Dose and delivery method impact cognitive outcome of Ethinyl Estradiol administration in the surgically menopausal rat

by

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ABSTRACT

Ethinyl estradiol, (EE) a synthetic, orally bio-available estrogen, is the most commonly prescribed form of estrogen in oral contraceptives (Shively, C., 1998), and is found in at least 30 different contraceptive formulations currently prescribed to women (Curtis et al., 2005). EE is also used in hormone therapies prescribed to menopausal women, such as Femhrt™ (Simon et al., 2003). Thus, EE is prescribed clinically to women at ages ranging from puberty through reproductive senescence.

Here, in two separate studies, the cognitive effects of cyclic or tonic EE administration following ovariectomy (Ovx) were evaluated in young, female rats. Study I assessed the cognitive effects of low and high doses of EE, delivered tonically via a subcutaneous osmotic pump. Study II evaluated the cognitive effects of low, medium, and high doses of EE administered via a daily subcutaneous injection. For these studies, the low and medium doses correspond to the range of doses currently used in clinical formulations, and the high dose corresponds to the range of doses prescribed to a generation of women between 1960 and 1970, when oral contraceptives first became available. For each study, cognition was evaluated with a battery of maze tasks tapping several domains of spatial learning and memory. At the highest dose, EE treatment impaired multiple domains of spatial memory relative to vehicle treatment, regardless of administration method. When given cyclically at the low and medium doses, EE did not impact working memory, but transiently impaired reference memory during the learning phase of testing. Of the doses and regimens tested here, only EE at the highest dose impaired several domains of memory; this was seen for both cyclic and tonic regimens. Cyclic and tonic delivery of low EE, a dose that corresponds to doses used in the clinic today, resulted in transient and null impairments, respectively, on cognition.

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INTRODUCTION

Ethinyl estradiol (EE), a synthetic form of naturally-circulating 17β-estradiol (E2), is by far the most commonly used form of estrogen in oral contraceptives (Shively, C., 1998), and is found in at least 30 different formulations (Curtis et al., 2005). A national survey on contraceptive use from the Centers for Disease Control found that the oral contraceptive pill was the top method of contraception in the United States; 10.7 million women were using hormonal contraceptives between 2006 and 2008, comprising around 18% of all women (Mosher and Jones, 2010). EE cannot be converted to estrone or other weaker estrogens, making EE more potent than the endogenous E2, which is converted to other estrogens (Prokai-Tatrai, K., et al., 2005, Kuhl, H. 2005).

EE is also found in hormone therapies (HTs) prescribed to menopausal women, such as Estinyl™ and Femhrt™ (Simon et al., 2003). Menopause is the complete, permanent termination of the menses via the depletion of ovarian follicles (Hawkes, K., 2003), during which, women undergo a drastic decrease in estrogens and progesterone. Though the life expectancy of women in the United States is steadily increasing, the age of menopause onset remains constant, at around 45-50 years of age (Hawkes, K., 2003). Thus, women are now spending a larger proportion of their lives in a menopausal state, and whether to take HT is a decision that many women will be faced with as aging ensues. Menopause has been linked with cognitive deficits amongst a multitude of other negative symptoms, including osteoporosis and cardiovascular disease (Notelovitz, M., 2006).

Although EE is among the most commonly prescribed forms of estrogen and is prescribed to women at ages ranging from puberty to post-menopause, most of the existing clinical and preclinical research on the cognitive impact of estrogens

does not include EE (for review see: Bimonte-Nelson et al., 2010). The few studies that have investigated the cognitive effects of EE have found mixed results. A study evaluating use of various EE-containing hormonal contraceptives in young adult women found no cognitive impact on a battery of five well-established psychometric tests measuring memory and concentration (Silber et al., 1987). However, another study found that the active pill phase enhanced verbal memory compared to the inactive pill phase; these effects were not shown on visuospatial memory, verbal fluency, visuospatial abilities, or attention (Mordecai et al., 2008). Importantly, because women in these studies were taking several different contraceptive formulations, the results cannot be attributed to any specific dose of EE or progestin component.

EE is also used in HT formulations, and there are studies investigating the cognitive effects of EE in older subjects. In a study of menopausal women using fMRI, EE-containing HT was found to increase frontal cortex activation during working memory testing, suggesting a possible cognitive benefit of EE-containing HTs on cognition (Smith, Y.R., et al., 2006). In aged, ovariectomized (Ovx) rhesus monkeys, EE improved spatial working memory (Lacreuse et al., 2002), but impaired performance on a face recognition task (Lacreuse and Herndon, 2003), and had no impact on executive function tasks, which involve higher-order cognition and decision-making processes (Lacreuse et al., 2004).

