats (Frye & Rhodes, 2005). Together, these data suggest a role of these steroids in the cortical-hippocampal circuitry underlying performance in this non-spatial, working memory task.

The present study confirms and extends previous reports on the effects of estrogens on object recognition memory and putative central mechanisms for these effects. Oral administration of E_2 to middle-aged mice enhances object recognition (Fernandez & Frick, 2004). In the present study, similar effects were observed in rats administered estrogens with different affinity for ER α and ER β . 17β - E_2 , which is a ligand for both ER α and ER β , produced consistent effects across experiments to enhance performance in the object recognition task when it was administered post-training compared to that observed in OVX, vehicle-administered rats. Furthermore, rats administered the ER β -selective ligand, PPT, or the ER α -selective ligand, DPN, had similar increases over that observed with vehicle-administration in the percentage of time spent exploring the novel object. We and others have demonstrated that cognitive enhancing effects may occur through actions at ER α and/or ER β using specific ligands for these receptors or knockout mice (Fugger et al., 2000; Gibbs et al., 2004; Luine et al., 2003; Lund & Lephart, 2001; Rhodes & Frye, in press; Rissman et al., 2002). The present study utilized post-training administration of 17β - E_2 in higher dosages than that observed to enhance object recognition in another study (Luine et al., 2003). It may be that higher dosages of these SERMs could eliminate the specificity of these ligands for ER α vs. ER β and this would be an important distinction to determine in future studies. The present results that 17β - E_2 , DPN, or PPT enhance object recognition performance confirm that actions at ER α and/or ER β may enhance cognitive performance.

The ability of progestins to modulate cognitive performance of female rodents was confirmed by the present study and others investigating the effects of progestins. In young rodents, evidence suggests that P_4 administration can have beneficial effects on cognitive performance (Sandstrom & Williams, 2001; Sato et al., 2004; Vongher & Frye, 1999). Furthermore in situations associated with degeneration, P_4 can enhance T-maze performance (Dohanich et al., 1994). In aged rats, with low endogenous progestin levels, the beneficial effects of P_4 in various tasks are less clear owing to the fact that basal levels of P_4 have not been addressed (as reviewed in Arnaiz et al., 2004; Bimonte-Nelson, Singleton, Williams, & Granholm, 2004; Blank, Nijholt, Kye, Radulovic, & Spiess, 2003; Kiray, Uysal, Sonmez, Acikgoz, & Gonenc, 2004; McEchron, Cheng, & Gilmartin, 2004; Warren & Juraska, 2000). It may be that beneficial effects of progestins to enhance cognitive performance depend on both ligand availability and/or activity of metabolism enzymes, both of which are different in the aging population (reviewed in, Chakraborty & Gore, 2004). Indeed, our results suggest that enhancing 3α , 5α -THP levels with SC administration of 3α , 5α -THP increases both P_4 and 3α , 5α -THP levels in the hippocampus (Table 2). Although the P_4 antibody used cross-reacts with 3α , 5α -THP and dihydroprogesterone (less than 4%) and has negligible binding to other pregnane and cortico-steroids, effects of other progestins as a source of increases in P_4 cannot be entirely ruled out (Purdy et al., 1990). A possibility more likely than artifactual increases in P_4 associated with radioimmunoassay is that P_4 was increased in the hippocampus due to enhanced de novo synthesis. Indeed, it has been demonstrated that exposure to a novel open field for behavioral testing can increase de novo production of progestins (Frye & Bayon, 1999; Frye & Rhodes, 2006). Alternatively, increased P_4 levels in the hippocampus following 3α , 5α -THP administration could be due to back-conversion of 3α , 5α -THP to its precursors. However, this is unlikely given that 3α , 5α -THP can be oxidized to dihydroprogesterone, but dihydroprogesterone does not typically

get back-converted to P_4 (as reviewed in Martini, Celotti, & Melcangi, 1996). Another point to consider in interpretation of these data is that progestins' trophic effects may confer a benefit and underlie enhanced cognitive performance. Indeed, our laboratory and others have shown that progestins, in particular 3α , 5α -THP, can have neuroprotective/trophic effects in the hippocampus in several models of neurodegeneration (Ciriza, Azcoitia, & Garcia-Segura, 2004; Djebaili, Hoffman, & Stein, 2004, 2005; Frye & Rhodes, 2005; Frye & Scalise, 2000; Garcia-Estrada, Del Rio, Luquin, Soriano, & Garcia-Segura, 1993; He, Hoffman, & Stein, 2004a; He, Evans, Hoffman, Oyesiku, & Stein, 2004b; Rhodes & Frye, 2004b; Rhodes, McCormick, & Frye, 2004; Vongher & Frye, 1999). Together, the present data extend previous findings on the role of progestins to suggest that enhanced performance in the object recognition task may be related to levels of 3α , 5α -THP in the cortex and hippocampus.

