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Estrodial effects on age-related shifts in learning and memory: A multiple memory systems approach

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Abstract

Many findings from our lab and others suggest that circulating estrogens as well as estrogen replacement after ovariectomy influence cognitive performance. Young female rats trained during proestrus, when estrogens are high, or with acute estradiol (E2) administration after ovariectomy perform better on the hippocampus-sensitive place task but worse on the striatum-sensitive response task (Korol et al., 2004; Korol and Kolo, 2002). Behavioral neuroscience studies tend to use male rodent models to avoid the complexities of the female reproductive cycle, producing a gap in our knowledge about neural mechanisms of learning and memory in females. As a consequence, age-related effects of estrogens on learning and memory have been poorly characterized despite the fact that women tend to outlive men and may live a substantial portion of their adult life post-menopausally. Our lab recently found that aged male rats shift from a preferred hippocampus-sensitive learning strategy to a striatum-sensitive learning strategy. To better assess estrogen sensitivity in aged female rats, the present study compared the performance of hormonally deprived young (N=17) and aged (N=19) female rats via ovariectomy, with and without estradiol administration on the response task. Rats were given estradiol injections 48 and 24 hrs before training. Young adult rats given the estradiol treatment were slower to acquire the response task though the difference did not reach statistical significance. Aged rats given the estradiol treatment were substantially worse at response learning than were the vehicle-treated rats. These data suggest that estradiol retains its ability to modulate learning and memory well into late age, raising questions about the mechanisms of estradiol action.

Executive Summary

Aging is a fundamental part of an organism's lifespan. Fifty percent of people will undergo menopause, which is the cessation of a woman's menstrual period. It can occur during a woman's 40s or 50s with the average in the United States being 51. Common symptoms include hot flashes and night sweat. Another common complaint is "brain fog," or problems with memory, confusion, and inability to focus or concentrate. In order to ameliorate these symptoms, many women seek hormone replacement therapy (HRT) in order to replace the lack of estrogens. However, there are negative side effects of treatment and the direction of the effects depends on the kind of estrogens and the start as well as the duration of the treatment. There are three naturally occurring estrogens: estrone, estradiol, and estriol. Estradiol is the one we focus on in the lab because it is the predominant estrogen during reproductive years in terms of absolute serum levels and estrogenic activity. Our lab has also shown that estradiol can improve certain types of learning, while impairing others, thus modulating not only what is learned, but also how it's learned. Normal aging is accompanied by brain alterations that can lead to impairments in information processing. The present study aims to better assess the effects of estradiol and hormone sensitivity on age-related changes to learning and memory. To do so we adopt a multiple memory systems approach. This idea posits that different kinds of information is stored in different parts of the brain. Our lab uses two learning tasks—place and response. During place, a rat learns to navigate and find the food reward using spatial cues. During response, the rat learns to navigate through the maze using body turns. Both have been dissociated into two different brain regions. Previous research has shown that place is hippocampus-sensitive and response is striatum-sensitive, meaning that lesions or any

manipulations to these regions would result in altered performance on these two tasks. In addition, previous research has shown that estradiol improves performance on place and impairs performance on response.

My model organism is the Fisher 344, which are readily provided by the National Institute on Aging (NIA). For this study, there are two age group—three and 24 month old female rats. They are further split by task (i.e. place or response) and then further split by treatment (i.e. estradiol or vehicle). Before we are able to begin testing, rats are ovariectomized in order to remove any endogenous estrogens. Then there is a wait period of 21 days. Seven days prior to testing, food restriction procedures are initiated in order to appropriately motivate the rats to perform the task and 48 and 24 hr prior to testing, they are injected with the desired treatment. The data presented here reflects training on the response task.

Similar to our previous findings, young rats treated with estradiol are impaired on response training and are outperformed by vehicle-treated rats. Surprisingly, the old rats treated with estradiol were also impaired on response. This challenged our original hypothesis, which was that old female rats would not be impaired on the striatum-sensitive response task due to estrogen insensitivity. The critical window hypothesis postulates that hormone replacement has positive effects on learning and memory when administered soon after the start of menopause for women and estropause for rats. Although the rats were well past their estropause phase, they continued to respond to estradiol treatments. As hormone replacement therapy gains attention as a possible treatment for preventing the cognitive changes that accompany menopause in women, this study carries important implications for the kinds of memory systems that are affected with hormone therapy and may also offer insight into the multifaceted mechanistic actions of estradiol across age.

