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Long term replacement of estrogen in combination with medroxyprogesterone acetate improves acquisition of an alternation task in middle aged female rats

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Abstract

Studies have shown that ovarian hormones protect against some of the cognitive deficits associated with aging. Although much of the literature in rodents has focused on hippocampal dependent tasks, studies suggest that tasks dependent on the prefrontal cortex are also influenced by ovarian hormones. The present study investigated the effects of ovarian hormone treatment during aging on a delayed alternation t-maze. Female Long Evans hooded rats were ovariectomized at middle age (11–12 months) and placed in one of 5 treatment groups: no replacement, chronic estradiol (E₂), cyclic E₂, chronic E₂ and progesterone, or chronic E₂ and medroxyprogesterone acetate (MPA). Following six months of hormone treatment, animals were trained to alternate in a t-maze. After reaching criterion, a series of delays from 5 to 90 seconds were introduced in random order. Rats receiving E₂ with MPA reached criterion significantly faster than animals not receiving treatment and those who received chronic or cyclic E₂ only. There was a nonsignificant trend for animals receiving E₂ and progesterone to reach criterion in fewer sessions than animals receiving E₂ only. Mode of administration, cyclic or chronic, did not affect performance. Hormones did not affect performance on the delayed alternation. This study, in combination with previous research, indicates that hormone effects cannot be generalized across tasks, age or duration, and long-term estrogen in combination with MPA can be beneficial for some tasks.

Keywords

aging; t-maze; MPA; progesterone; memory

Introduction

In humans, several studies have identified the prefrontal cortex (PFC) as a region that has greater decline in gray matter volume during aging than other brain areas (Raz et al. 2005; Resnick, Pham, Kraut, Zonderman, & Davatzikos, 2003). Also, the largest decline in

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metabolism during aging is localized to the medial prefrontal cortex (Pardo et al., 2007) and decreases in synaptic density, spine density, and dendritic arborization have been found in the aged human frontal cortex (de Brabander, Kramers, & Uylings, 1998; Huttenlocher, 1979; Jacobs, Driscoll, & Schall, 1997; Masliah, Mallory, Hansen, DeTeresa, & Terry, 1993). In addition to the anatomical changes, several studies have shown that the functions of the PFC, including behavioral flexibility, attention, memory, and language, are impaired during aging (reviewed in Stuss & Benson, 1984). For example, the aged have deficits in item recognition and source memory as well as delayed verbal recall (Wegesin & Stern, 2007). In addition, older adults performed worse on the Wisconsin card sorting task, a test of executive functioning, and on a free-recall task which was mediated more by executive functioning impairments than deficits in perceptual speed (Baudouin, Clarys, Vanneste, & Isingrini, 2009).

The rat medial prefrontal cortex (mPFC) also experiences age-related changes. For example, reductions in both spine density and arborization of dendrites have been found in the mPFC of aged animals (Grill & Riddle, 2002; Markham & Juraska, 2002; Wallace, Frankfurt, Arellanos, Inagaki, & Luine, 2007) and the density of axospinous synapses decreases with age (Nakamura, Kobayashi, Ohashi, & Ando, 1999). Age-related changes also occur in the levels and turnover of dopamine and its metabolites (Godefroy, Bassant, Lamour, & Weil-Fugazza, 1991) as well as decreases in the amount of dopaminergic innervation (Mizoguchi, Shoji, Tanaka, Maruyama, & Tabira, 2009), and NMDA receptor density (Castorina, Ambrosini, Pacific, Ramacci, & Angelussi, 1994; Magnusson & Cotman, 1993; Miyoshi, Kito, Doudou, & Nomoto, 1990). Similar to humans, the mPFC in rodents plays a role in many cognitive abilities, including behavioral flexibility and memory for temporal order (Birrell & Brown, 2000; de Bruin et al., 2000; Kolb, 1990) and these measures are impaired during aging. For example, aged rats showed decreased performance, as compared to young rats, on an object recognition task (Wallace et al., 2007) and attentional set shifting (Barense, Fox, & Baxter, 2002). Also, studies have found deficits in aged animals when tested on delayed alternation tasks using the T-maze (Ando & Ohashi, 1991; Mizoguchi et al., 2009) and operant versions (Neese, Korol, Katzenellenbogen, & Schantz, 2010).

