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Effects of long-term treatment with 17 β-estradiol and medroxyprogesterone acetate on water maze performance in middle aged female rats

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ABSTRACT

Although previous research has indicated that hormone replacement therapy benefits memory in menopausal women, several recent studies have shown either detrimental or no effects of treatment. These inconsistencies emphasize the need to evaluate the role of ovarian hormones in protecting against age-related cognitive decline in an animal model. The present study investigated the effects of long-term hormone treatment during aging on the Morris water maze. Female Long Evans hooded rats were ovariectomized at middle age (12–13 months) and were immediately placed in one of five groups: no replacement, chronic 17 β-estradiol only, chronic 17 β-estradiol and progesterone, chronic 17 β-estradiol and medroxyprogesterone acetate (MPA), or cyclic 17 β-estradiol only. 17 β-estradiol was administered in the drinking water in either a chronic or cyclic (3 out of 4 days) fashion. Progesterone and MPA were administered via subcutaneous pellets. Following 6 months of hormone treatment, animals were tested on the Morris water maze. Animals performed four trials a day for 4 days and after the final day of testing a subset of animals completed a probe trial. Across 4 days of testing, rats receiving 17 β-estradiol in combination with MPA performed significantly worse than all other groups receiving hormone replacement. In addition on the last day of testing, chronic 17 β-estradiol administration was more beneficial than cyclic administration and no replacement. Thus compared to other hormone-treated groups, long-term 17 β-estradiol treatment in combination with MPA results in impaired performance on the spatial Morris water maze.

Introduction

A decline in many cognitive abilities accompanies aging in humans. For women, this decline has been associated with the decrease in ovarian hormones that occurs during menopause (Sherwin, 1988; Nappi et al., 1999). Numerous studies have reported an improvement in cognitive performance of women receiving hormone treatment (reviewed by LeBlanc et al., 2001). For example, hormone treatment enhances measures of verbal memory and improves performance on tasks of spatial and verbal working memory (Kampen and Sherwin, 1994; Carlson and Sherwin, 1998; Duff and Hampson, 2000). However, other studies have found little or no cognitive benefit of hormone treatment in postmenopausal women (reviewed by Hogervorst et al., 2000). 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 (Rapp et al., 2003b; Shumaker et al., 2003, 2004; Espeland et al., 2004). These conflicting results emphasize the need to determine the factors influencing whether hormone replacement benefits or impairs cognition.

Studies investigating the association between hormone treatment and cognition in humans contain confounds which can be manipulated or controlled in a rodent model. Rodents experience age-related cognitive decline and thus may provide useful models for explaining the role ovarian hormones play in cognition during the aging process. However, most research investigating the effects of hormone treatment on cognition has used young animals (reviewed in Daniel, 2006; Juraska and Rubinow, 2008), which does not reflect the possible interaction of hormone treatment with aging. Indeed, studies suggest that the effects of hormone treatment in young female animals are often not the same as the effects of hormone treatment during aging. Our laboratory found that young adult females who were ovariectomized and given 17 β-estradiol (E_2) and progesterone were impaired in the acquisition of the Morris water maze (Chesler and Juraska, 2000), while replacement of E_2 and progesterone in middle aged animals facilitated performance of the same task (Markham et al., 2002). Other laboratories have also found hormone by age interactions in females within the same study (Foster et al., 2003; Talboom et al., 2008). Therefore, it is important to assess the effects of hormone treatment in middle aged or aged female animals, a model that more

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closely mimics hormone treatment during human menopause. Recently, studies have used middle aged or aged female animals when evaluating the cognitive effects of various types of hormone treatment. These studies have found improved performance after E_2 treatment on the radial arm maze, spontaneous alternation, and both delayed matching to position and delayed non-matching to position tasks (Miller et al., 1999; Gibbs, 2000; Markowska and Savonenko, 2002; Heikkinen et al., 2004). E_2 or estradiol benzoate treatment in both middle aged and aged female rodents often improves performance on the Morris water maze (Markham et al., 2002; Frick et al., 2002; Frye et al., 2005; Harburger et al., 2007). However, Foster et al. (2003) found no effect of estradiol benzoate on acquisition of the water maze in aged animals. Also, 5 weeks of oral E_2 administration in middle aged animals resulted in impairment on the radial arm maze (Fernandez and Frick, 2004). In addition, in aged animals, chronic E_2 had no effect on working memory errors in the radial arm maze (Gresack and Frick, 2006). The inconsistencies in the literature could be due to several factors including the mode of administration (chronic or cyclic replacement), pharmacokinetics of the route of administration, whether or not estrogens are combined with a progestagen, and the type of task that is used to assess the influence of hormone treatment on cognition.

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 study that has administered E_2 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 E_2 did not improve performance on the same task (Gresack and Frick, 2006). Therefore it is important to evaluate the effects of hormone treatment using a route of administration that is similar to the most common route used by post menopausal women.

