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Estrogen has mnemonic-enhancing effects in the inhibitory avoidance task

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Abstract

Gonadal hormones, such as estrogen, can alter cognitive performance. The present studies investigated the relationship between performance on the inhibitory avoidance task and endogenous fluctuations in ovarian hormones and estrogen replacement. In Experiment 1, proestrous or diestrous I female, or male, rats were trained in the inhibitory avoidance task. Following a 24-h intertrial delay, when female rats were tested in metestrus or diestrus II, no differences in crossover latencies were observed among groups. In Experiment 2, female rats in proestrus or diestrus I, and male rats, were trained in the inhibitory avoidance task and were tested following a 4-h intertrial delay (so that training and testing were accomplished in the same phase of the cycle). In this paradigm, proestrous rats had significantly longer crossover latencies than did either diestrous I or male rats. Posttraining administration of estrogen, but not progesterone, to ovariectomized rats increased crossover latencies compared to vehicle with a 4-h (Experiment 3) or 24-h (Experiment 4) intertrial delay. In Experiment 5, estrogen administration to ovariectomized rats immediately, but not 1, 2, or 3 h posttraining, increased crossover latencies compared to vehicle. Together, these data suggest that estrogen can have positive mnemonic effects in the inhibitory avoidance task.

Keywords: Learning; Proestrus; Estrogen; Nongenomic; Mnemonic

1. Introduction

Gonadal hormones may have activational effects on cognitive performance. Women taking estrogen replacement following surgical or natural menopause have enhanced performance on tasks of verbal ability compared to women not on estrogen therapy (Janowsky, 2002; Sherwin, 2003; Nappi et al., 1999). Estrogen can also enhance cognitive performance in a population with more profound cognitive deficits. Women with Alzheimer's disease administered estrogen via transdermal patches show significant improvement on a number of cognitive measures compared to age-matched controls with Alzheimer's disease not on estrogen (Asthana et al., 2001). In rodent models, estrogen can enhance cognitive performance as well. Estrogen administration to ovariectomized rats 72 and 48 h prior to training improves performance in the water maze compared to vehicle administration (Sandstrom and Williams, 2001). Acute or chronic estrogen treatment to ovariectomized rats enhances radial arm maze performance compared to vehicle treatment (Luine and Rodriguez, 1994, Luine et al., 1998). Moreover, estrogen's cognitive-enhancing effects have also been observed in aged rats (Markham et al., 2002) and young rats with hippocampal degeneration due to adrenalectomy (Frye, 2001). Together, these data suggest that estrogen can have activational effects to alter cognitive performance.

Although estrogen can have effects to improve cognitive performance in some paradigms, there are reports of decrements or no change in performance associated with endogenous variations in, or replacement with, estrogen. For example, some reports of spatial performance of women suggest that performance is enhanced during the follicular phase of the menstrual cycle, when lower levels of estrogen are present, compared to the luteal phase (Hampson, 1990). Spatial performance of diestrous rats, with low endogenous estrogen, has also been reported to be enhanced (Frye, 1995;

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Warren and Juraska, 1997; Diaz-Veliz et al., 1989) or unchanged (Berry et al., 1997; Singh et al., 1994; Stackman et al., 1997) compared to proestrous rats, with higher endogenous estrogen levels. Additionally, estrogen administration to young ovariectomized rats has been shown to have no effect on spatial reference memory in the water maze task when compared to vehicle administration (Chesler and Juraska, 2000). These data suggest that estrogen can have variable effects on cognitive performance of women and rodents.

The different effects of estrogen on cognitive performance discussed above may be due to several factors. The concentration of estrogen present at training and/or test time may influence estrogen's effects on cognitive performance. Estrogen replacement therapy that produces physiological estrogen concentrations enhances performance on spatial tasks, whereas very high or very low estrogen levels result in decrements in spatial performance (Hogervorst et al., 1999, 2000). Postmenopausal women treated with moderate estrogen levels have significant improvement on tests of verbal, visual, and semantic memory compared to that of postmenopausal women given placebo (Asthana et al., 2001; Linzmayer et al., 2001). Rats or voles with low physiological levels of estrogen have better performance in maze tasks compared to rats with high physiological levels of estrogen (Galea et al., 1995; Holmes et al., 2002). Additionally, ovariectomized rats administered estrogen regimen that produces moderate physiological estrogen levels have better performance than do rats administered estrogen regimen that produces low physiological estrogen levels or vehicle (Bimonte and Denenberg, 1999). These data support a curvilinear effect of estrogen on spatial performance. It should be noted, however, that other gonadal hormones, which also fluctuate across the estrous cycle, such as progestins and androgens, have also been demonstrated to alter cognitive performance (Frye and Lacey, 2000, 2001; Frye and McCormick, 2000; Diaz-Veliz et al., 1994).