In both humans and rodents, several studies have shown that the presence of ovarian hormones may protect against some of these behavioral deficits. Although much of the literature has focused on hippocampal dependent tasks, ovarian hormones during aging also influence tasks dependent on the prefrontal cortex. In postmenopausal women, performance on a tasks relying on the prefrontal cortex, including digit ordering, delayed recall and a Wisconsin card sorting task, was improved after both short-term and long-term estrogen treatment (Krug, Born, & Rasch, 2006; Wegesin & Stern, 2007). In addition, long-term estrogen only and estrogen and progestagen treatments enhance measures of verbal memory and improves performance on tasks of spatial and verbal working memory (Carlson & Sherwin, 1998; Duff & Hampson, 2000; Kampen & Sherwin, 1994). In rodents, chronic estradiol administered via injections and silastic capsules improves performance on a delayed-nonmatching-to-position task (Markowska & Savonenko, 2002; Miller et al., 1999) and long-term chronic estradiol alone or in combination with progesterone improves performance on a delayed-matching-to-position task (Gibbs, 2000). However, the Women's Health Initiative found that conjugated equine estrogen alone or administered with medroxyprogesterone acetate (MPA), the most common progestin given to women, failed to enhance cognition and increased the number of subjects diagnosed with either probable dementia or mild cognitive impairment in post menopausal women (Espeland et al., 2004; Rapp et al., 2003; Shumaker et al. 2003, 2004). However, hormone treatment in the Women's Health Initiative was started several years after the onset of menopause and a recent review of the existing literature found hormone treatment to be beneficial when

initiated close to the onset of menopause but not when initiated later (Rocca, Grossardt, & Shuster, 2011).

MPA is a synthetic analogue of progesterone and binds to progesterone receptors, as well as androgen and glucocorticoid receptors (Bamberger, Else, Bamberger, Beil, & Schulte, 1999; Bardin, Brown, Isomaa, & Janne, 1983). However, progesterone is readily metabolized to allopregnanolone (Majewska, Harrison, Schwartz, Barker, & Paul, 1986) and MPA inhibits the enzymes needed for this conversion (Jarrell, 1984; Lee, Miller, & Auchus, 1999; Penning, Sharp, & Krieger, 1985). Differences in mechanisms of action between these progestagens may result in divergent behavioral responses. Surprisingly, few studies have addressed the effects of MPA on cognition. Our lab has previously shown that long term treatment with estrogen in combination with MPA attenuates the beneficial effects of estrogen alone or estrogen in combination with progesterone on water maze performance in middle aged rats (Lowry, Pardon, Yates, & Juraska, 2010). In addition, MPA administered without estrogen impaired performance on the water radial arm maze and the spatial water maze (Braden et al., 2010). When these two studies directly compared the behavioral effects of MPA with those of progesterone, they both found that progesterone did not impair performance on the water maze, but progesterone and MPA both impaired performance on the water radial arm maze (Braden et al., 2010; Lowry, Pardon, Yates, & Juraska, 2010). Both of these studies used hippocampal dependant tasks and it is unknown how MPA would affect performance on tasks that are mediated by the mPFC.

Because tasks that are mediated by the mPFC are impaired during aging and because studies suggest that the presence of estrogen may prevent this, we chose to evaluate the effects of hormone treatment, including MPA, on age-related cognitive decline in the t-maze, a working memory task dependent on the mPFC. Middle aged female rats were ovariectomized, given ovarian hormone treatment, and subsequently tested on an alternation and delayed alternation task. We administered four different types of replacement: chronic replacement of estrogen alone, chronic replacement of estrogen and progesterone, chronic replacement of estrogen and MPA, or cyclic replacement of estrogen alone. Differences between groups of animals may suggest distinct roles for estrogen and progestagens during aging, and would indicate whether the effects vary with the type of progestagen administered. Whether treatment with estrogen in combination with MPA or progesterone will differentially affect performance on learned alternation or delayed alternation during aging is currently unknown.