One criticism of this study may be that having hormones present during testing may have contributed to other behavioral changes in rodents that masked the potential mnemonic effects of these compounds. We used a 4 h-inter-trial interval in this study. This testing procedure was utilized so that rats would be in the same estrous cycle phase when trained and tested in Experiment 1. When steroids were systemically administered to rats immediately after training, we found similar levels of steroids that were within a physiological range at testing time (4 h later) were produced concomitant with increased percentage of time spent exploring the novel object. We used this post-training steroid administration procedure to minimize potential effects of steroids interfering with training. We also investigated whether there was a critical time frame in which steroids had to be acting with the inclusion of Experiment 5. In this experiment, rats were administered vehicle or steroid hormone 1 h after training (i.e., after consolidation of memory occurs) and were tested 4 h after training. Although rats had similar levels of these steroids at testing in this experiment as in other experiments, performance in this task was not enhanced with a 1 h delay in post-training administration. Thus, these data suggest that ovarian steroids may have effects to enhance cognitive performance in this task that can be parsed out from their other effects during training.

There are other critical factors to consider when interpreting effects of estrogens and progestins for cognitive performance. Given that these effects have been investigated more thoroughly for estrogens (as reviewed in Korol, 2004), specific examples of each are discussed as follows, but similar factors likely influence effects of progestins for cognitive performance. These include the timing of E_2 administration, E_2 regimen, type of E_2 administered, and factors related to the history of the individual. First, effects of E_2 may be clearer when E_2 is administered after training in a learning task, because this precludes effects of E_2 interfering with training. E_2 has effects on motor behavior (Joyce & Van Hartesveldt, 1984; Morgan & Pfaff, 2001, 2002), and these effects could alter training. Second, E_2 's effects may also be concentration-dependent. There is a biphasic relationship between E_2 and cognitive performance, such that beneficial effects are clearest when E_2 levels are low and physiological (Frye & Rhodes, 2002; Gibbs, 1997; Gibbs, Burke, & Johnson, 1998; Luine et al., 1998; Wide, Hanratty, Ting, & Galea, 2004). Whereas, high levels and concentrations at nadir can be disruptive. Indeed, in the present study, higher cortical levels of E_2 appear to be disruptive to performance among cycling females in this task, which may be related to these regimen-dependent effects of E_2 . Third, the type of E_2 will also alter its effects. For example, conjugated equine estrogens that are typically used in hormone therapies for postmenopausal women are primarily composed of estrone, rather than the neuroactive E_2 , 17β - E_2 . We, and others, have demonstrated that various SERMs alter cognitive performance (Garey, Morgan, Frohlich, McEwen, & Pfaff, 2001; Gibbs et al., 2004; Luine et al., 2003; Lund & Lephart, 2001; Rhodes & Frye, in press). Specifically, as in the present study, both $ER\alpha$ and $ER\beta$ -specific SERMs have been shown to enhance

performance in the object recognition task (Luine et al., 2003). Fourth, an individual's history, such as environment and/or the duration of time spent in an E_2 -deficient state, may alter the response to subsequent E_2 exposure. In one study, long-term (6+ months) environmental enrichment, compared to standard housing, attenuated the effects of E_2 to enhance object memory, and impaired working memory in the water radial arm maze (Gresack & Frick, 2004). Length of time in an E_2 -deficient state before E_2 is reintroduced may also alter response to exogenous E_2 . For instance, long-term treatment with E_2 and/or P_4 was only effective at enhancing acquisition in the delayed matching-to-position tasks, when initiated 3 months, but not 10 months, post-OVX (Gibbs, 2000). Thus, there are many factors that can contribute to the effects of ovarian steroids for cognitive performance.