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Introduction

Gonadal steroids have robust effects on the structure and function of the adult brain. Substantial evidence has found that estrogens regulate learning and memory in human and nonhuman animals. However, when viewed across a large literature, the direction of the effect is diverse, with actions ranging from impairment to enhancement.

Estrogens impact learning and memory in a variety of cognitive tasks depending on the type of hormone, duration of hormone, stress levels, and specific task demands (Korol, 2004). Using a multiple memory systems approach, findings from our laboratory and from others show that young adult female rats trained during proestrus, the stage in the estrous cycle when estrogens and progesterone are highest, on an appetitive T-maze task use spatial or 'place' strategies effectively. On the other hand, female rats trained during estrus, a stage in the estrous cycle when hormones are lowest, tend to use egocentric or 'response' strategies on tasks that require the use of a body turn (i.e. left or right) (Korol, 2004). This dissociation in learning strategy is further supported by findings from numerous studies examining the effects of hormone administration after ovariectomy, Estrogen administration to ovariectomized rats enhance performance on hippocampus-sensitive tasks, while impairing performance on striatum-sensitive tasks (Korol and Kolo, 2002). Together, these results suggests that by biasing the learning strategy used to solve a task, estrogens not only modulate memory formation, but also change what and how information is learned. While this explanation appears to explain estrogens effects in young adult rats, the effects of estrogens on strategy selection across a lifespan remain unclear.

A hallmark of female reproductive senescence is the cessation of the menstrual cycle in humans and the estrous cycle in rodents. Aside from hot flashes and night sweats, another common complaint among women is "brain fog" that is characterized by confusion, forgetfulness and lack

of focus or mental clarity. As a result, many women often seek hormone replacement therapy in an attempt to ameliorate these symptoms. The large scaled Women's Health Initiative Study (WHI) conducted by the National Institute of Health found that hormone therapy with Conjugated Equine Estrogens (CEE), given to women 15 years past normal menopause did not improve cognition. However, estrogen replacement improves verbal memory and working memory among women who began treatment during, or soon after menopause (for review see Sherwin and Henry, 2008), suggesting that a critical period or window of opportunity may exist for positive estrogen effects. The corollary is that long-term ovarian deprivation decreases the effectiveness of treatment (Gibbs, 2000).

Normal aging is accompanied by brain alterations both at the functional and physiological levels (for review see Peters, 2006). A growing literature indicates that these alterations eventually lead to impairments in information processing such as the ability to store, retrieve, and integrate newly acquired information (for review see Gold and Korol, 2014). For example, learning and memory in aged subjects is comparable to young adults when tests are administered soon after training but when tests are administered at later times, aged subjects display poorer memory (Barnes, 1991; Korol, 2002). In addition, recent findings from our lab suggest that in male rats, there is an age-related shift in preferred learning strategy from hippocampus-sensitive learning toward striatum-sensitive learning. While young rats display superior performance on the place task compared to the response task, the opposite appears to be true in the aged subjects. Moreover, Markowska (1999) found that the onset of age-related spatial memory decline takes place earlier in female rats compared to male rats.

Fluctuations in estrogens, either by hormone replacement or naturally across the reproductive cycle in intact females, lead to a series of morphological, neurochemical, and

electrophysiological changes in various brain regions believed to participate in learning and memory. Rats given estradiol replacement following an ovariectomy display an increase in number of spines and synaptic boutons in the CA1 region of the hippocampus relative to those that lack the estrogen replacement (Woolley and McEwen, 1992, 1993). Fluctuations in dendritic spine density are also present across the rat's estrous cycle. Dendritic spine density on CA1 pyramidal neurons appears to decrease during estrus, when estradiol levels are lowest, and increase during proestrus, when estradiol levels are highest (Woolley and McEwen, 1992).

The present study was undertaken to understand the effects of estradiol on age-related shifts in learning and memory. To do so we trained young and aged ovariectomized rats with and without estradiol administration on a food-motivated response task. Similar to our previous studies, we expected young ovariectomized rats treated with a vehicle to perform better than young rats with estradiol replacement. Due to previous research showing that some cognitive functions become impaired with normal aging while others improve and prolonged hormone deprivation resulting in hormone insensitivity, we hypothesized that aged female rats would perform better on the response task, similar to the aged male rats.