Methods

Subjects

Subjects were 51 female Long Evans hooded rats purchased from Charles River Laboratories as retired breeders at the age of 11–12 months. Due to the large number of subjects and limited availability from the supplier, animals were run in three experimental cohorts. Animals from the same group were pair- or, if necessary, triple housed, in clear Plexiglass cages in a temperature-controlled environment on a 12:12-hr light–dark cycle. Food and water were available *ad libitum* to all animals, except during behavioral procedures during which the animals were maintained at 85–90% of their normal body weight. All rats were handled, weighed, and checked for health problems (tumors) once a week. At sacrifice, both body and uterine weights were measured. Animals from two of the cohorts were used in another behavioral experiment following completion of the present task (Lowry et al., 2010). Animal care and experimental procedures were in accordance with National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee.

Hormone Treatment

The ovaries were removed from all subjects at 12–13 months. Subjects were anesthetized with 4% isoflurane and ovariectomized via bilateral incisions. Animals were administered the analgesic, carprofen (0.05 mg/kg delivered intraperitoneally), prior to surgery and again 12 hours later, in accordance with animal care policy. Subjects were housed individually for five days following surgery to allow for recovery and then returned to pair- or triple-housed conditions. Hormone administration was initiated the day of surgery and continued throughout the behavioral procedures. Animals were randomly assigned into the following five groups: no replacement (NR) (n = 12), chronic 17 β -estradiol (E₂) (n = 11), E₂ and MPA (E₂ + MPA) (n = 12), E₂ and progesterone (E₂ + P) (n = 7) or cyclic E₂ (n = 9).

17 β -estradiol (E₂) Administration—As in Lowry et al. (2010), all groups receiving hormones were given E₂ in their drinking water. Acyclic intact females continue to secrete estrogen at levels between 25–30 pg/ml and progesterone at 10–40 ng/ml depending on estropausal state (Markham & Juraska, 2002; Warren & Juraska, 2000; Wise & Ratner, 1980; Miller & Riegle, 1980). In a pilot study, we found that middle aged female rats drink a steady amount of water whether individually or group housed, and an aqueous E₂ dose of 70 μ g/kg/day produced estrogen levels averaging 46 pg/ml. In order to keep the dose in the physiological range for this age group, we decreased the dose to 47 μ g/kg/day. E₂ was first dissolved in 95% ethanol (2mg/ml) and then dissolved in water as described previously (Gordon, Osterburg, May, & Finch, 1986). Water consumption was measured weekly for each cage and remained between 120–140 ml/kg/day throughout the experiment for all groups. The dose of E₂ was calculated by taking the amount of water consumed by a cage and dividing by the sum of the weights in that cage. This value was then multiplied by the E₂ concentration in the water.

Chronic Hormone Treatment—Estradiol was available in the drinking water every day for the three chronic hormone treatment groups: E₂, E₂ + P, and E₂ + MPA. On the day of OVX, one hormone pellet of either P or MPA was inserted in the appropriate groups through a small incision in the nape of the neck. The progesterone pellets were made from silastic tubing (Dow Corning) packed with crystalline hormone. Plugs were made using short wooden applicator sticks and the implants were sealed with silicone sealant, type A (Dow Corning). Studies have shown that 40 mm implants produce hormone levels between those found in aging female rats in persistent estrus and persistent diestrus (Liu et al., 1997). The MPA pellets (1.5mg) were purchased from Innovative Research of America. Using 1.5mg 90 day release pellets results in a dose similar to those in women taking 2.5 mg per day when expected daily release and average weight are factored in. Progesterone and MPA pellets were replaced every 90 days. All other groups received sham surgeries at the time of pellet replacement.

Cyclic Hormone Treatment—E₂ was administered in the drinking water three out of every four days in the group receiving cyclic E₂ treatment. On the fourth day, the water did not contain E₂ and on the following day, the four day cycle was repeated. Due to a circadian drinking pattern, the highest elevation of estrogen levels occurs during peak nocturnal drinking periods and levels return to near baseline fifteen hours later (Gordon, Osterburg, May, & Finch, 1986) which indicates that a day without E₂ resulted in levels near baseline.