Further characterization of ovarian steroids' effects and putative mechanisms for cognitive performance are important for greater understanding of how estrogens and progestins may act in concert in brain areas, such as the PFC and hippocampus, to produce functional cognitive effects. For instance, our regression analyses suggest that the line of best fit explains only some of the unexplained error (i.e., 23% for 3α , 5α -THP in the cortex, 36% for E_2 in the hippocampus, and 46% for 3α , 5α -THP in the hippocampus). These results suggest that there are other variables strongly affecting performance in the object recognition task. Notably, other steroids that alter cognitive performance, such as glucocorticoids and androgens, may have produced some of this variance (as reviewed in Rhodes & Frye, 2002). Future studies could investigate effects of glucocorticoids and/or androgens as well as the mechanisms of E_2 and P_4 for modulation of learning and memory. There is an extensive literature on the pharmacology of memory consolidation, which supports interplay between GABAergic, glutamatergic, noradrenergic, dopaminergic, and serotonergic systems and their downstream signal transduction pathways in the hippocampus (as reviewed in Izquierdo & Medina, 1997). Other studies using cognitive performance or sexual receptivity as behavioral assays have demonstrated the importance of ovarian steroids' effects through these pathways, which may also underlie the effects observed in the present study. For instance, E_2 , which can be pro-excitatory, enhances NMDA receptor function for some of its cognitive performance effects (Bi, Foy, Vouimba, Thompson, & Baudry, 2001; El-Bakri et al., 2004; Gureviciene et al., 2003). P_4 's effects to enhance sexual receptivity of female rodents requires its metabolism to 3α , 5α -THP in the ventral tegmental area and its subsequent actions at GABA_A receptors (reviewed in Frye, 2001; Frye et al., 2006). Future studies could investigate these and downstream pathways that underlie the effects of ovarian steroids to enhance object recognition.

In summary, this study demonstrated that administration of 17β - E_2 and/or P_4 , or 3α , 5α -THP to OVX rats produced similar results to enhance performance in the object recognition task and increase levels of these steroids in serum, the cortex, or hippocampus as was observed in behavioral estrous rats. Further, administration of ER α - or β -specific ligands similarly enhanced performance, suggesting involvement of these receptors for these effects. Lastly, similar behavioral responses of rats administered vehicle, 17β - E_2 , P_4 , or 17β - E_2 and P_4 1 h after training suggest that these ovarian steroids need to be present during memory consolidation for their beneficial effects for object recognition to occur. Thus, these data suggest that ovarian steroids may have actions in the cortex and/or hippocampus to enhance object recognition memory.

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Fig. 1. Percentage of time spent exploring the novel object during testing of intact, cycling rats. Rats in behavioral estrus ($n = 6$) spent a significantly higher percentage of time exploring the novel object than did estrous ($n = 7$) or diestrous rats ($n = 5$; **). Estrous rats spent a significantly higher percentage of time exploring the novel object than did diestrous rats (*).

Fig. 2.

Scatter plots depicting regression analyses of cortical and hippocampal E_2 , P_4 , and 3α , 5α -THP concentrations and the percentage of time spent by cycling rats exploring the novel object. Black circles represent data of behavioral estrous rats. Gray circles represent data of estrous rats. White circles represent data of diestrous rats.

Fig. 3.

Percentage of time spent exploring the novel object during testing of OVX, hormone-primed rats. Rats administered 17β -E₂ ($n = 14$), P₄ ($n = 16$), or 17β -E₂ + P₄ ($n = 16$) immediately post-training had a significantly higher percentage of time exploring the novel object than did rats administered vehicle ($n = 15$; *).

Fig. 4. Percentage of time spent exploring the novel object during testing of OVX rats administered ER ligands. Rats administered 17β -E₂, the ER α ligand (PPT), the ER β ligand (DPN) immediately post-training had a significantly higher percentage of time exploring the novel object than did rats administered vehicle ($n = 15/\text{group}$; *).

Fig. 5. Percentage of time spent exploring the novel object during testing of OVX, progestin-administered rats. Rats administered P_4 ($n = 14$) or $3\alpha, 5\alpha$ -THP ($n = 14$) immediately post-training had a significantly higher percentage of time exploring the novel object than did rats administered vehicle ($n = 11$; *).

Fig. 6. Percentage of time spent exploring the novel object during testing of OVX rats administered 17β -E₂ ($n = 5$), P₄ ($n = 6$), or 17β -E₂ + P₄ ($n = 5$), or vehicle ($n = 7$) 1 h after training. All rats in this experiment had similar percentages of time exploring the novel object that were not above chance levels.