The cognitive effects of EE alone have not yet been methodically evaluated in a rodent model and the cognitive effects of EE remain unclear. Animal models allow the unique opportunity to evaluate the cognitive impact of EE without a progestin component at several doses and using multiple methods of administration in a model with rigorous control. Here, two modes of delivery are tested to model tonic

and cyclic administration regimens. In women taking daily oral contraceptives, serum levels differ from those of women using alternative methods such as transdermal patches or vaginal inserts. Specifically, oral contraceptives produce a rise and fall of serum EE levels throughout the day, with serum concentrations highest following ingestion of the pill, and falling throughout the rest of the 24 hour cycle (van den Heuvel et al., 2005; Devineni et al., 2012). Transdermal patches and vaginal inserts produce lower, steady serum levels of EE, unlike the rise and fall pattern produced by daily oral ingestion. There is accumulating evidence that tonic estrogen-containing treatment regimens can provide cognitive benefits. For example, in randomized, placebo controlled studies looking at women with mild-moderate probable Alzheimer's disease, treatment with E2 using a transdermal delivery method has been repeatedly shown to a positively affect multiple measures of cognitive function, such as attention, verbal memory, visual memory and semantic memory (Asthana et al., 1999; Wharton et al., 2011). A study evaluating the effects of transdermal versus oral contraceptive use on general quality of life measures in women found no difference in quality of life, side effects or impact on regularity of the menses, however patch users were more likely to report that their contraceptive had a favorable impact on "daily activities" (Sucato et al., 2011). Finally, there is evidence for other health-related benefits with transdermal over oral delivery. For example, metabolism by the liver following oral administration of various estrogens is linked to numerous markers associated with increased risk of thromboembolic side effects whereas transdermal administration is not (Scarabin et al., 1997; Decensi et al., 2002; Post et al., 2003).

Study I evaluates the effects of two doses of EE, delivered tonically, on cognition in young, Ovx rats, relative to vehicle treatment, using a battery of spatial

memory tasks. Study I will be performed to evaluate the cognitive effects of EE, administered via subcutaneous pumps that deliver a steady rate of hormone throughout the day. This administration regimen most closely resembles the tonic pattern of EE delivery resulting from a transdermal patch or vaginal insert in humans. A low dose of EE, corresponding to the most popular dose currently prescribed to women will be evaluated as well as a high dose that is outside of the range of doses currently prescribed to women but is representative of the high doses of EE that were previously prescribed in contraceptives, before the health benefits of lower doses were known.

Study II was performed to test the cognitive effects of cyclically administered EE, given via a daily injection, to model daily oral contraceptive use. The characteristic daily flux in serum EE levels seen with cyclic EE use may exert cognitive effects distinct from tonic effects, possibly due to estrogen receptor (ER) cycling. Several studies have shown that cyclic treatment with E2 produces a decrease in number of cytoplasmic ERs one hour after E2 administration, accompanied by an increase in nuclear ER levels. From two to four hours after administration, nuclear ER levels decrease 60-70 percent but cytoplasmic ER levels remain constant, resulting in an overall decreased level of ERs. This is followed by an overall increase in ER number 12 hours after administration, with cytoplasmic and nuclear ER levels returning to control levels 16 hours after treatment administration (Sarff and Gorski, 1971; Kassis and Gorski, 1981; Blaustein et al., 1993, Rosser et al., 1993). This ER cycling is likely to influence the cognitive impact of estrogen treatment, however, there have been no studies evaluating how administration method impacts the cognitive effects of EE treatment.

Additionally, in Study II a medium dose of EE was assessed, equivalent to the highest dose of EE currently available in contraceptives, in order to encompass the entire range of doses currently prescribed to women (Curtis et al., 2005). The purpose of the current studies is to evaluate the cognitive effects of cyclically and tonically delivered EE at several doses, modeling both tonic administration methods, such as with a transdermal patch or vaginal insert, as well as cyclic administration methods, such as with daily oral ingestion. I hypothesize that dose and administration method impact the cognitive effects of EE treatment and predict that the high dose of EE will impair performance on spatial memory tasks, regardless of method of administration, whereas the cognitive effects of low and medium doses may depend on administration method.

METHODS

Study I

Subjects

Subjects were 29 female, Fischer-344 rats raised at the National Institute on Aging colony at Harlan Laboratories (Indianapolis, IN). Animals were three months old at the beginning of the study, four months old at maze test, and five months old at sacrifice. After arrival, animals were pair-housed, had food and water ad-lib, and were maintained on a 12-h light/dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee and adhered to National Institutes of Health standards (Appendix A).

Experimental Design and Hormone Treatments

At three months of age, all animals received Ovx surgery. Rats were anesthetized via isoflurane inhalation, underwent bilateral dorsolateral incisions in the skin and peritoneum, and ovaries and tips of the uterine horn were ligatured and removed. Muscle and skin were then sutured closed. During surgery, rats received a single injection of Rimadyl™ (5mg/ml/kg) for pain and saline (2ml) to prevent dehydration.