Furthermore, the types of hormones prescribed to post menopausal women are different from those that are most commonly studied in animal models. Conjugated equine estrogen, the estrogen that was used in the Women's Health Initiative, is the most commonly prescribed estrogen, but very few animal studies have evaluated the effects of this estrogen on cognition. Recently, one study found that conjugated equine estrogen administration to middle aged female rats prevented overnight forgetting in the Morris water maze (Acosta et al., 2009). This is similar to other studies that have used E_2 and found improved performance on the Morris water maze (Markham et al., 2002; Bimonte-Nelson et al., 2006; Talboom et al., 2008). Also, many animal studies that have evaluated the effects of progestagens on cognition have used progesterone rather than MPA, the most commonly prescribed progestin. While MPA is a synthetic analogue of progesterone and is an agonist at progesterone receptors, it also binds to androgen and glucocorticoid receptors (Bardin et al., 1983; Bamberger et al., 1999). A recent study found that MPA administered without estrogen impaired performance on the water radial arm maze and the spatial water maze (Braden et al., 2010). To our knowledge, no published studies have investigated the effects of E_2 in combination with MPA on cognition in an aging animal model.

The present study was designed to evaluate the effects of different modes of hormone treatment (chronic and cyclic) and different regimens of hormone treatment on a reference memory task that is hippocampal-dependent, the spatial Morris water maze. Middle aged female rats were ovariectomized, immediately given hormone treatment for 6 months, and subsequently tested on the Morris water maze. Because much of the animal research to this point has used E_2 rather than conjugated equine estrogen, we used E_2 in order to more directly compare our results. Four types of treatment were administered: chronic E_2 alone, chronic E_2 and progesterone, chronic E_2 and MPA and cyclic E_2 alone. Directly comparing the groups with progesterone to those with MPA should help determine if these progestagens differ in preventing the cognitive decline associated with aging. Comparing these hormone treatments with E_2 only treatments will indicate if the addition of a progestagen impacts the outcome of hormone treatments on age related cognitive decline. We predicted that middle aged females receiving hormone treatment would perform better than animals not receiving hormones. Furthermore, we hypothesized that cyclic E_2 treatment, which is closer to the natural cycle, would be more beneficial for this task than chronic E_2 treatment.

Methods

Subjects

Subjects were 47 female Long Evans hooded rats purchased from Charles River Laboratories as retired breeders at the age of 10–12 months. Due to the large number of subjects and groups, animals were run in two 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 a delayed T-maze task during which the animals were maintained at 85–90% of their normal body weight. Prior to being tested on the water maze, food was made available *ad libitum* for 2 weeks and animals returned to their normal body weight.

All rats were handled once a week and checked for health problems (tumors). Body weight was recorded weekly and uterine weight was measured after sacrifice. 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

At 12–13 months, all subjects were anesthetized (4% isoflurane) and ovariectomized (OVX) via bilateral incisions. In accordance with animal care policy, animals were administered the analgesic, carprofen (0.05 mg/kg delivered intraperitoneally) prior to surgery and again 12 hours later. Following surgery, subjects were housed individually for 5 days to allow for recovery and then returned to pair- or triple-housed conditions. Hormone administration was initiated immediately after surgery. Animals were randomly assigned into the following five groups for the first cohort: no replacement (NR) ($n=3$), chronic 17 β-estradiol (E) ($n=4$), E and MPA (E + MPA) ($n=2$), E and progesterone (E + P) ($n=4$) or cyclic 17 β-estradiol (CYE) ($n=2$) and for the second cohort: no replacement (NR) ($n=8$), chronic 17 β-estradiol (E) ($n=6$), E and MPA (E + MPA) ($n=8$), E and progesterone (E + P) ($n=2$) or cyclic 17 β-estradiol (CYE) ($n=8$).

17 β-estradiol (E_2) administration

All groups receiving hormones were given E_2 in their drinking water. Previous work from our lab has found that middle aged, acyclic intact females have circulating estrogen levels averaging 25–30 pg/ml (Warren and Juraska, 2000; Markham and Juraska, 2002). In a pilot study, we found that an E_2 dose of 70 μg/kg/day produced estrogen levels averaging 46 pg/ml. In order to keep the dose relatively low and in the physiological range for this age group we decreased the dose to 47 μg/kg/day. E_2 was first dissolved in 95% ethanol (2 mg/ml) and then dissolved in water as described in Gordon et al. (1986). Water consumption was checked weekly for each cage and remained between 60 and 80 ml/kg/day for each rat throughout the experiment for all groups. The dose of E_2 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 E_2 concentration in the water.