The demand characteristics of the task utilized may also influence estrogen's effects on cognitive performance. First, motor demand of the task examined may alter estrogen's effects on performance. It is well known that rats in proestrus exhibit increased motor behavior compared to rats in other phases of the estrous cycle (Beatty, 1992; Frye et al., 2000). Tasks that require more gross motor responses may be more difficult for proestrous rats to show optimal performance because the adaptive response in such a situation may be to further increase motor behavior, which then makes proestrous rats perform poorly due to the additional load on motor function. Notably, physiological regimen of estrogen can improve performance in tasks without a high motor demand. For example, in the hippocampal-dependent, classical eye-blink conditioning task, performance is better in proestrus compared to other phases of the cycle (Shors et al., 1998; Wood et al., 2001). Second, stress load associated with some cognitive tasks may also alter estrogen's effects on cognitive performance. Indeed, prior exposure to the test apparatus, which may be adequate to reduce the stress load of a task, can alter subsequent performance. Proestrous rats that have pretraining exposure to the water maze have enhanced performance compared to rats tested in other phases of the estrous cycle (Healy et al., 1999); pretraining in the water maze affects has been shown to affect subsequent performance of male (Bannerman et al., 1995) or female (Frye, 1995) rats. As well, the aversive versus appetitive nature of the task being utilized may influence hormonal modulation of spatial performance. For example, the water maze task, which is widely used to assess spatial performance, may be an especially aversive task for rodents (Hodges, 1996). However, using tasks that involve more appetitive stimuli, such as food reward, is problematic because estrogen can alter food intake (Hrupka et al., 2002). The effects of increased motor behavior in proestrus may be compounded by tasks that have high motor demand and/or are particularly stressful, which may result in detrimental performance of rats.

The present studies were designed to address these factors so that estrogen's mnemonic effects could be investigated. First, based on prior reports, estrogen's performance-enhancing effects may be observed more readily when low or moderate estrogen levels are present. Thus, effects of endogenous variations in, or replacement with, physiological estrogen regimen were examined. Second, estrogen's known effects on motor behavior may influence its effects on cognitive performance. For this reason, we utilized the inhibitory avoidance task, which is a low motor demand task. Indeed, in order for rats to have increased crossover latencies (an indication of learning), motor activity must be suppressed. Thus, the inhibitory avoidance task is a valuable cognitive task in which to examine estrogen's mnemonic effects. Finally, estrogen can alter stress responsiveness. Indeed, there are differences in response to aversive stimuli across the estrous cycle and following estrogen administration (Frye et al., 1993). Thus, in our extirpation and replacement studies, a posttraining estrogen regimen was utilized to minimize effects of estrogen on stress responses to the shock stimulus, and other possible confounds. Estrogen may have mnemonic effects that can be masked by other factors. Thus, the present experiments were designed to test the hypothesis that estrogen can have mnemonic effects in the inhibitory avoidance task.

2. Methods

These methods were preapproved by the Institutional Animal Care and Use Committee at the University at Albany-SUNY.

2.1. Animals and housing

Female ($n = 135$) and male ($n = 30$) Long–Evans rats were bred and raised in the animal facility at SUNY-Albany

from stock obtained from Taconic Farms (Germantown, NY). Rats were housed in groups of four in polycarbonate cages (45 × 24 × 21 cm) in a temperature-controlled room (21 ± 1 °C) in the Laboratory Animal Care Facility. The rats were maintained on a 12:12-h reversed light cycle (lights off 8:00 a.m.) with continuous access to Purina Rat Chow and tap water in their home cages.

2.2. Estrous cycle determination (Experiments 1 and 2)

Samples of vaginal epithelium were obtained daily by lavage approximately 1 h prior to lights off and were immediately examined using low-power light microscopy. Rats that had vaginal epithelium characterized by large round nucleated cells that are associated with proestrus (Long and Evans, 1922) were subsequently manually palpated (1–2 h after lights out) thrice to determine lordosis responsiveness (Hardy and DeBold, 1971). Rats were considered in proestrus if they had nucleated vaginal epithelium and demonstrated lordosis responses on at least one of three palpations. Rats in proestrus were trained 4–5 h after lights out. Based upon our previous data (Frye and Bayon, 1999; Vongher and Frye, 1999), this is when estrogen levels are moderate (26.9 ± 7.3 pg/ml) and prior to the peak in progesterone concentrations (15.2 ± 7.3 ng/ml).