Our findings show that ovariectomized rats treated with the vehicle outperformed rats treated with estradiol. In addition, old rats took a longer time to learn and acquire the task. This suggests that although there is a decrease in the levels of endogenous estradiol, aged female rats still remain sensitive to estrogen replacement. This may also offer insight into the mechanistic actions of estradiol across age.

Methods

Subjects and experimental design

Three- and 22- to 24-month-old female Fischer-344 (F344) were obtained from the National Institute on Aging (NIA) colony at Harlan Laboratories (Oregon, WI). The Syracuse University Animal Care and Institutional Animal Care and Use Committee (IACUC), accredited by the Association for Assessment and Accreditation of Animal Care (AAALAC), approved all procedures. The rats were housed individually and maintained on a 12:12-h light:dark cycle. All rats had free access to food and water until food-restriction procedures were initiated.

Rats were allowed one week to acclimate to the vivarium before any experimental procedures were initiated. Vaginal smears were taken daily for 10-14 days to determine whether young rats were cycling normally and whether old rats had ceased cycling. All rats were ovariectomized one week after vaginal smears were complete. Prior to the start of behavioral procedures, rats were handled daily during head-to-toe health checks, and were placed on a food-restricted diet for seven days until they reached 80% of the *ad libitum* feeding weight. Each day of food restriction, rats were given one Frosted Cheerio® to familiarize them with the food reward used during training. Rats with physically limiting characteristics (e.g. cataract) or poor physical health were excluded from the study. Any tumors found were excised during surgery with permission from the veterinarian. Training took place 21 days after ovariectomy.

Young and old rats were randomly assigned to receive either estradiol (E; N = 8 young, N = 9 old) or sesame oil vehicle (oil; N = 9 young, N = 9 old), which served as the control. They were trained on the response learning task, creating a 2 X 2 age (young vs old) by treatment (oil vs E) design within each task.

Vaginal Smears

Estrous cycle status prior to ovariectomy and efficacy of ovariectomy and estradiol treatment post-operatively were determined with vaginal smears. Smears were taken daily for 10-14 days. They were done by gently inserting a small sterile swab (Fisher Scientific, Pittsburgh, PA) soaked in sterile saline into the vagina. The swabbed cells were applied to a clean slide, fixed with EtOH, and stained with toluidine blue. Slides were staged by a single investigator.

Ovariectomy

All rats were ovariectomized under isoflurane anesthesia three weeks prior to testing on the response tasks. Rats were given NSAID flunixin (1ml/kg, s.c.) and penicillin (1/3 of flunixin, i.m.). Brief, dorsolateral incisions were made in the skin, followed by a blunt dissection of the muscle to expose the ovary. The ovary and its blood supply were sutured at the oviduct after which the ovary was excised and examined for abnormal growths. The muscle was sutured and the wound closed with stainless steel clips and treated with topical antibiotic (Bacitracin). Rats were given saline (10ml, s.c.) immediately after surgery to prevent dehydration. To reduce postoperative discomfort, the analgesic, ibuprofen, was added to the rats' drinking water (2.35ml) for 24 hours. All rats were allowed at least 1 week to recover after surgery prior to initiation of handling procedures.

Estradiol Administration

Nineteen days following ovariectomy, rats were randomly assigned to either the estradiol (E) or vehicle group (oil). For the E group, two subcutaneous injections of 45µg/kg 17β-estradiol

benzoate (Sigma, St. Louis, MO) in isolated sesame oil (Sigma) were made 48hr and one 24hr prior to training. Vehicle-treated rats received subcutaneous injections (1ml/kg) of oil alone at the same time as the E-injected rats. All injections were made between 1400-1600.

Training Procedures

Training apparatus and environment. The training apparatus was a symmetrical plus-shaped maze with four arms, each 45cm long × 12.5cm wide, with 7.5-cm-high walls, constructed from black Plexiglas® and mounted on a platform. At the end of each arm was a perforated food boat that had inaccessible food reward (Frosted Cheerio® crumbs) placed underneath to exclude the use of odor cues to find the reward. For response training, any extramaze visual cues that were present in the room were covered with beige curtains.