Chronic hormone treatment

The three chronic hormone treatment groups had E_2 in their drinking water every day: E, E + P, and E + 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 approximating the peak circulating levels of progesterone achieved during the normal estrous cycle in the adult female rat (Smith et al., 1975; Hope et al., 1992). The MPA pellets (1.5 mg) were purchased from Innovative Research of America. Using 1.5 mg 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. Average weight for animals (.4 kg) was calculated at the start of the experiment and the average weight used for humans was 60 kg. Using these values the expected dose of MPA would be 41.7 micrograms/kg in rat and 41.6 micrograms/kg in humans. Both the progesterone and MPA pellets were replaced every 90 days. At the time of pellet replacement, all other groups received a sham surgery.

Cyclic hormone treatment

The group with cyclic E_2 treatment received E_2 in their drinking water three out of every 4 days. On the fourth day, the water did not contain E_2 and on the following day, the 4-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 et al., 1986). Thus, it is likely that on the fourth day, when the animals were not receiving any E_2, levels of estrogen were not detectable.

Behavioral procedures

The first day of testing occurred after approximately 6 months of hormone treatment, so that subjects were 18–19 months old at the start of testing. Hormone treatment continued throughout the testing period.

Pretraining

Apparatus

The maze used during pretraining was a small wading pool (122 cm in diameter, 36 cm deep), located in a room other than the one used for testing. It had a sheet metal liner inserted to provide a vertical perimeter, and the visible escape platform (19 cm tall, 9 cm in diameter) was placed in the center of the pool. The pool was filled to 1 cm below the surface of the escape platform. The temperature of the water was maintained at 26 °C ± 0.5 °C. The experimenter remained at one of four start locations around the pool for all pretraining trials.

Procedure

Pretraining began 2 days prior to the first day of testing. Subjects received four consecutive pretraining trials, each beginning from one of four start locations. Order of the start location was the same for all subjects. Rats were placed in the water while facing the edge of the pool and allowed 60 s to find the escape platform. If the platform was not located at the end of 60 s, the animal was guided to the platform by the experimenter. The subject remained on the platform for 15 s and was then removed from the maze and dried off using a towel. After completion of pretraining, rats were returned to their home cages.

Testing

Apparatus

The circular water maze testing tank (175 cm in diameter and 74 cm deep) was located in a room different than the one used for pretraining. A variety of extra-maze cues were present in the testing room. The maze was filled to a depth of 60 cm, 2 cm higher than the escape platform (58 cm tall and 10 square cm). White nontoxic paint was used to turn the water opaque, and the water was maintained at a temperature of 26 °C ± 0.5 °C.

Procedure

Subjects were tested across 4 days, with four trials per day (16 trials total). The location of the escape platform remained the same throughout the experiment. At the start of each trial, the subject was placed in the water, facing the edge of the tank, from one of four start locations. Start location order was the same for all animals. For each trial, the rat was given 60 s to find the platform, and the time to reach the platform was recorded. If the subject was unable to locate the platform by the time 60 s had elapsed, the experimenter guided the subject to the platform. The subject remained on the platform for 15 s before being removed from the maze and dried off using a towel. The intertrial interval varied between 13 and 15 min. At the end of testing on Day 4, the platform was removed and a probe trial lasting 60 s was performed on the second cohort of animals. A video camera was mounted above the center of the tank and all trials were recorded. The experimenter recorded latency times with a stopwatch. After testing was complete, the pathlengths taken by the subjects to reach the platform were traced and then scanned into a computer and pathlengths were calculated using Image J (National Institutes of Health). Swim speed was calculated by dividing pathlength by latency.

Statistical analysis

Body weights and uterine weights were analyzed using a two-way ANOVA (5 Treatment × 2 Cohort). Pretraining latency was analyzed using a two-way (5 Treatment × 4 Trial) ANOVA with repeated measures on trial. For testing, latency and pathlength data were analyzed with each cohort of animals as a factor using a three-way (5 Treatment × 2 Cohort × 4 Day) ANOVA with repeated measures on day. Since there were no main effects of cohort and no cohort by treatment interaction, the two cohorts were then combined for analysis using a two-way (5 Treatment × 4 Day) ANOVA with repeated measures on day. Furthermore, post-hoc t-tests of treatment at each of the 4 days of testing were performed for both latency and pathlength because we expected differences between hormone groups in the later trials (Warren and Juraska, 2000). In addition, each day of testing was divided into two blocks of two trials for analysis because previous work in our lab indicated that differences in water maze performance may be due to the animals forgetting the task after a one-hour delay or overnight (Warren and Juraska, 1997; Markham et al., 2002). Therefore preplanned comparisons of Block 2 versus 3, 4 versus 5, and 6 versus 7 were performed to compare performance at the beginning of each day with performance at the end of the previous day and determine whether animals forgot the task overnight. At the request of a reviewer, linear trend analyses were conducted to investigate whether the rate of improvement over trials differed between the groups. Swim speed during testing was analyzed using a two-way ANOVA (5 Treatment × 4 Day). Time spent in the target quadrant during the first half of the probe trial (30 s) and during the entire probe trial (60 s) was analyzed separately using a

one-way ANOVA. Two-tailed t-tests were used for all post hoc comparisons.