Eighteen days after Ovx, all animals received a subcutaneous Alzet Osmotic pump to deliver vehicle or hormone treatment. Rats were anesthetized via isoflurane inhalation and a small incision was made in the dorsal scruff of the neck, where a subcutaneous pocket was created. A pump filled with vehicle or one of two EE doses was inserted into the subcutaneous pocket, and the skin was then closed with surgical staples. Rats were randomly assigned to one of three treatments: vehicle (propylene glycol) (n=10), low EE (0.125µg per day) (n=9), or high EE

(0.3μg per day) (n=10). EE (Sigma, St. Louis, MO) was dissolved in propylene glycol and released at a rate of 2.5μl per hour throughout the remainder of the study. The low EE dose was based on a 30-35μg per day regimen that an average woman weighing 60-70kg would be prescribed in an oral contraceptive, adjusted to the weight of a rat (about 0.25kg) (Curtis et al., 2005). Since EE is 10 times as potent as 17β-estradiol (E2) (Fotherby, 1996), the high EE dose was one tenth of a dose of E2 previously shown to enhance performance on spatial tasks (Talboom et al., 2008). The high dose of EE also corresponds to a 70-80μg/day dose of EE in a 60-70kg woman, which is within the range of what was previously available in contraceptives for women during the early 1960's, before the health benefits of lower-dose formulations were known (Chadwick et al., 2012).

Water Radial-Arm Maze

Eighteen days after the start of injections, subjects were tested for 13 days on the eight-arm, win-shift Water Radial-Arm Maze (WRAM) to evaluate spatial working and reference memory, as previously described (Bimonte and Denenberg, 1999; Bimonte et al., 2000, 2002, 2003; Bimonte-Nelson et al., 2003; Bimonte-Nelson et al., 2004). The maze was an eight-arm apparatus (each arm 38.1 x 12.7cm) filled with opaque, room temperature water. Four of the eight arms contained a hidden platform (10cm diameter) just beneath the surface of the water and spatial cues were present to aid the animals in spatial navigation. Each subject was assigned different platform locations that remained fixed across all days of testing. A subject was released from the start arm and given 3 minutes (min) to locate a platform. Once a platform was found, the animal remained on it for 15 seconds (s), then was returned to its heated testing cage for a 30s inter-trial interval (ITI).

During the ITI, the just-found platform was removed from the maze and the water was cleaned to remove any debris and to obscure olfactory cues. The animal was then placed back into the start arm and given another 3min to locate a platform. Each animal received four trials per day for 13 days, with the number of remaining platforms reduced by one on each subsequent trial. Thus, the working memory system was increasingly taxed as trials progressed within a day, allowing working memory load to be assessed. On the 13th day of testing, a six-hour delay was instilled between trials 2 and 3 to test delayed memory retention.

Errors were quantified using orthogonal measures of working and reference memory, as done previously (Jarrard et al., 1984; Bimonte et al., 2000, 2002; Hyde et al., 2000; Acosta et al., 2009, 2010; Braden et al., 2010, 2011). Working Memory Correct (WMC) errors were defined as all entries into any arm that previously contained a platform, Reference Memory (RM) errors were first entries into any arm that never contained a platform and Working Memory Incorrect (WMI) errors were all subsequent entries into arms that never contained a platform. An arm entry was counted when the tip of a rat's snout crossed a mark on the outside of the arm (not visible from inside the maze; 11 cm into the arm).

Morris water maze

One day after completion of the WRAM, spatial reference memory was evaluated using the Morris Water Maze (MM). The apparatus was a round tub (188cm diameter) filled with opaque, room temperature water containing a submerged platform (10cm diameter) in the North East quadrant. The platform remained in a fixed location across all days and trials with spatial cues available to aid the animals in spatial navigation, testing spatial reference memory (Morris et al.,

1982). Testing consisted of six trials per day for three days. At the beginning of each trial, animals were dropped off from one of four starting points (North, South, East or West), varying semi-randomly. Animals had 60s to locate the platform, where they remained for 15s before being placed back into a heated cage for an ITI of 5-8min. To evaluate whether animals spatially localized the platform, a seventh probe trial was given on the third day of testing, during which the platform was removed and animals were given 60s to swim freely in the maze. A video camera and tracking system tracked and measured each rat's swim path (Ethovision; Noldus Instruments, Wageningen, The Netherlands).

Visible platform task

After completion of behavioral testing, motor and visual competence was evaluated using the visible platform task. This was a non-spatial adaptation of the cue-navigation version of the spatial MM task, previously used to dissociate visual and motor acuity from place memory (Morris et al., 1982). This task is ideal for this purpose due to its similarity to other spatial water-maze tasks with respect to motor and visual requirements, the only difference being that animals are not required to associate the location of the platform with distal spatial cues. The apparatus was a rectangular tub (100 x 60cm) filled with clear room temperature water. A black platform (10cm wide) was positioned 4cm above the surface of the water, following previously published methods (Hunter et al., 2003). A ring of opaque curtains surrounded the maze, blocking all spatial cues to prevent spatial navigation. Animals were given six trials in one day. The drop off location remained the same across trials; however the platform location varied semi-randomly across three

locations. Each rat had 90s to locate the platform, where it remained for 15s before being placed back into a heated cage for an ITI of 5-8min.

Markers of Peripheral Stimulation

To verify Ovx and subsequent hormone treatment, vaginal smears were taken at four months of age for four days, after animals were given hormone treatment. Smears were classified as proestrus, estrous, metestrus, or diestrus (Goldman et al., 2007; Acosta et al., 2009b; Engler-Chiurazzi et al., 2011, 2012). At sacrifice, uteri of all subjects were removed and trimmed of visible fat, and wet uterine weight (grams) was measured, as done previously (Westerlind et al., 1998; Acosta et al., 2009b; Engler-Chiurazzi et al., 2011, 2012). Osmotic pumps were removed and visually inspected upon sacrifice for visible cracks or tops that had come off of the pumps.