Training protocol. Training took place between 1400-1600 to avoid the confound of circadian rhythms and inter-day changes in circulating estrogen levels. Rats were placed in a clean holding cage with free access to water in the training room for 20min and allowed to acclimate to the training environment prior to being tested on the maze. For each training trial, the rat was placed into a start arm and allowed to enter one arm to find the food reward (½ of a Frosted Cheerio). A choice was made when all four paws were inside an arm. Each trial had a maximum latency of two minutes, with a 30s intertrial interval, during which time the rat was in the holding cage. If no choice was made within the two minutes, the rat was returned to its holding cage and an 'omission' was recorded. After every trial, the maze was rotated 90˚ to avoid the use of any intramaze cues, using preset stops built into the maze platform. Rats were trained to criterion (9/10 correct choices with 6 consecutive correct choices) or to a maximum of 75 trials.

Response training. For response learning, both young (N = 17) and old (N = 18) rats were trained to make a left or right turn to find the food reward. The start arm was randomized and counterbalanced across all four arms with the goal arm assigned (i.e. left or right) and counterbalanced across all rats within treatment conditions (Figure 1).

Post-training Procedures

Immediately after training, rats were overdosed with pentobarbital (50mg/kg, i.p.). Blood was collected in order to measure levels of circulating estradiol. The brain was harvested and dissected to measure levels of BDNF, and glycogen in future analyses. The uterine horns were collected and analyzed for length and weight.

Data Analysis and Statistics

Measures. The main measure taken during training was arm choice. The main dependent variables generated from this measure include number of trials-to-criterion that reflects how quickly rats learned and percent correct choices in 15 trial blocks or in the first vs the second half of training, reflecting accuracy of performance more generally. Additional measures included choice latency, number of omissions, testing day body weight, uterine horn weight, and estrous status before ovariectomy.

Statistical analysis. Because of the 75-trial maximum, the nonparametric Mann-Whitney *U* tests were performed on trials-to-criterion to evaluate the main effect of treatment within each age group for the response task. A repeated measures analysis of variance (ANOVA) was used to assess the variables of treatment condition (oil vs. E), of block (learning) effect, and differences in aging. Interactions between treatment and age were assessed as well Choice latency was analyzed

with a two way ANOVA with the data grouped into 15-trial blocks. An unpaired t test was used to measure differences in omissions, testing day body weights, and uterine horn weights.. All tests were run with an alpha level of 0.05.

Results

Task Acquisition

In accordance with previous findings, estradiol administration had a notable effect on learning. Across all rats without regard to age, rats given the acute estradiol treatment were impaired when compared to oil-treated animals. This effect, however, was more robust in the old rats, with most not reaching criterion levels of learning within the 75-trial maximum and with a lack of accuracy across training.

The number of trials to reach criterion in aged rats was significantly different for oil-treated and estradiol-treated groups (Figure 2). The estradiol-treated rats took significantly more trials to reach criterion: median trials to criterion = 75 for E and 42 for oil; U (9,9) = 15, $p \leq 0.05$. Although not statistically significant, this similar trend was also seen in the young estradiol-treated rats: median trials to criterion = 38.5 for E and 25 for oil; U (8,9) = 31, $p > 0.05$.

Similar findings were obtained for changes in accuracy (i.e. percent correct) across trials, with estradiol-treated rats in both age groups showing slower learning across training blocks compared to oil-treated rats. All rats did show learning across training blocks, $F(4,124) = 16.40$, $p < 0.001$ (Figure 3). There was a main effect of treatment, $F(1,31) = 13.64$, $p < 0.05$ and main effect of age, $F(1,31) = 7.61$, $p < 0.05$. In addition, there was a main effect of age across trials, $F(4,124) = 2.54$, $p = 0.043$. Upon further analysis, there was no interaction between treatment and age. There was also a significant difference when comparing the first and the second half of testing

(Figure 4). Again, all rats showed learning across the first and second half of training, $F(1,31) = 49$, $p < 0.001$. There was also a main effect of age across trials, $F(1,31) = 9$, $p < 0.05$ and with no age X treatment interaction $F(1,31) = 0.061$, $p = .807$.