Results

Body weight

There was a significant effect of treatment on body weight (F (4, 41) = 4.258, $p<0.007$). Post-hoc t-tests revealed that the no replacement group weighed significantly more than all groups that received hormone treatment except the group receiving cyclic E_2 treatment for which there was a non-significant trend. (E: $p<0.01$; E + MPA: $p<0.003$; E + P: $p<0.001$; CYE: $p<0.08$). No other comparisons reached significance (Table 1).

Uterine weight

There was a significant effect of treatment (F (4, 36) = 4.149, $p<0.01$) and cohort (F 1, 36) = 11.169, $p<0.01$) on uterine weight but there was no treatment × cohort interaction (F 4, 36) = 0.597, $p = 0.667$). Post-hoc t-tests revealed that uterine weight in the no replacement group was significantly less than all groups that received hormone treatment except the group receiving chronic E_2 and progesterone treatment for which there was a trend. (E: $p<0.01$; E + MPA: $p<0.01$; CYE: $p<0.01$; E + P: $p<0.058$;), indicating that hormone treatment was physiologically effective (Table 1).

Pretraining

There was no significant effect of treatment on pretraining performance nor was there an interaction between treatment and pretraining trials (Fig. 1).

Latency

There was a significant effect of day (F (3, 126) = 58.954, $p<0.001$) as latency decreased as the animals became skilled at the water maze task. There was also a main effect of treatment (F (4, 42) = 2.683, $p<0.05$). The treatment by day interaction was not significant. Post-hoc t-tests revealed that the chronic E_2 and MPA group performed significantly worse than all other groups receiving hormone treatment (E: $p<0.007$; E + P: $p<0.03$; CYE: $p<0.03$), but they were not significantly different from the no replacement group (Fig. 2A). No hormone treated group was significantly different than the no replacement group. Analysis by 2-trial block indicated that none of the groups forgot the task overnight (Fig. 3A). At the suggestion of a reviewer we conducted a linear trend analysis of block to determine whether the rate of improvement over trials differed between the treatment groups. All groups improved performance across training as seen with the significant linear trend for block (F(1, 42) = 181.427, $p<0.001$), but treatment groups did not differ in their rates of improvement.

Table 1
Body and uterine weights for all groups.

Hormone group	Mean body weight (g)	Mean uterine weight (g)
No replacement	584.5 ± 20.6	0.10 ± 0.01
Chronic estrogen	498.2 ± 22.2 *	0.17 ± 0.01 *
Chronic estrogen and progesterone	479.0 ± 32.4 *	0.15 ± 0.02 ψ
Chronic estrogen and MPA	466.8 ± 27.6 *	0.17 ± 0.02 *
Cyclic estrogen	526.6 ± 23.4 †	0.16 ± 0.01 *

Body weight (grams) and uterine weights (grams) for all animals. * indicates significant ($p<0.01$) difference while ψ ($p<0.06$) and † ($p<0.08$) indicate trends for a difference between hormone treated and no replacement groups.

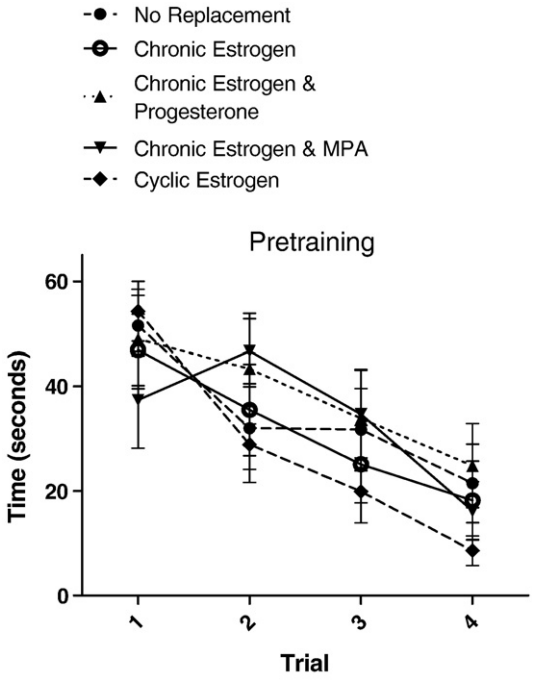

Fig. 1. Mean latency to find the visible platform during four trials of pretraining. There were no significant differences between groups.