Rats that had been in proestrus on the previous day and had vaginal epithelium that contained cornified cells were considered in estrus. Rats that responded to manual palpation uniformly with lordosis ratings of 0 and had vaginal epithelium containing neither nucleated cells nor cornified cells were considered in metestrus, diestrus I, or diestrus II, depending upon the previous and subsequent occurrence of proestrus and estrus.

2.3. Surgery and hormone replacement (Experiments 3, 4, and 5)

Rats were anesthetized with Rompun (12 mg/kg) and Ketaset (80 mg/kg) and were ovariectomized 1 week prior to behavioral testing. Ovariectomized rats were subcutaneously administered vehicle (sesame oil, 0.2 cc), progesterone (500 μg/0.2 cc), or estradiol benzoate (10 μg/0.2 cc) posttraining in the inhibitory avoidance task. This estrogen regimen produces physiological levels of circulating estrogen 4 h (21 ± 8 pg/ml) and 24 h (45 ± 8 pg/ml) following administration. The progesterone regimen produces physiological levels of circulating progesterone 4 h following administration (46 ± 1 ng/ml; Vongher and Frye, 1999).

2.4. Inhibitory avoidance task

The step-through inhibitory avoidance procedure was used to assess hippocampal-dependent memory (Bianchin et al., 1994). The apparatus consisted of a two-compartment (24 × 18 × 19 cm each) stainless steel box similar to that described by Venault et al. (1987). One chamber was brightly lit from above and painted white. The other was painted black and covered to block out light. A sliding door separated the two chambers.

Habituation and training—Rats were placed in the white chamber; after 5 s, the door was lifted and rats were allowed to explore the entire box for 2 min. Twenty minutes later, rats were placed in the white chamber for 5 s or until they faced the door. When the door was lifted, the rats entered the black compartment and the door was closed behind them. Rats then received a mild shock (0.25 mA, 2-s duration).

Testing—Rats were placed in the white chamber for 5 s. The door was lifted and the latency to enter the dark chamber was recorded (300 s maximum).

2.4.1. Experiment 1

In Experiment 1, proestrous ($n = 13$), diestrous I ($n = 13$), or male ($n = 15$) rats were trained in the inhibitory avoidance task. Twenty-four hours following training, rats were tested for crossover latencies.

2.4.2. Experiment 2

In Experiment 2, proestrous ($n = 16$), diestrous I ($n = 14$), or male ($n = 15$) rats were trained in the inhibitory avoidance task. Four hours following training, rats were tested for crossover latencies.

2.4.3. Experiment 3

Ovariectomized rats in Experiment 3 were trained in inhibitory avoidance and administered subcutaneously with vehicle ($n = 10$), estradiol benzoate ($n = 10$), or progesterone ($n = 10$) immediately posttraining. Four hours following training, rats were tested for crossover latencies.

2.4.4. Experiment 4

Ovariectomized rats in Experiment 4 were trained in inhibitory avoidance and administered subcutaneously with vehicle ($n = 8$), estradiol benzoate ($n = 8$), or progesterone ($n = 8$) immediately posttraining. Twenty-four hours following training, rats were tested for crossover latencies.

2.4.5. Experiment 5

Ovariectomized rats in Experiment 5 were trained in inhibitory avoidance and subcutaneously administered vehicle ($n = 5$) or estradiol benzoate immediately ($n = 5$), 1 ($n = 5$), 2 ($n = 5$), or 3 ($n = 5$) h posttraining. Twenty-four hours following training, rats were tested for crossover latencies.

2.5. Statistical analyses

One-way analyses of variance (ANOVAs) were used to examine effects of estrogen to increase crossover latencies on test day in the inhibitory avoidance task. Where appropriate, ANOVAs were followed by Fisher's Least Significant Difference post hoc tests to determine differences among groups. Alpha level for the determination of statis-

tical significance was $P < .05$. In the absence of significance with an overall omnibus ANOVA, t tests were used to examine whether estrogen increased crossover latencies compared to vehicle (Experiment 4).

3. Results

No differences among the groups were observed on training day crossover latencies. Thus, the results presented are crossover latencies on test day. Notably, a similar pattern of effects was observed in each experiment when a difference score was calculated (i.e., test day latency – training day latency).