Behavioral Procedures

The first day of testing occurred after approximately five months of hormone treatment, so that subjects were 17–18 months old at the start of testing. Hormone treatment continued throughout the testing period and all pellets were at approximately 55 days into their 90 day

period. Food deprivation began five days prior to introduction to the maze. Animals were food deprived to 85–90% of their free feeding body weight.

Apparatus—The t-maze consisted of an approach alley (15 cm wide × 45 cm long) and two goal arms (15 cm wide × 45 cm long). The start box (15 cm wide and 26 cm long) opened into the approach alley. The walls of the maze were 15 cm high and were constructed of black plexiglass. Manually operated sliding doors were positioned at the entrance to each goal arm. The orientation and position of the maze were not changed during the experiment.

Testing Procedure—A previous study in our lab ran adolescent animals twice a day in order to complete testing within the adolescent time period (Koss, Franklin, & Juraska, 2011). We used the same procedure as that study; therefore in the present study, animals completed two sessions per day during all phases of the task.

Habituation: Animals were exposed to the maze during four 10-minute sessions over 2 days. During this time, each arm was baited with sunflower seeds and animals were allowed to roam the maze freely for 10 minutes.

Forced Alternation: Animals were placed in the maze for 10 forced alternation trials twice per day for three days for a total of 6 sessions. During each trial, the animal had access to only one open arm, which was baited with a sunflower seed reward and the opposite arm was blocked. Once the animals had eaten the sunflower seed they were returned to the start box. The arm previously visited was then blocked, restricting access during the next run to only the alternate arm. For all trials, the animals remained in the chosen arm until they had eaten the sunflower seed or until 15 seconds had elapsed.

Alternation: On the first choice run, animals were placed in the start box and rewarded for choosing either arm. During the next 10 trials, animals were rewarded only when they entered the alternate arm. Each animal completed two sessions of 11 trials a day (1 free choice trial followed by 10 test trials) until a criterion of 80% over three consecutive sessions was achieved.

Delayed Alternation: All animals that reached criterion were then run for two sessions where they were introduced to short delays of 5, 10, and 15 seconds to reduce the level of stress associated with delays. Then the delayed alternation portion of the task was introduced. Delays of 5, 10, 15, 30, 60, 90 seconds were presented in a semi-randomized order. The procedures were the same as the alternation, except that after a trial, animals were returned to the start box where they would experience one of the delays. Each animal completed two sessions of 11 trials a day for a total of 15 sessions. This resulted in 25 trials at each delay. If an animal made an incorrect choice and then continued to choose the same arm, this was classified as a perseverative error in both the no-delay and delayed alternation portions of the task.

Post-behavior procedures: All animals were returned to *ad libitum* food. Two weeks after the completion of the present study, two of the three cohorts were run on a spatial water maze that has been previously reported (Lowry, Pardon, Yates, & Juraska, 2010). At 19–20 months of age, they were sacrificed for neuroanatomical studies and body weight and uterine weights were recorded. Hormone treatment continued until sacrifice.

Statistical Analysis

Body weights and uterine weights were analyzed using a one-way ANOVA with cohort as a covariate. The number of sessions required to reach criterion was analyzed using a one-way ANOVA with cohort as a covariate. Performance on delayed alternation was analyzed using a two-way (5 Treatment $\times$ 6 Delay) ANOVA with repeated measures on delay and cohort as a covariate. Cohort was not significant for any of the analyses. Fisher's LSD tests were used for all post hoc comparisons.

Results

Body Weight

There was a significant effect of treatment on body weight ($F(4, 45) = 6.470, p < .001$). Post-hoc tests revealed that the no replacement group weighed significantly more than all groups that received hormone treatment ($E_2: p < .001$; $E_2 + \text{MPA}: p < .001$; $E_2 + P: p < .01$; Cyclic $E_2: p < .02$) (Table 1).