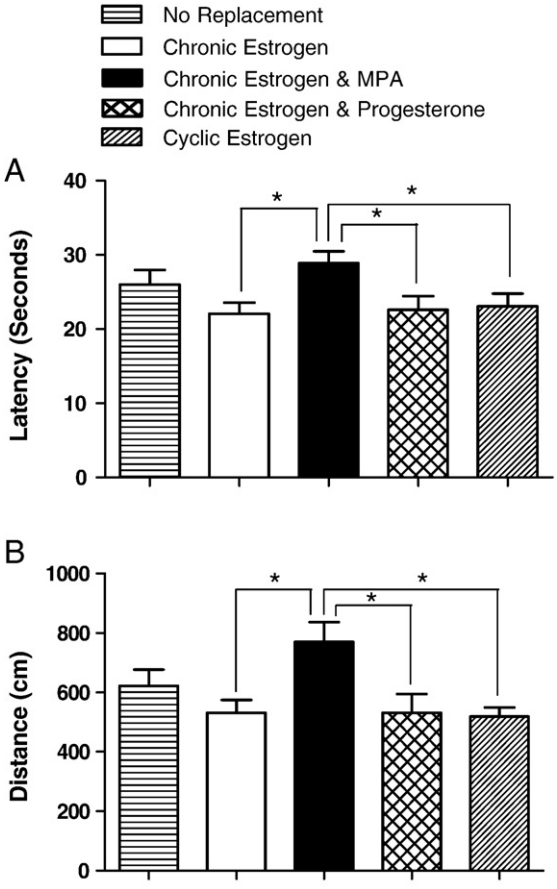

Fig. 2. Mean ± SEM latency (A) and pathlength (B) to find the submerged platform averaged across 4 days of testing for all groups. The E + MPA group had significantly longer latencies (E: $p<0.01$; E + P: $p<0.03$; CYE: $p<0.03$) and pathlengths (E: $p<0.01$; E + P: $p<0.04$; CYE: $p<0.01$) than all other groups receiving hormone replacement.

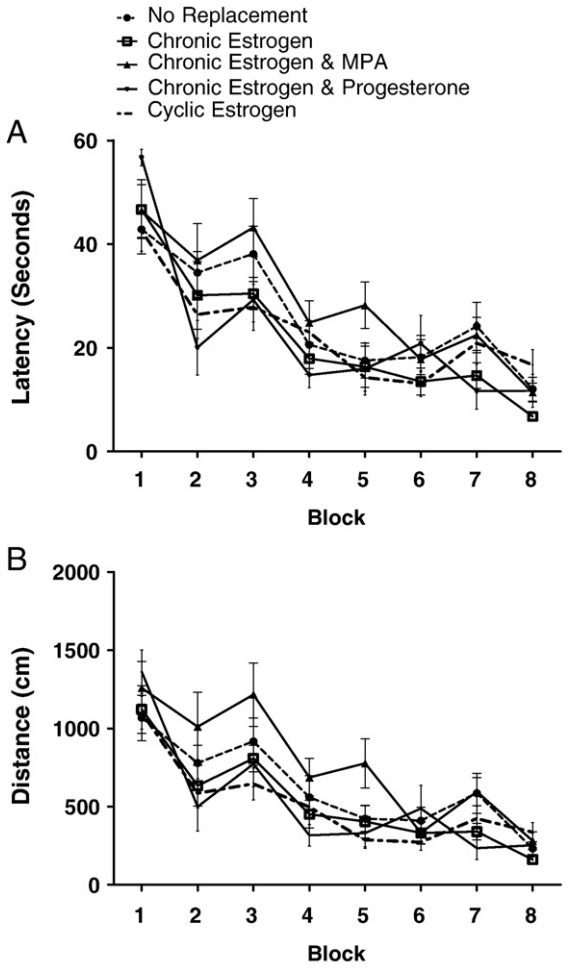

Fig. 3. Mean ± SEM latency (A) and pathlength (B) to find the submerged platform for eight blocks (each consisting of two trials) of testing for all groups. Preplanned comparison of Block 2 versus 3, 4 versus 5, and 6 versus 7 found that none of the groups forgot the task over night except for E + MPA between Block 6 and 7 ($p<0.04$).

Furthermore, preplanned comparisons of treatment at each of the 4 days of testing found that the animals receiving chronic E_2 had significantly shorter latencies than no replacement ($p<0.05$) and cyclic E_2 ($p<0.02$) treated animals on the last day of testing (Fig. 4A). Importantly, there were no differences between any of the groups on the first day of testing.