3.1. Experiment 1

There was no significant difference in test day crossover latencies among the groups when tested 24 h posttraining [$F(2,40) = 0.38$, $P = .69$]. Crossover latencies on test day did not differ for females trained in proestrus and tested in metestrus (37 ± 12 s), females trained in diestrus I and tested in diestrus II (61 ± 38 s), or male rats (33 ± 11 s).

3.2. Experiment 2

Crossover latencies in the inhibitory avoidance task on test day differed significantly among groups that were tested 4 h following training [$F(2,42) = 4.01$, $P = .03$]. Rats trained and tested in proestrus had significantly longer crossover latencies than did either diestrous or male rats (see Fig. 1).

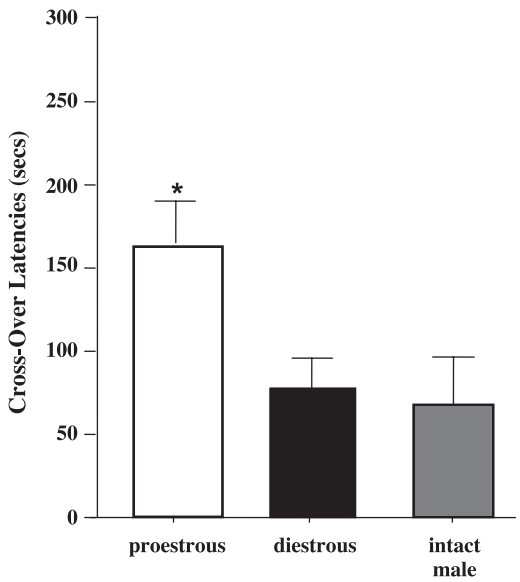

Fig. 1. Represents crossover latencies of female rats trained in proestrus (open bar; $n = 16$) or diestrus (black bar; $n = 14$), or male rats (striped bar; $n = 15$) and tested 4 h later. * Indicates significant difference from all other groups at $P < .05$.

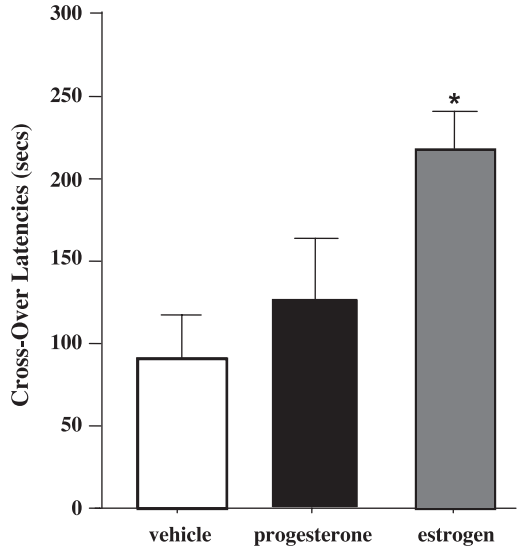

Fig. 2. Represents crossover latencies of ovariectomized rats administered vehicle (open bar; $n = 10$), progesterone (black bar; $n = 10$), or estrogen (striped bar; $n = 10$) immediately posttraining and tested 4 h later. * Indicates significant difference from all other groups at $P < .05$.

3.3. Experiment 3

There were significant differences in crossover latencies of hormone-primed rats tested 4 h posttraining [$F(2,27) = 3.83$, $P = .03$]. Rats administered estrogen immediately posttraining and tested 4 h later had significantly longer crossover latencies than did rats administered vehicle or progesterone (see Fig. 2).

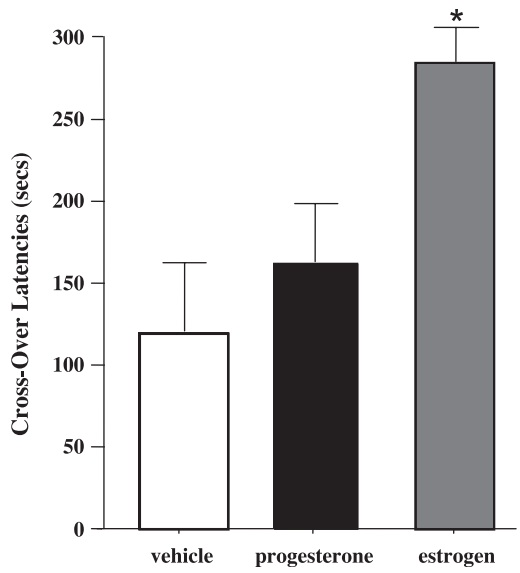

Fig. 3. Represents crossover latencies of ovariectomized rats administered vehicle (open bar; $n = 10$), progesterone (black bar; $n = 10$), or estrogen (striped bar; $n = 10$) immediately posttraining and tested 24 h later. * Significantly different from vehicle.