Serum, cortical, and hippocampal (hippo) concentrations of E_2 , P_4 , and 3 α , 5 α -THP, when rats in different endogenous hormonal milieu are tested in the object recognition task

Table 1

Condition	Estrogen levels		Progesterone Levels				3 α , 5 α -THP levels			
	Serum (pg/ml)	Cortex (ng/ml)	Hippo (ng/mg)	Serum (ng/ml)	Cortex (ng/g)	Hippo (ng/g)	Serum (ng/ml)	Cortex (ng/g)	Hippo (ng/g)	Hippo (ng/g)
Diestrus	4.9 $\pm$ 1.2	6.0 $\pm$ 0.8	2.1 $\pm$ 0.5	5.3 $\pm$ 1.6	2.6 $\pm$ 1.2	9.1 $\pm$ 4.5	1.2 $\pm$ 2.00	4.7 $\pm$ 0.5	3.2 $\pm$ 0.3	
Behavioral Estrus	19.1 $\pm$ 1.9 ^{a,b}	3.8 $\pm$ 0.8	4.9 $\pm$ 0.8 ^a	21.1 $\pm$ 1.9 ^{a,b}	12.9 $\pm$ 2.6 ^a	27.3 $\pm$ 7.2	11.5 $\pm$ 0.9 ^a	9.6 $\pm$ 1.4 ^a	13.7 $\pm$ 0.8 ^{a,b}	
Estrus	16.6 $\pm$ 0.4	4.1 $\pm$ 0.5	3.2 $\pm$ 0.5	12.8 $\pm$ 1.2 ^a	9.0 $\pm$ 2.8	18.9 $\pm$ 3.4	8.7 $\pm$ 0.5	7.3 $\pm$ 0.7	5.3 $\pm$ 1.0	

^aDifferent from diestrus ($p < 0.05$).

^bDifferent from estrus ($p < 0.05$).

Table 2

Serum, cortical, and hippocampal (hippo) concentrations of E_2 , P_4 , and 3 α , 5 α -THP at test-time of OVX rats administered vehicle, E_2 and/or P_4 , or 3 α , 5 α -THP subcutaneously

Condition	Estrogen levels			Progesterone Levels			3 α , 5 α -THP levels		
	Serum (pg/ml)	Cortex (ng/ml)	Hippo (ng/mg)	Serum (ng/ml)	Cortex (ng/g)	Hippo (ng/g)	Serum (ng/ml)	Cortex (ng/g)	Hippo (ng/g)
OVX Vehicle SC	0	0.1 $\pm$ 0.1	0	0.6 $\pm$ 0.6	1.2 $\pm$ 0.1	0.9 $\pm$ 0.3	1.6 $\pm$ 1.6	0.5 $\pm$ 0.1	0.9 $\pm$ 0.3
OVX E_2 SC	17.2 $\pm$ 2.9 ^{a,d,e}	3.0 $\pm$ 1.2 ^{a,e}	1.5 $\pm$ 0.2 ^{a,d,e}	4.2 $\pm$ 1.5	1.3 $\pm$ 0.4	2.7 $\pm$ 1.3	6.1 $\pm$ 4.4	1.3 $\pm$ 0.4	1.2 $\pm$ 0.1 ^{c,d,e}
OVX E_2 + P_4	13.0 $\pm$ 6.7 ^{a,d,e}	1.3 $\pm$ 0.1	1.3 $\pm$ 0.2 ^{a,d,e}	50.9 $\pm$ 18.3	15.4 $\pm$ 5.0 ^{a,b,e}	6.7 $\pm$ 1.3	5.9 $\pm$ 0.5	7.1 $\pm$ 0.5 ^{a,b}	9.3 $\pm$ 1.0 ^{a,b}
OVX P_4 SC	0	1.1 $\pm$ 0.9	0.1 $\pm$ 0.0	59.9 $\pm$ 28.6	9.3 $\pm$ 3.8	18.6 $\pm$ 4.3 ^{a,b,e}	10.2 $\pm$ 2.3	6.7 $\pm$ 0.9 ^{a,b}	9.6 $\pm$ 1.1 ^{a,b}
OVX 3 α , 5 α -THP SC	0	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	2.2 $\pm$ 1.1	6.0 $\pm$ 2.5	14.9 $\pm$ 6.0 ^{a,b}	6.1 $\pm$ 0.6	9.0 $\pm$ 0.9 ^{a,b,c,d}	11.9 $\pm$ 2.1 ^{a,b}

^aDifferent from vehicle (p .05).

^bDifferent from E_2 (p .05).

^cDifferent from E_2 + P (p .05).

^dDifferent from P (p .05).

^eDifferent from THP (p .05).