Pathlength

Differences in pathlength were very similar to those found for latency. There was a significant effect of day on pathlength (F (3, 126) = 57.715, $p<0.001$) such that pathlength became shorter as testing progressed. There was also a main effect of treatment ($F(4,42) = 4.198$, $p<0.007$). The treatment by day interaction was not significant. Post-hoc t-tests revealed that the chronic E_2 and MPA group had significantly longer pathlengths than all other groups receiving hormone treatment (E: $p<0.008$; E + P: $p<0.04$; CYE: $p<0.004$), but they were not significantly different from the no replacement group (Fig. 2B). No hormone treated group was significantly different than the no replacement group. Analysis by 2-trial block indicated that none of the groups forgot the task overnight, except for E + MPA between blocks 6 and 7 ($p<0.04$) (Fig. 3B). Similar to latency, all groups improved performance across training as seen with the significant linear trend for block ($F(1, 42) = 137.055$, $p<0.001$), but treatment groups did not differ in their rates of improvement.

Furthermore, preplanned comparisons of treatment at each of the 4 days of testing found that on the last day of testing, the chronic E_2-treated animals had significantly shorter pathlengths than the cyclic E_2-treated animals ($p<0.05$) and a trend in comparison to no replacement animals ($p<0.09$) (Fig. 4B). There were no differences between any of the groups on the first day of testing.

Swim speed

Swim speed decreased across the 4 days of training ($F(3, 126) = 4.668$, $p<0.01$). There was no effect of treatment on swim speed, as well as no treatment by day interaction (Table 2).

Probe trial

Treatment did not significantly influence the amount of time spent in the target quadrant during the full 60 s or during the first 30 s of the probe trial (Fig. 5).

Discussion

The present study found that in middle aged female rats, long-term administration of chronic E_2 with MPA resulted in worse performance on the Morris water maze than all other types of hormone treatment but not the group with no replacement. Also by the fourth (and last) day of training, chronic E_2 treatment improved performance on the maze, but this effect was not seen earlier in training nor was it seen in the cyclic E_2 group. It should be noted that hormone treatment did not alter swim speed and there were no differences in pretraining latencies which suggests no motivational differences between groups.

The most striking finding is that middle aged rats receiving E_2 in combination with MPA, while not different than no replacement, performed significantly worse on the Morris water maze than all other groups receiving hormone treatment. To our knowledge, no published studies have investigated the effects of E_2 in combination with MPA, the most common progestin given to women, on cognition in an aging animal model. However, a recent study found that MPA administered without estrogen impaired performance on the water radial arm maze and the spatial water maze (Braden et al., 2010). In addition, the Women's Health Initiative found that conjugated equine estrogen in combination with MPA increased the number of subjects diagnosed with either probable dementia or mild cognitive impairment in post menopausal women (Rapp et al., 2003b; Shumaker et al., 2003, 2004; Espeland et al., 2004). Although the dose of MPA was calculated to be similar to that in humans, the route of administration in humans is oral, while the route used in this study was subcutaneous. As previously noted, routes of administration alter rates of metabolism and therefore future studies need to assay levels of MPA in similarly treated animals of the same age.

The known neural effects of MPA are mixed. In vitro studies have found that E_2 protected against glutamate toxicity while E_2 in combination with MPA did not (Nilsen et al., 2006). In addition, progesterone protected against kainic acid-induced neuronal loss while MPA failed to do so (Ciriza et al., 2006). However, in male brain-injured rats, MPA resulted in a reduction of cerebral edema (Wright et al., 2008). Also, chronic treatment with conjugated equine estrogen alone or in combination with MPA resulted in an increase in the number of synapses in the hippocampus in young female rats (Silva et al., 2000). At present, too little is known about long-term neural effects of hormone treatments during normal aging to predict behavioral outcomes. Furthermore, this task is a measure of spatial reference memory and it is possible that the outcome would be different in other tasks that depend on other types of memory (e.g., working, nonspatial), and this should thus be further evaluated.