Statistical Analyses

WRAM testing was blocked into learning (days 2-7) and asymptotic (days 8-12) phases, based on prior studies (e.g., Bimonte & Denenberg, 1999; Bimonte et al., 2000, 2003; Hyde et al., 2000). Data were analyzed separately for each type of error using repeated measures ANOVA, with treatment as the between-groups variable and number of errors on each trial as the repeated measure. Group differences on the lattermost portion of WRAM testing have been observed previously, with most pronounced effects on trial 4, the highest working memory load trial (Bimonte & Denenberg, 1999; Bimonte-Nelson et al., 2003, 2004; Braden et al., 2010); therefore, interactions between treatment and working memory load (trials) were analyzed(α=0.05). Fisher PLSD post hoc tests were used. Alpha level was 0.05.

MM data were analyzed using repeated measures ANOVA, with treatment as the between-groups variable and distance to the platform as the repeated measure. Probe trial data were analyzed with repeated measures ANOVAs with percent distance in the Northeast (platformed) and Southwest (diagonally opposite) quadrants as the repeated measure.

Visible platform data were analyzed using repeated measures ANOVA, with treatment as the between-groups variable and latency to reach the platform on each trial as the repeated measure.

Study II

Subjects

Subjects were 36 female, Fischer-344 rats raised at the National Institute on Aging colony at Harlan Laboratories (Indianapolis, IN). Animals were three months old at the beginning of the study, four months old at maze test, and five months old at sacrifice. After arrival, animals were pair-housed, had food and water ad-lib, and were maintained on a 12-h light/dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee and adhered to National Institutes of Health standards.

Experimental Design and Hormone Treatments

Following arrival, experimental procedures were identical to those in Study I through Ovx surgeries. Eighteen days after Ovx, animals started receiving daily, subcutaneous injections at a volume of 0.1ml, continuing until sacrifice. Rats were randomly assigned to one of four treatment groups (n=9 per group): vehicle (sesame oil), EE low (0.125μg per day), EE med (0.18μg per day), or EE high (0.3μg

per day). EE (Sigma, St. Louis, MO) was dissolved in sesame oil at the appropriate dose at the beginning of the study, then aliquoted into daily quantities and stored in the refrigerator (2-4°C) until needed. Behavioral testing and sacrifice procedures were identical to those in Study I. Statistical analyses were identical to those in Study I.

RESULTS

Study I

Water Radial Arm Maze

There were no effects of EE treatment on WMC, WMI or RM errors during the learning phase of testing. During the asymptotic phase of testing, there was a Trial x Treatment interaction for both WMC [$F_{(4,48)}$=3.22; p<.05] and WMI [$F_{(6,72)}$= 5.28; p<.01] errors (fig 1). Two group planned comparisons showed that the high EE group committed more WMC [$F_{(2,36)}$=3.38; p<.05] and WMI [$F_{(3,54)}$=5.06; p<.01] errors than the vehicle group as working memory load increased. Post hoc analyses showed that high EE animals also made more WMI and WMC errors than low EE animals on the trial with the highest working memory load (Fisher; p<.05) (fig 1). There was also a main effect of Treatment for WMI errors across all trials [$F_{(2,24)}$= 4.99; p<.05] during the asymptotic portion of testing. Planned comparisons showed the high EE group made more errors than the vehicle group [$F_{(1,18)}$= 4.38; p=.05], and post hoc analyses showed that the high EE group also made more errors than the low EE group (Fisher; p<.05) across all trials. There were no differences between the low EE and vehicle groups and there were no differences in number of RM errors (fig 1).

Morris Water Maze

There were no Treatment main effects for days 1-3 (fig 2a). For the probe trial, a higher percent distance was spent in the previously platformed vs. the opposite quadrant [$F_{(1, 24)}$=12.87, p<0.01], with no Percent Distance by Treatment interaction, indicating that all groups spatially localized the platform by the end of testing (fig 2b).

Visible platform task

There were no Treatment effects for Latency ($p>0.05$), indicating that all treatment groups possessed a similar level of capability to solve a water maze task (fig 3). Levene's homogeneity of variance F-test showed that Homogeneity of variance was violated in this analysis with the high EE group exhibiting greater variance than both the vehicle group ($F=10.51$, $p<0.0001$) and the low EE group ($F=6.23$, $p<0.0001$), across all six trials. Closer inspection of the data revealed an outlier in the high EE group; one animal had latencies far outside of two standard deviations of the mean from the rest of the high EE group across trials 1-4 of visible platform testing. However, for trials 5 and 6 of the visible platform task, this animal was no longer outside of two standard deviations of the rest of the high EE group and homogeneity of variance was not violated for trials 5 or 6 of visible platform testing, according to Levene's test for each trial. Also, upon closer inspection of all behavioral data, this animal was determined not to be an outlier for any of the other behavioral measures. Since this animal was able to perform the visible platform task by the end of the testing session and did not demonstrate difficulties during any of the memory tasks, this suggests the animal possessed the motor and visual capacities necessary to perform a water maze task and, thus, this animal was not excluded from any of the behavioral analyses.