Choice latencies per trial block appear to decrease across training for both treatment groups in the young age group (Figure 5), but remain consistent in the old age group, with E-treated rats requiring more time to make a choice. A repeated measures ANOVA discovered a main treatment effect, $F(1,31) = 10.23$, $p < 0.05$. There was also a main effect of age, $F(1,31) = 30.37$, $p < 0.001$, but not interaction between the two, $F(1,31) = 5.52$, $p = 0.25$. More importantly, a two-way ANOVA showed that latencies on the first choice made were not significantly different across treatment, $F(1,31) = 0.452$, $p = 0.59$, but old rats did take longer to make a choice, $F(1,31) = 5.95$, $p < 0.05$ (Figure 6).

An unpaired *t* test showed that there was no significant difference between E-treated rats and oil-treated rats in average testing day body weight: $t(16) = 0.334$, $p = 0.749$ or by percent *ad lib*. weight: $t(15) = 1.309$, $p = 0.219$ in the old age group or the young age group: average testing day body weight, $t(15) = 0.489$, $p = 0.39$; percent *ad lib*. weight, $t(15) = 0.46$, $p = 0.65$ (Figure 7). There was also no significant difference in average number of omissions between E-treated rats and oil-treated rats in the young age group; $t(15) = 0.23$, $p = 0.8233$, but a significant difference between treatment groups in the old rats; $t(16) = 3.25$, $p = 0.005$ (Figure 8). In addition, the uterine horn weights for E-treated rats was significantly higher compared to oil-treated rats in both age groups—young : $t(8) = -0.13$, $p < 0.0001$, old: $t(4) = -0.07$, $p = 0.0011$ (Figure 9). Lastly, histology shows that prior to ovariectomy, all young rats appeared to have a consistent estrous cycle of 4-6 days. Vaginal smears revealed that old rats were postestropausal, with histology showing old rats in persistent estrus (N = 7), persistent diestrus (N = 7), or having irregular cycles (N = 4). When

compared with their trials-to-criterion, there appeared to be no correlation with prior estrous status and their performance on the maze.

Discussion

Consistent with previous findings, it appears that estradiol continues to bias learning and memory—young and old rats treated with estradiol 48 and 24 hr before being tested on response performed worse than did the oil-treated rats. In addition, this observed treatment effect was more robust in the old animals with E-treated rats requiring more time to reach criterion.

Several lines of evidence suggest that the direction of the estrogenic regulation of learning and memory rely on factors such as duration of treatment, dose, and cognitive and task demands. Studies using the swim task to test spatial memory or the use of the hippocampus-sensitive fear conditioning tasks with female rodents often observe a deficit in performance (Frye, 1995; Markus and Zcevic, 1997), due to possible interactions between estrogens, stress, and learning and memory (Shors et al., 1998). Because we were consistent with the use of the maze, room, and pre-training regimen, all rats appear to be matched for motivation and stress variables. Findings from task acquisition across blocks and from the first half vs. the second half reveal that in the old age group, estradiol (E)-treated rats learned more slowly. However old rats acquired the task nonetheless—some even reaching performance levels comparable to oil-treated rats once criterion was achieved. While the same appears to be true in the young age group, the first half vs. the second half reveals that rats treated with estradiol are impaired in the beginning, but learn more than those treated with sesame oil.

From the choice latency data, one might speculate that the estradiol may act on locomotion or sensorimotor function. While there was no significant difference between treatments in the latencies of the young age group, there was a significant difference in the overall latencies of the old age group. However, based on the first choice latency, which was used to determine whether or not estradiol treatment predisposed an animal to longer choice latencies in general, and training day weight (percent from *ad lib*.), it appears that the response task has comparable stress and motivation levels for young and old rats. Together this suggests that the pattern of choice latencies across training may not only reflect motivational state, but instead may reflect an interaction between age, treatment, and the strategy being used. For example, an E-treated rat using an inappropriate strategy may take longer to acquire the task during the start of testing, but may learn more than an oil-treated rat by the end. In addition, old E-treated rats had significantly more omissions and significantly heavier uterine horn weights, suggesting that old rats were highly sensitive to the assigned estradiol treatment dose, which could have contributed to their observed performance. Lastly, there were no correlations with estrous status prior to ovariectomy and performance on the maze, suggesting that the possible mechanisms underlying these observed effects on the response task are not heavily influenced by prior estrous status upon removal of endogenous hormones. However, this does not exclude the possibility that the hormone status of a rat during estropause could influence its responsiveness to hormone replacement and performance on a learning and memory task.