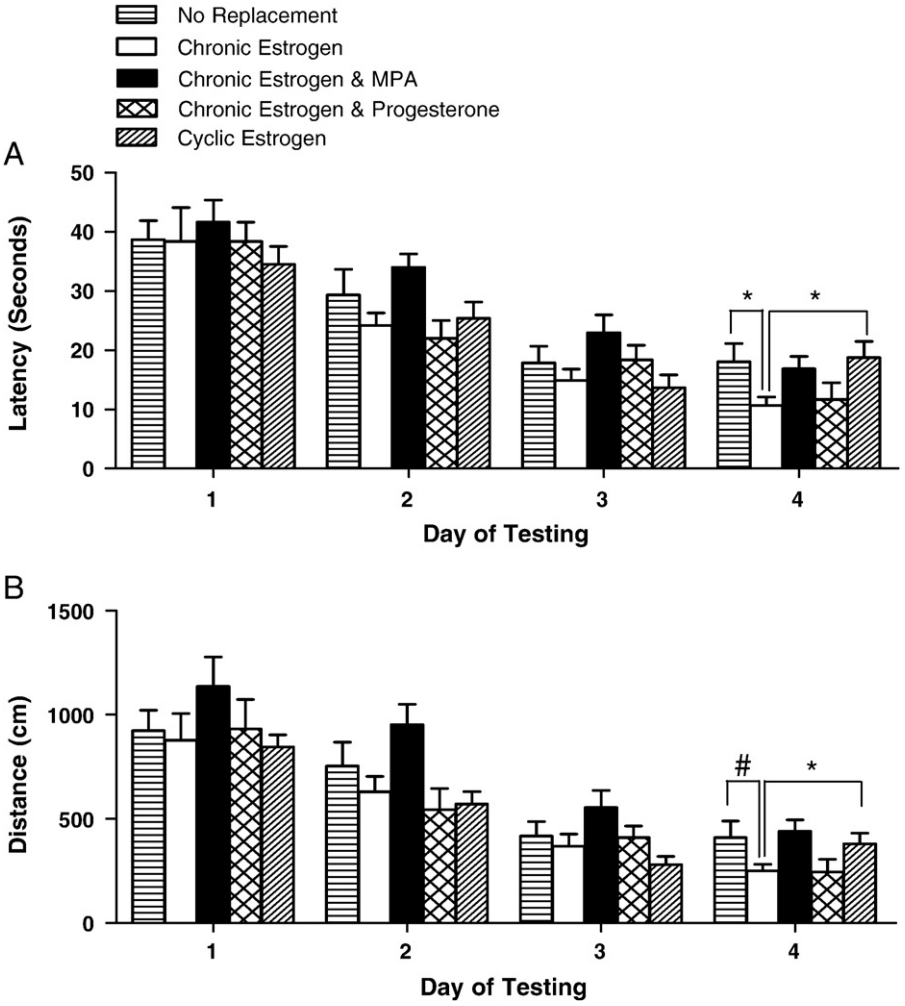

Fig. 4. Mean ± SEM latency (A) and pathlength (B) to find the submerged platform for each of the 4 days of testing for all groups. The group receiving chronic 17 β-estradiol performed better on Day 4 than both NR and CYE groups. * ($p<0.05$); # indicates a trend ($p<0.09$).

Our finding that E_2 improved water maze performance on the fourth day of testing is consistent with previous work from our lab and others showing that chronic treatment with E_2 or estradiol benzoate benefited acquisition and retention of the water maze in middle age female rats (Markham et al., 2002; Foster et al., 2003; Talboom et al., 2008). Although we did not see significant differences on our probe trial, it is possible that the trends seen would have been significant with a larger sample size. Studies have also found that chronic E_2 treatment in middle aged OVX rats enhanced performance on the several other tasks including, the T-maze (Gibbs, 2000), object recognition (Vaucher et al., 2002), and spontaneous alternation (Miller et al., 1999). However, not all studies in middle aged animals have found beneficial effects of hormone treatment (Fernandez and Frick, 2004; Gresack and Frick, 2006). Since there is increasing evidence that hormone treatment may not be beneficial if initiated after long-term deprivation of hormones all studies discussed previously initiated hormone treatment within 1 month of ovariectomy. Although, the length of hormone deprivation fails to explain the differing results of the previous studies, other variables to consider

Table 2
Average swim speed for all groups.

Hormone group	Day 1	Day 2	Day 3	Day 4
No replacement	21.39	26.61	22.82	21.47
Chronic estrogen	23.98	24.57	23.57	22.26
Chronic estrogen and progesterone	25.44	26.51	25.92	25.45
Chronic estrogen and MPA	27.60	26.71	25.81	24.86
Cyclic estrogen	25.59	23.93	19.57	21.28

Mean swim speed (cm/s) for each of the 4 days of testing. Swim speed decreased across the 4 days of testing ($p<0.01$). There were no differences between treatment groups and no treatment by day interaction.

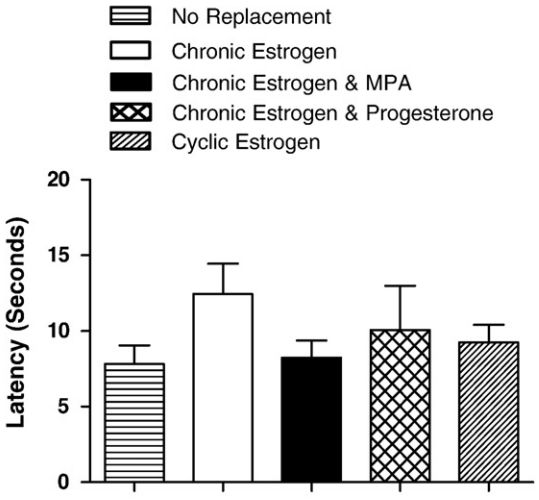

Fig. 5. Mean ± SEM time spent in the target quadrant during the first 30 s of the probe trial for all groups. There were no significant differences between groups.

include the route of administration, the type of task used, and species and strain differences. In addition, a recent study found that reproductive experience alters responsiveness to estrogens (Barha and Galea, 2009), and it is possible that the use of retired breeders in the present study influenced our results. Furthermore, animals in the present study were exposed to a behavioral experiment prior to the present one which could have influenced performance.