Uterine Weight

There was a significant effect of treatment ($F(4, 45) = 5.902, p < .001$) on uterine weight. Post-hoc tests revealed that uterine weight in the no replacement group was significantly lower than all groups that received hormone treatment ($E_2: p < .001$; $E_2 + P: p < .01$; $E_2 + \text{MPA}: p < .001$; Cyclic $E_2: p < .03$), indicating that hormone treatment was physiologically effective (Table 1).

Alternation—A one way ANOVA revealed a main effect of treatment on the number of sessions to reach criterion ($F(4, 45) = 2.731, p < .05$). Post hoc Fisher's LSD revealed that the group receiving $E_2 + \text{MPA}$ required significantly fewer sessions to reach criterion than animals in three groups: no replacement ($p < .04$), chronic E_2 ($p < .02$), and cyclic E_2 ($p < .02$). There were non-significant trends for animals that received $E_2 + P$ to require fewer days to meet criterion than animals receiving chronic E_2 ($p < .09$) and cyclic E_2 ($p < .08$) (Figure 1). The groups did not differ in the number of perseveration errors during alternation.

Delayed Alternation—One animal that had met criterion in the learned alternation task failed to leave the start box during the delayed alternation task and was not included in analysis. There was a significant effect of delay ($F(5, 220) = 3.135, p < .01$) (Figure 2) such that increasing the intertrial delay significantly reduced performance in all treatment groups. Hormone treatment was not significant ($F(4, 44) = .959, p = .440$), nor was there a significant interaction between treatment and delay. The groups did not differ in the number of perseveration errors during delayed alternation.

Cyclic E_2 —No effects were found within the cyclic E_2 group between days when E_2 was administered in the drinking water and when it was not.

Discussion

Long term chronic administration of E_2 in combination with MPA enhanced acquisition of an alternation task in middle aged female rats. Animals receiving E_2 in combination with MPA reached criterion in fewer sessions than controls and animals who received chronic or cyclic E_2 only. Performance on delayed alternation decreased as the intertrial delay increased; however, hormone treatment failed to alter performance on the delayed portion of the task. These results are in contrast to our previous study in which 80% of the animals used in the present study were tested on the water maze and estradiol in combination with

MPA impaired performance as compared to other hormone treated groups (Lowry et al., 2010).

Lesions of several different brain regions, including the hippocampus, striatum and prefrontal cortex, impair alternation behavior (reviewed in Lalonde, 2002) so that identifying a single brain region responsible for the behavioral outcome in this study is difficult. Furthermore, although animals can use allocentric spatial relationships to alternate in the t-maze, a study that manipulated several aspects of the maze environment found that performance on this task does not rely exclusively on these extramaze cues (Dudchenko, 2001). The beneficial effect of E₂ in combination with MPA found in the present study differs from previous studies examining the effects of MPA on cognition. Chronic MPA alone or in combination with E₂ impaired performance on the water maze in middle aged animals (Braden et al., 2010; Lowry et al., 2010). Performance on the water maze relies heavily on the hippocampus, which suggests that MPA might impair functioning of this brain region. Although MPA seems to impair hippocampal functioning, there is no evidence to suggest that this is true for other brain regions which may become engaged while solving the task used in the current study.

Interestingly, E₂ in combination with progesterone resulted in a more variable but similar pattern of fewer trials to acquire the alternation task as E₂ in combination with MPA. In agreement with this finding, weekly injections of E₂ in combination with progesterone improved t-maze performance in aged rats (Gibbs, 2000). It is unknown if progesterone and MPA have similar neural effects because few studies have directly compared them. Braden et al. (2010) found that chronic MPA significantly and progesterone marginally decreased levels of glutamic acid decarboxylase in the hippocampus when administered via osmotic pumps. However, a single injection of progesterone transiently down regulated mRNA expression of GABA_A subunits, whereas MPA did not (Pazol, Northcutt, Patisaul, Wallen, & Wilson, 2009). If MPA and progesterone result in different neural outcomes, they may differentially affect cognition. Indeed our previous study found that while long-term E₂ in combination with MPA impaired performance when compared to other hormone treated groups on the water maze, long-term E₂ in combination with progesterone did not (Lowry et al., 2010). However, the current study found similar effects of the two progestagens, emphasizing the importance of the task used to evaluate learning and memory.