Many studies investigating the impacts of hormone replacement on aging have focused on hippocampus-sensitive memory. It is interesting to note that while the hippocampus memory system is positively influenced by estrogens, it is also susceptible to the effects of aging (Gallagher and Rapp, 1997; Barnes 1979). The dissociation between place and non-place learning strategies

has been well established with hormone replacement in young rats (Korol and Kolo, 2002), across the estrous cycle (Korol et al., 2004), and with hippocampus (Chang and Gold, 1999) and striatal inactivation (Chang and Gold, 2001). Together, these findings suggest that aside from competing for cognitive resources, these memory systems can also work independently during learning and memory. The hippocampus / striatal dissociation with regards to age has shown that there is a spatial memory deficit with age (Barnes, Nadel, and Honig, 1980). Barnes et al. (1980) has shown that in a cue-rich task that required rats to move away from a brightly lit surface to find a goal tunnel, old rats made more errors, taking a longer time to find the goal. This is also reflected in the data reported here that the old female rats required more trials to learn the task. In a two-choice discrimination task, Barnes et al. (1980) also found there to be no difference between age groups in the number of trials to criterion, but upon examining the way in which the rats solved the task, old rats used the response strategy more frequently. These findings are supported with data from our lab where old male rats take fewer trials to learn the response task as opposed to the place task (Korol et al., 2014). While male rats demonstrate an age-related shift in their preferred learning strategy from place in young to response in old, it appears that old female rats learn the response task more slowly than young rats do.

In contrast to our initial hypothesis that our old rats may fall outside the sensitive window for estrogenic modulation of learning and memory we found that in fact the old rats appeared to be more sensitive than the young rats to the impairing effects of estradiol.

Long-term ovarian hormone deprivation impairs the ability of subsequent estradiol exposure to enhance hippocampus-sensitive memory in aged female rats (Gibbs, 2000). Middle-aged rats that began chronic estradiol replacement three months after ovariectomy showed enhanced performance on a delayed match-to-position maze task when tested at 23 months

compared to rats that began chronic estradiol replacement 10 months after ovariectomy. This suggests that the start of hormone treatment after induced menopause or cessation of a regular cycle is critical for positive effects of estradiol. In this present study, all rats were 22 to 24 months of age at the time of ovariectomy. While they had all gone through estropause based on our vaginal smear data, one possibility for our observed estradiol effect could be due to the ovaries remaining intact so late in life, which could have helped maintain or prevent a significant decrease in the presence or distribution of estrogen receptors. Moreover, aging results in a decreased ER-α expression, which may contribute to this decreased responsiveness with age (Adams et al., 2002; Mehra et al., 2005). Roger et al. (2010) found that middle-aged rats that received a 40-day estradiol treatment, where it was then discontinued for seven months, (followed by testing) was associated with increased ER-α expression and improved memory in older rats. This further implicates ER-α with the etiology of age-related memory decline.

The cellular mechanisms underlying estrogen's effects on learning strategy remain unclear. Estrogen's potential mode of action has traditionally been characterized as binding to the nuclear receptors ER-α and ER-β and regulating gene expression via estrogen response elements. Our data suggest that estradiol may act through other mechanisms that may not solely rely on the presence of this classical estrogen receptor. Current research has focused on characterizing signaling cascades originating at the neuronal membrane. These rapid actions of estradiol have also been shown to occur in brain regions that express little to no estrogen receptors such as the auditory region NCM (Remage-Healy et al., 2009). Membrane-impermeant estrogen analogs are also able to stimulate rapid effects on cAMP response element-binding protein. (Boulware et al., 2005). Classical estrogen receptors can be transactivated by metabotropic glutamate receptors (mGluRs), which are a family of G-protein coupled receptors that are activated upon binding to glutamate.