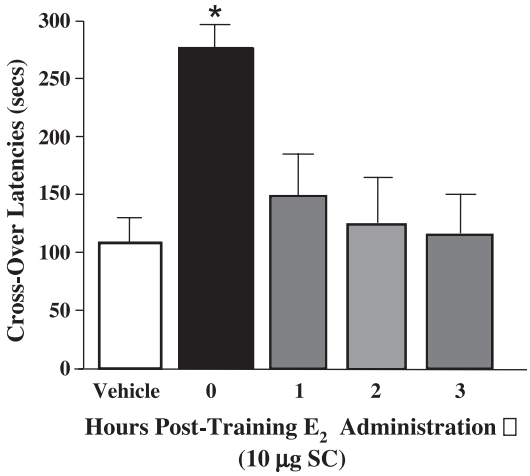

Fig. 4. Represents crossover latencies of ovariectomized rats administered vehicle (open bar; $n=5$) or estrogen immediately (black bar; $n=5$), 1 h (diagonally striped bar; $n=5$), 2 h (vertically striped bar; $n=5$), or 3 h (gray bar) posttraining and tested 24 h later. * Indicates significant difference from all other groups at $P<.05$.

3.4. Experiment 4

Two-group comparisons revealed that estrogen administration increased crossover latencies 24 h after training. Rats administered estrogen immediately posttraining and tested 24 h later had significantly longer crossover latencies than did rats administered vehicle [$t(14)=-2.77$, $P=.02$; see Fig. 3]. There were no differences in crossover latencies of rats administered vehicle or progesterone [$t(14)=-0.75$, $P=.47$].

3.5. Experiment 5

Crossover latencies of rats administered estrogen at different time points following training were significantly different among the groups [$F(4,20)=1.39$, $P=.01$]. Crossover latencies of rats administered estrogen immediately, but not 1, 2, or 3 h posttraining, had significantly longer crossover latencies than did vehicle-administered rats (see Fig. 4).

4. Discussion

Results of the present studies support our hypothesis that estrogen has mnemonic effects in the inhibitory avoidance task. First, proestrous rats had increased crossover latencies, when trained and tested in proestrous. Second, posttraining administration of estrogen increased crossover latencies. Finally, delayed posttraining administration of estrogen was not effective at increasing crossover latencies. These data suggest that physiological regimen of estrogen administered within 1 h of training can have mnemonic effects in the inhibitory avoidance task 24 h later.

Data from the present studies are consistent with previous reports in both the clinical and basic research literature that estrogen can enhance cognitive performance. Estrogen-replacement therapy can enhance cognitive performance of women, with and without severe cognitive dysfunction (Sherwin, 2003; Asthana et al., 2001; Nappi et al., 1999). Estrogen administration to ovariectomized rats improves performance in several memory tasks (Frye, 2001; Gibbs, 1999, 2000; Frye and Rhodes, 2002; Bimonte and Denenberg, 1999; Korol and Kolo, 2002; Sandstrom and Williams, 2001; Daniel et al., 1997; Dohanich et al., 1994; Fader et al., 1999; Fugger et al., 2000; Luine et al., 1998; O'Neal et al., 1996). Thus, our present data from Experiment 2 that proestrous rats have longer crossover latencies compared to diestrous or male rats suggest that levels of estrogen present at training and testing may be important for estrogen's enhancing mnemonic effects.

Notably, there was a striking difference in results of Experiments 1 and 2. One possible explanation for the lack of a difference in performance between groups in Experiment 1 may have been due to the longer intertrial delay (24 h versus 4 h in Experiment 2), making the task too difficult for the animals to learn. However, previous investigations from our laboratory and data from subsequent experiments suggest that rats are readily able to learn the association between the dark compartment and a mild shock, and to retain this information for 24 h. A more plausible explanation for the disparate results between Experiments 1 and 2 involves state-dependent learning. In Experiment 1, females were trained in one phase of the cycle, but tested in another. Thus, their inability to learn the task when trained and tested during different phases of the cycle may reflect some component of state-dependent learning (Reus et al., 1979). Retention of a task may be modulated by alteration of an animal's internal state. An animal trained while under the influence of a particular drug will not perform well if testing occurs after the drug has worn off or if another drug is injected (Overton, 1964; Reus et al., 1979). However, an animal trained following administration of a drug and tested under the influence of the same drug performs similarly to nondrugged rats (Overton, 1964). Thus, to retrieve information, it may be necessary for the central nervous system to be in the same physiological state as during acquisition (Overton, 1964). Thus, the present experiments suggest that gonadal hormones may produce state-dependent learning effects, similar to those produced by psychoactive drugs (Gray, 1975; Overton, 1964; Gauvin et al., 2001; Nishimura et al., 1990; Stewart et al., 1967).