The t-maze task used in the present study requires the animal to recall information that varies from trial to trial and therefore tests working memory (Markowska, Olton, & Givens, 1995). This study is the first to examine the effects of long term treatment of E₂ in combination with MPA on working memory. Two previous studies have found that chronic MPA administered alone impairs working memory on the water radial arm maze (Braden et al. 2011; Braden et al 2010). However, hormone treatment therapies contain MPA in combination with estrogen and the cognitive effects of these hormones may differ when administered alone. Although the present study found that E₂ in combination with MPA improved performance on a working memory task, future studies should examine if this generalizes to other working memory tasks. Interestingly, long-term chronic oral E₂ failed to alter performance on this task. Similar to our findings, chronic E₂ administered both orally and via daily injections did not affect the number of working memory errors in the radial arm maze (Fernandez & Frick, 2004; Gresack & Frick, 2006), and although chronic treatment with E₂ minipellets decreased the number of reference memory errors in the radial arm maze there was no effect on working memory errors (Heikkinen, Puloivali, & Tanila, 2004). Studies using the operant versions of the delayed spatial alternation task consistently find that E₂ administered chronically via silastic capsules during aging impairs performance (Neese et al. 2010; Wang, Neese, Korol, & Schantz, 2009). Lesion studies indicate that the operant version of this task relies heavily on the mPFC (Sloan, Good, & Dunnett, 2006),

whereas performance on maze versions is altered by lesioning several different brain regions (Lalonde, 2002), indicating a fundamental difference between operant and maze versions. However, some studies have found improved performance after E₂ treatment on working memory tasks. Long-term chronic E₂ benefited acquisition of a delayed matching to position task, but similar to the present study, did not influence performance during delays (Gibbs, 2000). In addition, silastic capsules containing E₂ improved performance on a spontaneous alternation task in aged mice (Miller et al., 1999). Importantly, the duration of hormone treatment in Miller et al. (1 month) differed greatly from the present study in which hormones were administered for approximately six months prior to behavioral testing which might explain the differences between these two studies.

There is somewhat more agreement among studies evaluating the effects of estrogens on reference memory during aging. Several studies have found that E₂ or estradiol benzoate treatment in both middle aged and aged female rodents improves performance on the Morris water maze (Frick, Fernandez, & Bulinski, 2002; Frye, Rhodes, & Dudek, 2005; Harburger, Bennett, & Frick, 2007; Lowry et al., 2010; Markham, Pych, & Juraska, 2002; however see Foster, Sharrow, Kumar, & Masse, 2003). It has become apparent that it is often not possible to generalize hormonal effects across different tasks and varying modes and lengths of administration (reviewed in Frick, 2009; Juraska & Rubinow, 2008).

Most studies evaluating the neural effects of hormone treatments have administered hormones acutely. Acute E₂ interacts with neurotransmitters systems, increases synaptophysin in the hippocampus, and increases neurotrophins in the entorhinal cortex (Bimonte-Nelson, Nelson, & Granholm, 2004; Frick, Fernandez, & Bulinski, 2002; Gibbs, 1997). However, the few studies that have examined the neural outcomes of chronic long term hormone treatment have found that changes observed with acute treatment return to baseline after chronic exposure. Chronic long-term treatment with E₂ down-regulates estrogen receptors (Brown, Scherz, Hochberg, & MacLusky, 1996), and levels of choline acetyltransferase increase after acute E₂ treatment but return to normal after chronic treatment in the basal forebrain (Gibbs, 1997). Furthermore, acute treatment with estrogen and progesterone resulted in decreases in GABA receptor subunit expression 12 hours, but not 24 hours after treatment (Pazol et al., 2009). Therefore long term chronic hormone treatment during aging might lead to a down regulation of estrogen receptors in cognitive brain regions and different behavioral outcomes than short term treatments.