The oral route of administration was used here to model the most common route of administration of hormone replacement in post menopausal women. In rodents, E_2 in the drinking water has been shown to produce levels that fluctuate throughout the day with the highest levels two hours after the onset of the dark cycle and then falling back to baseline during the following light cycle (Gordon et al., 1986). The only other study to use this route of administration found improved performance on object recognition but impaired reference memory on a water-escape motivated 8-arm radial arm maze (Fernandez and Frick, 2004). In contrast, another study from the same lab found that daily injections of E_2 did not improve performance on object recognition (Gresack and Frick, 2006). A possible explanation for the differing results is that the stress of injections may have interfered with the ability of E_2 to improve performance on object recognition. Chronic oral administration of hormones removes the stress associated with daily injections, thereby decreasing the possibility of an interaction of hormones and stress on the behavioral outcome. Furthermore, the oral route allows for first pass metabolism of the hormone which may affect the outcome of the treatment. Although we were not able to measure blood levels of estrogen, the group differences in body weights and uterine weights indicate that the treatment was physiologically effective. Future studies should assay levels of estradiol (and MPA) in these treated groups. It should be noted that because estrogen was dissolved in ethanol, the hormone treated groups were exposed to a small amount of ethanol (0.014 g/kg/day) in their drinking water, while the no replacement animals were not. However, a previous study found no effect on the water maze when adult rats were exposed to 0.5 g/kg of ethanol, and ethanol levels of 2.0 g/kg were required for impairments on water maze performance in adult animals (Acheson et al., 2001).

Unlike chronic E_2 treatment, there were no indications that cyclic E_2 treatment benefited performance on the Morris water maze. On the fourth day of testing, the chronically treated E_2 group performed significantly better than the animals treated cyclically. Animals in the cyclic E_2 group were on the third day of their 4-day cycle of hormone treatment and thus received E_2 on this day of testing. The studies that have investigated the effects of repeated cyclic hormone treatment are inconsistent. Weekly injections of E_2 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 et al., 2003a). Two other studies have found that both continuous and intermittent E_2 significantly improved task acquisition in middle aged and aged animals (Markowska and Savonenko, 2002; Bimonte-Nelson et al., 2006). However, cyclic administration of estradiol benzoate did not improve performance in middle-aged female rats on a 12-arm radial arm maze (Ziegler and Gallagher, 2005). Furthermore, while continuous E_2 treatment had no effect on spatial working or reference memory in the radial arm maze, intermittent E_2 treatment impaired spatial reference memory (Gresack and Frick, 2006). Although the results of these studies differ, none of the studies found that cyclic treatment of hormones was more beneficial for task performance than chronic treatment. It is important to note that most of the studies that have attempted to mimic the natural cycle of hormones have only simulated certain aspects of the cycle, so that the inconsistent results are not surprising. For example, estrogen cyclicity was mimicked in the current study, but not the fluctuation in progesterone levels. It is possible that 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.

While E_2 in combination with progesterone did not benefit this task, it did not impair performance as did E_2 in combination with MPA. The few studies that have administered estrogens in combination with progesterone have resulted in equivocal findings. Some studies have found beneficial effects of estrogens given with progesterone. Gibbs (2000) found that weekly injections of E_2 in combination with progesterone improved T maze performance in aged rats. Furthermore, in middle aged rats, E_2 plus progesterone enhanced performance on the Morris water maze as well as E_2 alone (Markham et al., 2002). In contrast, another study found that progesterone reversed the beneficial effects of estradiol on this same task in middle aged rats (Bimonte-Nelson et al., 2006). However, similar to the present study, Bimonte-Nelson et al. (2006) found that while long-term treatment with estradiol and progesterone does not benefit the water maze, animals receiving this combination are not impaired on the task when compared to those not receiving hormone treatment.

Performance on the Morris water maze is dependant to a large degree on the hippocampus (Redish and Touretzky, 1998). Several studies have reported that estrogens influence the morphology and functional connectivity of the hippocampus in young adult females (e.g. Woolley and McEwen, 1992; Warren et al., 1995; Woolley et al., 1997). However, E_2 treatment does not increase spines in aged animals (Adams et al., 2001). Interestingly, chronic MPA exposure results in an increase in synapses in the hippocampus of young animals (Silva et al., 2000), although it is not known if this occurs in aged animals. The neural mechanisms underlying the behavioral effects of hormone treatment may be different in young and aged animals. In future studies it will be imperative to examine the neural effects of MPA, the progestin most commonly prescribed to menopausal women, in an aging female model.

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