That estrogen may have state-dependent effects to alter learning should not be surprising given that estrogen has been demonstrated to alter affective states in both the clinical and animal literature. Indeed, in animal models of anxiety, anxiolysis is associated with the proestrous phase of the cycle, when estrogen levels are physiological (Diaz-Veliz et al., 1997; Frye et al., 2000; Marcondes et al., 2001). As well, administration of estrogen to ovariectomized rats

reduces anxiety behavior (Frye and Walf, 2004; Young et al., 2001). Thus, dissociating estrogen's mnemonic effects from its effects on anxiety is an important area of estrogens' effects that requires further investigation.

Our current findings also extend previous reports of estrogen's effects on cognition. The few investigations of effects of posttraining estrogen in cognitive tasks have reported enhancing effects on learning (Packard, 1998; Packard and Teather, 1997; Luine et al., 2003). Our data, that posttraining estrogen administration increases crossover latencies, are congruous with these reports and extend them to suggest that estrogen has positive mnemonic effects in the inhibitory avoidance task, which has a lower motor demand, involves less training, and may be less stressful than tasks examined in previous reports. Further evidence for estrogen's mnemonic effects involves the time dependency of its effects. Previous reports have shown that administration of estrogen 2 h posttraining is not effective at enhancing learning in the water maze task (Packard and Teather, 1997). Our data are consistent with this and extend it to suggest that even a 1-h delay in estrogen administration after training can obviate its mnemonic effects in the inhibitory avoidance task.

Our present data also extend previous data to suggest possible mechanisms of estrogen for its mnemonic effects. The hippocampus is a target for estrogen and is known to be important for learning (Wong and Moss, 1991; Eichenbaum et al., 1992; Gould et al., 1990). Previous data have demonstrated that estrogen administered directly to the hippocampus can enhance learning in the water maze task (Packard and Teather, 1997). Our data that estrogen can enhance learning in the inhibitory avoidance task, which is mediated in part by the hippocampus (Izquierdo and Medina, 1997; Izquierdo et al., 1992), further suggest that the hippocampus is an important site of action for estrogen's mnemonic effects. As well, posttraining estrogen has to be present less than 1 h following training for consolidation to occur. Ligand-dependent actions of estrogen at intracellular estrogen receptors typically occur with latencies of hours to days. Thus, the finding that estrogen must be present within 1 h of training, suggests a more rapid onset for estrogen's actions for its mnemonic effects in the inhibitory avoidance task. What these mechanisms are for estrogen's mnemonic effects has not been investigated, but some putative substrates may involve actions at estrogen receptors, membrane receptors, and/or via various second messenger pathways (Kelly and Levin, 2001; Nilsen et al., 2002; Wade et al., 2001; Watters et al., 1997).

Although the present results provide novel and important evidence of estrogen's mnemonic effects, there are limitations that should be noted. First, estrogen can modulate stress responsiveness and these effects may vary considerably across or within tasks (Figueiredo et al., 2002). In the present studies, estrogen was administered posttraining and thus could not have had effects on training. However, the estrogen regimen utilized would still have been present 24 h later, during test time, and could have influenced testing. Although this is possible, the expected effects of estrogen would be to increase motor behavior, which would likely be demonstrated by decreased, instead of increased, crossover latencies. Second, in the present studies, only one behavioral endpoint was utilized. As a result, we are unable to directly address task-demand by comparing across multiple behavioral assays with different performance requirements. However, there were clear effects of estrogen in the inhibitory avoidance task, which suggests that this is a useful model to begin to investigate the mechanism(s) of estrogen's mnemonic effects.

The present data have important implications due to their clear clinical relevance. In the last century, the life expectancy of women has risen to >80 years; however, the age of onset for menopause has remained relatively constant. Because of this change in life expectancy, women spend an ever-increasing proportion of their lives in a hypoestrogenic state. As such, further characterization of the effects of estrogen on cognitive performance is important to better target the therapeutic effects of estrogen-replacement therapy (Sherwin, 2003).

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