Markers of Peripheral Stimulation

Thirty-one days after pump insertion surgeries, all vehicle-treated rats exhibited diestrous smears, indicating a lack of uterine stimulation, while animals treated with any dose of EE alternated between estrous and metestrous smears,

with each smear showing numerous cornified cells indicating uterine stimulation (Goldman et al., 2007). For wet uterine weight, there was a significant effect of hormone treatment [$F_{(1,15)}$= 71.03; p <.0001], with planned comparisons showing that that uteri of vehicle treated rats weighed less than uteri of EE low [$F_{(1,17)}$= 114.29; p <.0001] and EE high [$F_{(1,17)}$= 161.59; p <.0001] treated rats (fig 4). Pump inspection at sacrifice revealed that all pumps were intact with no visible cracks.

Study II

Water Radial Arm Maze

When delivered via daily subcutaneous injection, there were no effects of treatment on WMC, WMI or RM during the learning portion of testing. During the asymptotic phase of testing, similar to effects with tonic EE treatment, there was a Trial x Treatment interaction for WMC errors [$F_{(6,64)}$= 2.82; p<.05] with the high EE treated animals making more errors than vehicle treated animals as working memory load increased [$F_{(2,32)}$= 5.78; p<.01] (fig 5). Post hoc analyses also showed that the high EE group committed more WMC errors than the med EE and low EE animals on the trial with the highest working memory load (Fisher; p<.05)(fig 5). There were no differences in WMC errors between the vehicle group and the low or med EE group and there were no differences for WMI or RM errors during the asymptotic phase of testing (fig 5).

Morris Water Maze

For day 1 of MM testing, there was a main effect of Treatment [$F_{(3,32)}$=3.22; p<.05], with planned comparisons showing that the vehicle group performed better than the low EE [$F_{(1,16)}$= 6.84; p<.05], med EE [$F_{(1,16)}$= 8.51; p<.05] and high [$F_{(1,16)}$=

9.47; p<.01] groups (fig 6a). There were no effects of treatment for days 2-3 of MM testing. A higher percent distance was spent in the previously platformed vs. the opposite quadrant [$F_{(1,32)}$ =374.33; p<.0001] for the probe trial, with no percent distance by treatment interaction, indicating that all groups spatially localized the platform by the end of testing (fig 6b).

Visible platform task

There were no treatment effects for latency (fig 7), indicating that all animals possessed similar procedural capabilities to solve a water maze task (p>0.05).

Markers of Peripheral Stimulation

Fourteen days after the start of injections, all vehicle-treated rats exhibited diestrous smears, indicating a lack of uterine stimulation, while animals treated with any dose of EE alternated between estrous and metestrous smears, with each smear showing numerous cornified cells indicating uterine stimulation (Goldman et al., 2007). One uterus was lost due to experimental error and was not included in these analyses. For wet uterine weight, there was a significant effect of hormone treatment [$F_{(3,31)}$=29.88; p<0.0001], with uteri of vehicle-treated rats weighing less than EE low [$F_{(1,15)}$= 62.17; p <.0001], EE med [$F_{(1,16)}$= 117.36; p <.0001], and EE high [$F_{(1,16)}$= 109.10; p <.0001] (fig 8).

DISCUSSION

The present studies are the first to investigate the cognitive effects of cyclically- and tonically- administered EE in a rodent. In study I, high tonic EE treatment impaired performance on a multiple domains of a spatial working memory task when working memory load was highest. Tonic treatment with EE did not impact reference memory performance at any dose, as measured by the Morris Water Maze. Importantly, tonic low EE treatment did not affect performance on any task. This is encouraging, as the low EE treatment corresponds to the low end of available doses currently prescribed to women in contraceptive formulations, when corrected for body weight. The high dose of EE produced working memory impairments. However, it is important to note that the high dose, when corrected for body weight, corresponds to a higher dose in women than what is currently available in contraceptives. Although the high EE dose is higher than what women currently take, this dose is within the range of doses prescribed to women between 1960 and 1970, when contraceptives first became available. Importantly, treatments for this study were delivered via subcutaneous osmotic pumps, which release hormone at a steady rate throughout the day, with no diurnal fluctuation in hormone release rate.

Similar to results seen in Study I, Study II found that cyclic EE treatment at the highest dose impaired spatial working and reference memory relative to vehicle treatment. When given cyclically, low and medium doses of EE did not impact spatial working memory, but did produce impairments in spatial reference memory, although this impairment was only present during the learning phase of MM testing and was not evident by the second or third days of the task. Collectively, these results suggest that both dose and method of delivery impacts the cognitive effects

of EE. Specifically, cognitively neutral doses of EE delivered tonically can produce cognitive impairments when delivered in a daily cyclic regimen.

Results from Study I and Study II suggest that the contraceptive regimen that may be optimal for cognition could involve low doses of EE (30-35μg EE/day or less) delivered tonically, such as with a transdermal patch or a vaginal ring, rather than delivered via a daily pill. In addition to modulating the cognitive impact of EE, method of delivery can alter the dose necessary to halt ovulation; contraceptives released in a tonic manner can be given at lower doses, as the resulting range of serum concentrations is more precise than with a daily cyclic regimen (van den Heuvel et al., 2005).