Mermelstein et al. (1996) found that some rapid estrogen effects were sensitive to G protein manipulation and that mGluR1/mGluR2 were localized in the hippocampus and mGluR5/mGluR3 were localized in the striatum. In addition, hippocampal and striatal neurons, activation of ER-α with group I mGluRs triggers CREB phosphorylation and activation of ER-α and ER-β with group II mGluRs inhibits L-type calcium channel mediated CREB phosphorylation (Grove-Strawser et al., 2010). Upon further investigation, estrogen receptors and mGluRs were found to be organized into microdomains called caveoli and to be bound by specific caveolin (CAV) protein. Caveolin 1 associates with mGluR1/5 and is thought to be involved in up-regulation of CREB phosphorylation through MAPKinase signaling pathways while caveolin 3 associates with mGluR 2/3 and appears important for down-regulating CREB signaling (Warwick et al., 2005; Boulware et al., 2005; Boulware et al., 2007; Francesconi et al, 2009). This mode of transmembrane activation offers a possible explanation for the actions of estradiol that would help explain the increased estradiol sensitivity and continued impairment seen in old female rats on this striatum-sensitive response task.

Future work from this study will include Western blot analyses to compare the presence of CAV proteins in young and old rats as well as the ratios of both classical estrogen receptors and group I and II mGluRs to determine if transmembrane activation involved in the estradiol mechanism of action. Due to the robust effect observed in the old rats treated with estradiol, it is possible that there may be a higher amount of CAV present in old rats that make it possible to preserve this bias away from learning striatum-sensitive tasks. Other work will also involve testing young and old females treated with estradiol or vehicle control on the place training task. Given the findings from this experiment, it would be interesting to see whether or not E-treated rats will

outperform oil-treated rats on this hippocampus-sensitive task since an overwhelming amount of literature suggests that the hippocampus is particularly sensitive to functional declines with age.

The effects of estrogens on learning and memory are diverse and robust. Convergent evidences across laboratories suggest that estrogen replacement has a positive effect on cognition when administered shortly after the cessation of a woman's menstrual cycle. Additional studies are required to elucidate the mechanisms underlying these effects, which will aid in understanding how estrogens affect the aging brain should lead to its use as a valuable therapeutic agent in the maintenance of neuronal function. As hormone replacement therapy gains considerable attention as a possible treatment for preventing the cognitive changes that accompany menopause in women, this study carries important implications for the kinds of memory systems that are affected with hormone therapy.

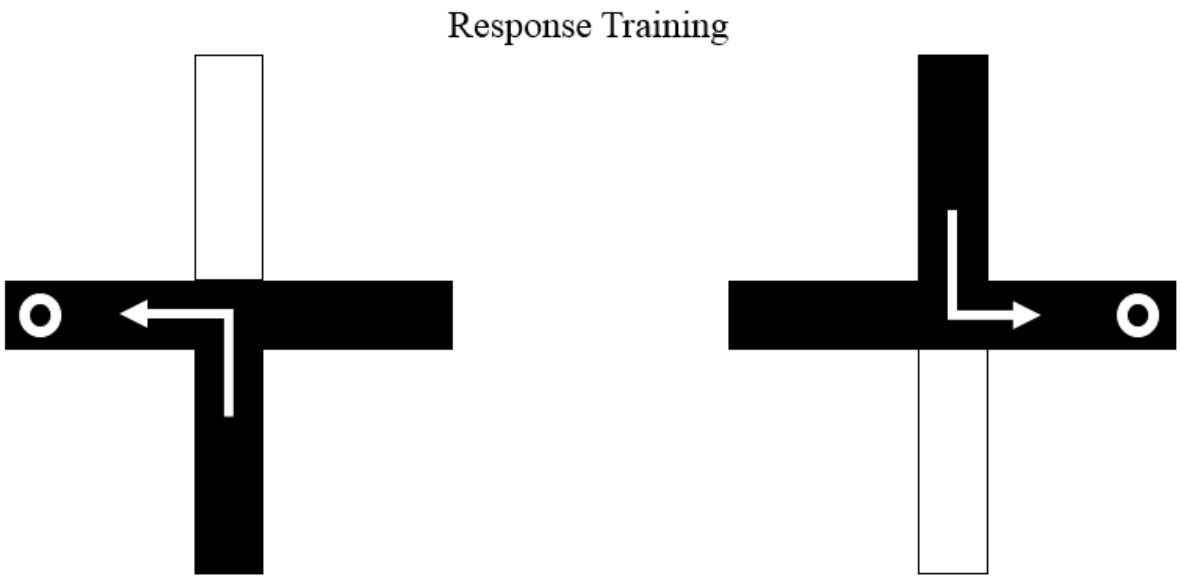


Figure 1. Graphic representation of training protocol for the response task on the T-maze. The arm opposite of the start arm was blocked off across all trials. Response training requires rats to locate food reward by making a left or right (not shown here) turn in the absence of extramaze cues. The goal arm was randomly assigned across training but maintained its position relative to the start arm with respect to the correct turn.