It has been suggested that that hormone treatments that more closely mimic the natural cycle of hormones might be more beneficial for cognition (Frick 2009). The present study found that E₂ administered cyclically resulted in a similar rate of acquisition to E₂ administered chronically. Similar to the present study, continuous E₂ treatment had no effect on spatial working or reference memory in the radial arm maze, while intermittent E₂ treatment impaired spatial reference memory (Gresack & Frick, 2006). Cyclic administration of estradiol benzoate failed to improve performance on a 12-arm radial arm maze in middle aged female rats (Ziegler & Gallagher, 2005). However, weekly injections of E₂ plus progesterone enhanced performance of aged female rats on a delayed match-to-position T-maze task (Gibbs, 2000) and in aged rhesus monkeys, cyclic estradiol cypionate injections improved spatial working memory performance (Rapp, Morrison, & Roberts, 2003). Continuous and intermittent E₂ improved task acquisition in middle aged and aged animals (Bimonte-Nelson, Francis, Umphlet, & Granholm, 2006; Markowska & Savonenko, 2002). Although these studies found beneficial effects of cyclic treatment as compared to controls, none of the studies found that cyclic treatment was more beneficial for task performance than chronic treatment. However, most of these studies only simulated certain aspects of the cycle. For example, estrogen cyclicity was mimicked in the current study, but not the fluctuation in progesterone levels. More closely simulating the natural cycle by including

fluctuations in both estrogen and progesterone levels may be more beneficial than the chronic treatment of ovarian hormones.

The route of administration used in a study can influence the outcome. Oral administration is the most common route of administration of hormone treatment in post menopausal women. Animal studies more commonly use injections or silastic capsules to deliver hormone treatments. These routes of administration have different pharmacokinetics than oral administration and do not allow for first pass metabolism of the hormone which may result in different outcomes of hormone treatment. Indeed, the only other study that has administered estradiol orally and examined effects on cognition in an animal model found improved performance on object recognition (Fernandez and Frick, 2004), while another study from the same lab found that daily injections of estradiol did not improve performance on the same task (Gresack and Frick, 2006). In the present study estradiol was administered orally both to model human treatment and to dose animals both cyclically and chronically. Although blood levels of estrogen are not available, group differences in body and uterine weights indicate that the treatment was physiologically effective, and preliminary results from a neuroanatomical study indicate treatment effects on brain structure (Packard, Lowry, Koss & Juraska, 2011).

After the Women's Health Initiative found that hormone treatment in postmenopausal women failed to enhance cognition and increased the number of subjects diagnosed with either probable dementia or mild cognitive impairment (Espeland et al., 2004; Rapp et al., 2003; Shumaker et al., 2003; Shumaker et al., 2004), researchers suggested that the presence of MPA in these hormone treatments might be responsible. However the current study found that E₂ in combination with MPA is beneficial for certain tasks. Taken together with our previous studies, these data emphasize the importance of avoiding generalizations between tasks, age, and length of replacement as well as time without estrogen. Elucidating the neural effects of long-term hormone treatments might help elucidate what determines the behavioral outcome.

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Figure 1.

Mean $\pm$ SEM number of sessions to criterion for no delay alternation trials. Animals receiving E_2 + MPA required fewer sessions to reach criterion than animals in three groups: no replacement ($p < .04$), chronic E_2 ($p < .02$), and cyclic E_2 ($p < .02$). There were non-significant trends for animals that received E_2 + P to require fewer days to meet criterion than animals receiving chronic E_2 ($p < .09$) and cyclic E_2 ($p < .08$). * = $p < .05$

Figure 2.

Mean number correct at each alternation delay. There was a significant effect of Delay ($p < .01$) such that increasing the intertrial delay significantly reduced performance in all treatment groups. Hormone treatment was not significant.

Table 1

Body and Uterine Weight

Hormone Group	Mean Body Weight (g)	Mean Uterine Weight (g)
No replacement	573.1 ± 24.4	.09 ± .01
Chronic E ₂	469.8 ± 22.5 *	.15 ± .02 *
Chronic E ₂ & P	470.6 ± 19.93 *	.13 ± .01 *
Chronic E ₂ & MPA	466.8 ± 27.6 *	.16 ± .02 *
Cyclic E ₂	526.6 ± 23.4 *	.16 ± .01 *

*
p < .05 compared to No replacement