It is crucial to note that EE-containing contraceptives also require a progestin component (Curtis et al., 2005). One commonly prescribed progestin, Medroxyprogesterone Acetate (MPA) (Curtis et al., 2005), when delivered alone, has been shown to impair spatial memory both during treatment as well as several months later, even when MPA levels are no longer detectable in serum (Braden et al., 2010, 2011); EE has yet to be methodically tested for cognition along with specific progestins.

Future directions include comparing the cognitive effects of EE to those of E2 and its metabolites, combining optimal doses of EE with currently available progestins, and investigating ultra-low doses of EE. In particular, I would like to investigate the long-term cognitive impact of EE along with several progestins to determine how contraceptive use throughout aging may impact the trajectory of cognitive changes associated with both age and changes in hormone levels with menopause. The broad goal of this research is to elucidate the impact that clinically prescribed hormones have on cognitive function and, ultimately, to optimize

contraceptives and HTs for cognitive health throughout aging. I hope the results of the current studies will set the stage for a series of future methodical investigations into the effects these clinically-prescribed hormones have on the brain and related memory processes.

Figures

Figure 1. During the asymptotic phase of testing, there was a Trial x Treatment interaction for both WMC [$F_{(4,48)}$=3.22; $p<0.05$] and WMI [$F_{(6,72)}$= 5.28; $p<0.01$] errors. The high EE group committed more WMC [$F_{(2,36)}$=3.38; $p<0.05$] and WMI [$F_{(3,54)}$=5.06; $p<0.01$] errors than the vehicle group as working memory load increased. High EE animals also made more WMI and WMC errors than low EE animals on trial 4, the trial with the highest working memory load ($p<0.05$). There was also a main effect of Treatment for WMI errors across all trials [$F_{(2,24)}$= 4.99; $p<0.05$] with the high EE group making more errors than the vehicle group [$F_{(1,18)}$= 4.38; $p=0.05$], and the low EE group (Fisher; $p<0.05$). There were no differences between the low EE and vehicle groups. There were no differences in RM errors.

Figure 1

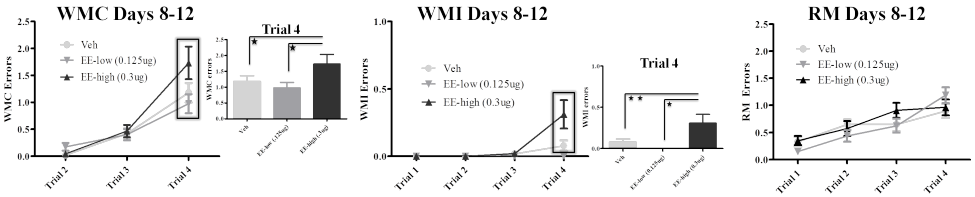

WMC Days 8-12
WMC Errors
Veh
EE-low (0.125ug)
EE-high (0.3ug)
Trial 2
Trial 3
Trial 4
Trial 4
WMI Days 8-12
WMI Errors
Veh
EE-low (0.125ug)
EE-high (0.3ug)
Trial 1
Trial 2
Trial 3
Trial 4
Trial 4
RM Days 8-12
RM Errors
Veh
EE-low (0.125ug)
EE-high (0.3ug)
Trial 1
Trial 2
Trial 3
Trial 4

Figure 2. a) There were no differences for day 1-3 of MM testing ($p>0.05$). b) For the probe trial, a higher percent distance was spent in the previously platformed vs. the opposite quadrant [$F_{(1, 24)}=12.87$, $p<0.01$] with no percent distance by treatment interaction, indicating that all groups spatially localized the platform by the end of testing.

Figure 2.

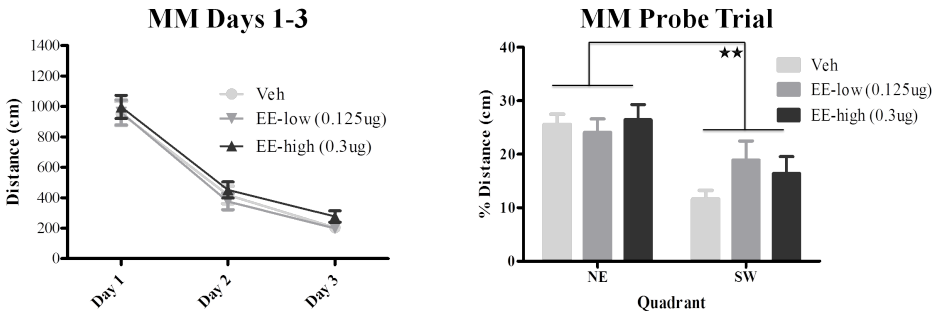

MM Days 1-3
Distance (cm)
1400
1200
1000
800
600
400
200
0
Day 1
Day 2
Day 3
Veh
EE-low (0.125ug)
EE-high (0.3ug)
MM Probe Trial
% Distance (cm)
40
30
20
10
0
NE
SW
Quadrant
★★
Veh
EE-low (0.125ug)
EE-high (0.3ug)

Figure 3. Tonic EE treatment does not impact swim ability. There were no treatment effects for latency on the visible platform task [$F_{(2,24)}$= 1.10, p= 0.35 NS].

Figure 3.

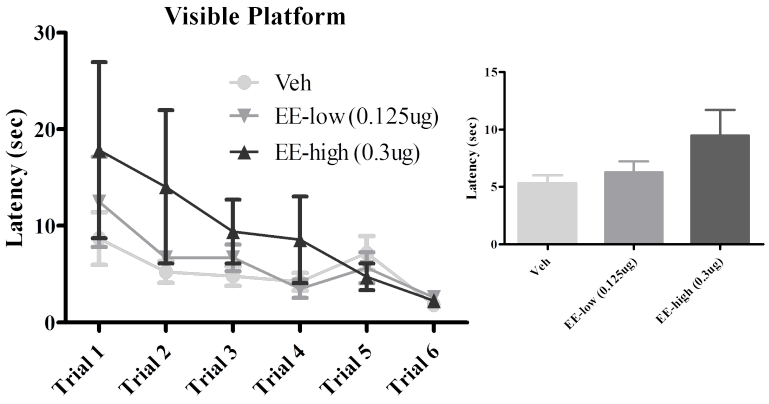

Visible Platform
Latency (sec)
30
20
10
0
Veh
EE-low (0.125ug)
EE-high (0.3ug)
Trial 1
Trial 2
Trial 3
Trial 4
Trial 5
Trial 6
Latency (sec)
15
10
5
0
Veh
EE-low (0.125ug)
EE-high (0.3ug)

Figure 4. Tonic EE treatment increased uterine weights. There was a main effect of treatment [$F_{(1,15)}$= 71.03; p<0.0001]. Uteri of vehicle treated rats weighed less than uteri of EE low [$F_{(1,17)}$= 114.29; p<0.0001] and EE high [$F_{(1,17)}$= 161.59; p<0.0001] treated rats.

Figure 4.

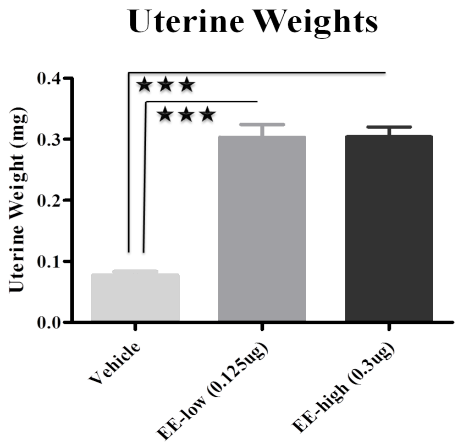

Uterine Weights
Uterine Weight (mg)
0.4
0.3
0.2
0.1
0.0
★★★
★★★
Vehicle
EE-low (0.125ug)
EE-high (0.3ug)

Figure 5. During the asymptotic phase of testing, there was a Trial x Treatment interaction for WMC errors [$F_{(6,64)}$= 2.82; $p<0.05$] with the high EE treated animals making more errors than vehicle treated animals as working memory load increased [$F_{(2,32)}$= 5.78; $p<.01$]. Post hoc analyses also showed that the high EE group committed more WMC errors than the med EE and low EE animals on trial 4, the trial with the highest working memory load [$F_{(3,32)}$= 2.89; $p=0.05$]. There were no differences in WMC errors between the vehicle group and the low or med EE group. There were no differences for WMI or RM errors.

Figure 5.

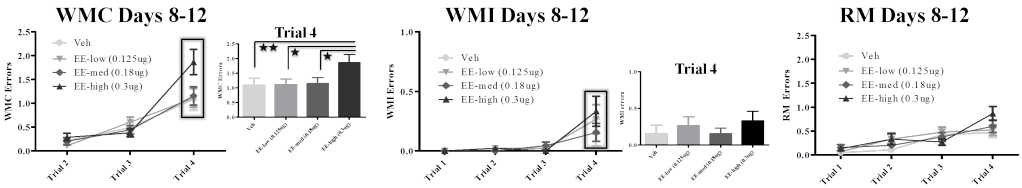

WMC Days 8-12
Trial 4
WMI Days 8-12
Trial 4
RM Days 8-12
Veh
EE-low (0.125ug)
EE-med (0.18ug)
EE-high (0.3ug)
WMC Errors
WMI Errors
RM Errors
Trial 1
Trial 2
Trial 3
Trial 4

Figure 6. a) There was a main effect of Treatment [$F_{(3,32)}$= 3.22; $p<0.05$] for day 1. The vehicle group performed better than the low EE [$F_{(1,16)}$= 6.84; $p<0.05$], med EE [$F_{(1,16)}$= 8.51; $p<0.05$] and high EE [$F_{(1,16)}$= 9.47; $p<0.01$] groups. There were no effects of treatment for days 2-3 of MM testing. b) A higher percent distance was spent in the previously platformed vs. the opposite quadrant [$F_{(1,32)}$= 374.33; $p<0.0001$] for the probe trial, with no percent distance by treatment interaction, indicating that all groups spatially localized the platform equally by the end of testing.

Figure 6.

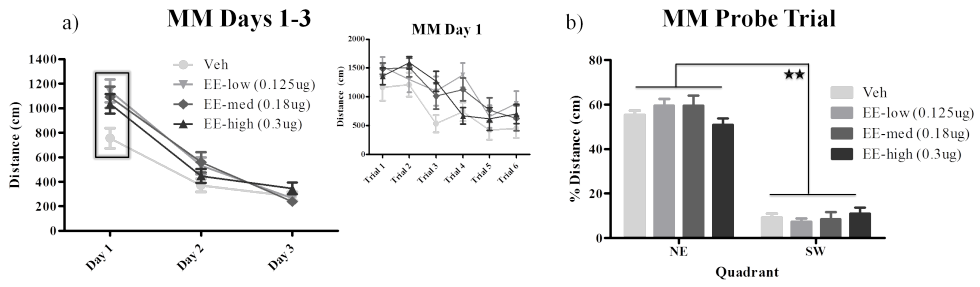

a)
MM Days 1-3
Veh
EE-low (0.125ug)
EE-med (0.18ug)
EE-high (0.3ug)
Distance (cm)
Day 1
Day 2
Day 3
MM Day 1
Trial 1
Trial 2
Trial 3
Trial 4
Trial 5
Trial 6
b)
MM Probe Trial
% Distance (cm)
NE
SW
Quadrant

Figure 7. There were no treatment effects for latency on the visible platform task [$F_{(3,32)}$= 0.72, p= 0.55 NS].

Figure 7.

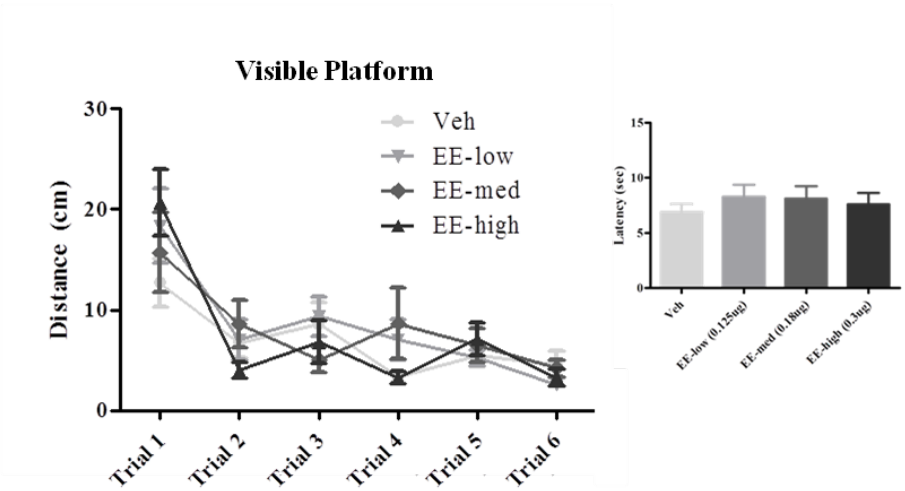
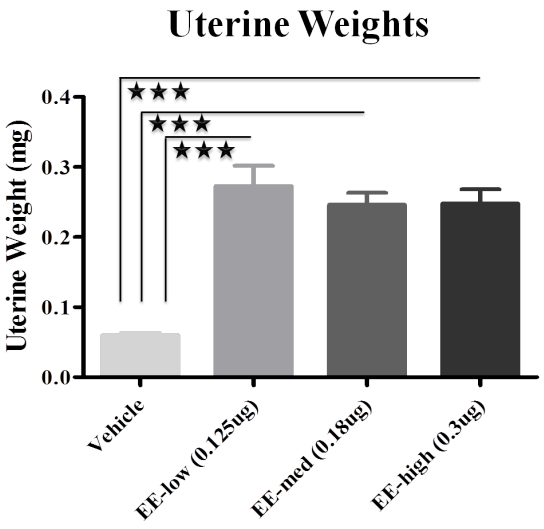

Visible Platform
Distance (cm)
30
20
10
0
Veh
EE-low
EE-med
EE-high
Trial 1
Trial 2
Trial 3
Trial 4
Trial 5
Trial 6
Latency (sec)
15
10
5
0
Veh
EE-low (0.125ug)
EE-med (0.18ug)
EE-high (0.3ug)

Figure 8. There was a main effect of Treatment [$F_{(3,31)}$=29.88; p<0.0001]. Uteri of vehicle-treated rats weighed less than those of EE low [$F_{(1,15)}$= 62.17; p<0.0001], EE med [$F_{(1,16)}$= 117.36; p<0.0001], and EE high [$F_{(1,16)}$= 109.10; p<0.0001] treated rats.

Figure 8.


Uterine Weights
Uterine Weight (mg)
0.4
0.3
0.2
0.1
0.0
★★★
★★★
★★★
Vehicle
EE-low (0.125ug)
EE-med (0.18ug)
EE-high (0.3ug)

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APPENDIX A
IACUC CERTIFICATION

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE AT ARIZONA STATE UNIVERSITY

CERTIFIES THAT

SARAH MENNENGA

as of this date

6/4/2012

Has completed animal care training for

IACUC Basic and Rat

Certificate Number

5864