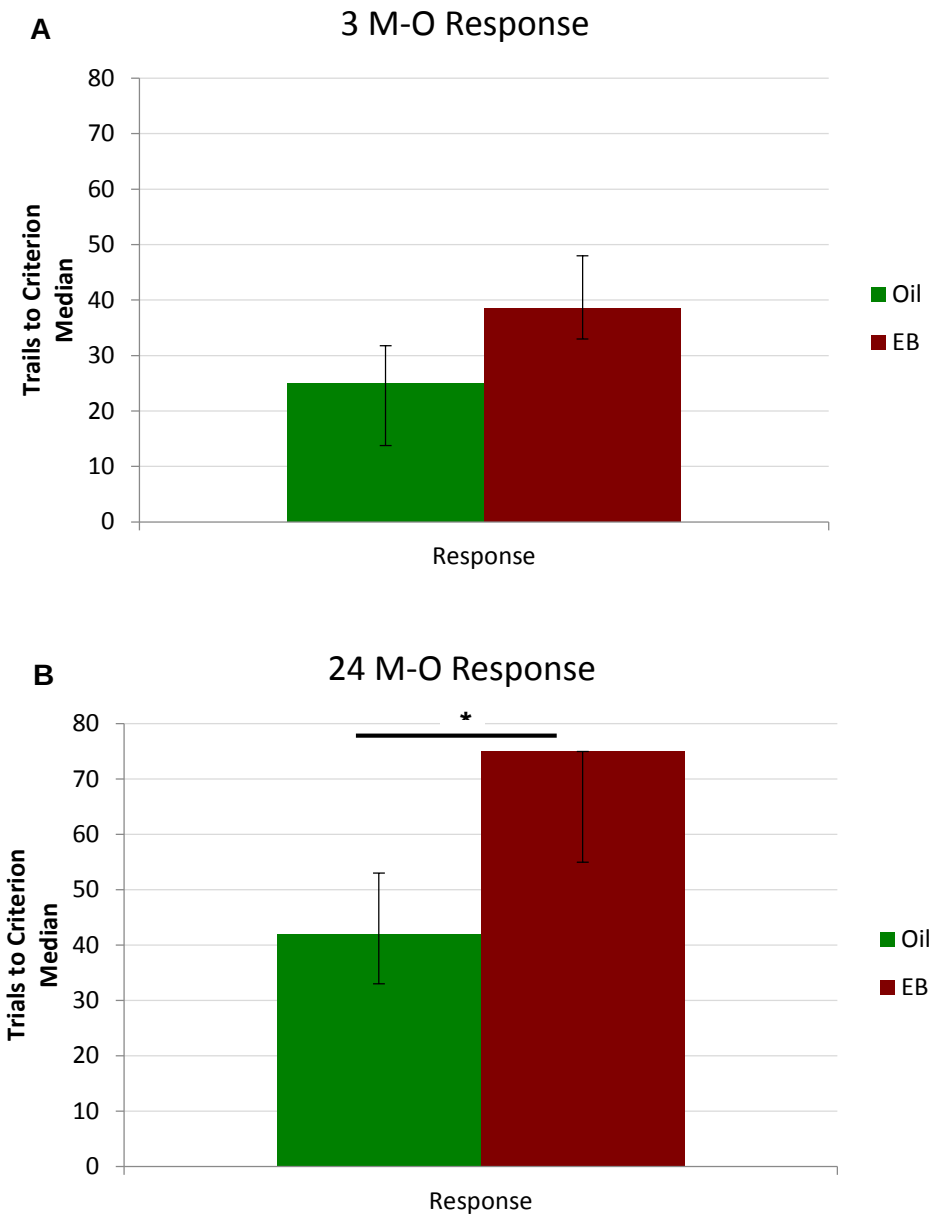


Figure 2. The number of trials to reach criterion for the response task. Old and young estradiol (E)-treated rats were impaired (more trials to criterion) than did the oil-treated rats. (A) Depicts the trials to criterion for young rats and (B) depicts the trials to criterion for old rats. Data are presented as medians + IQR.

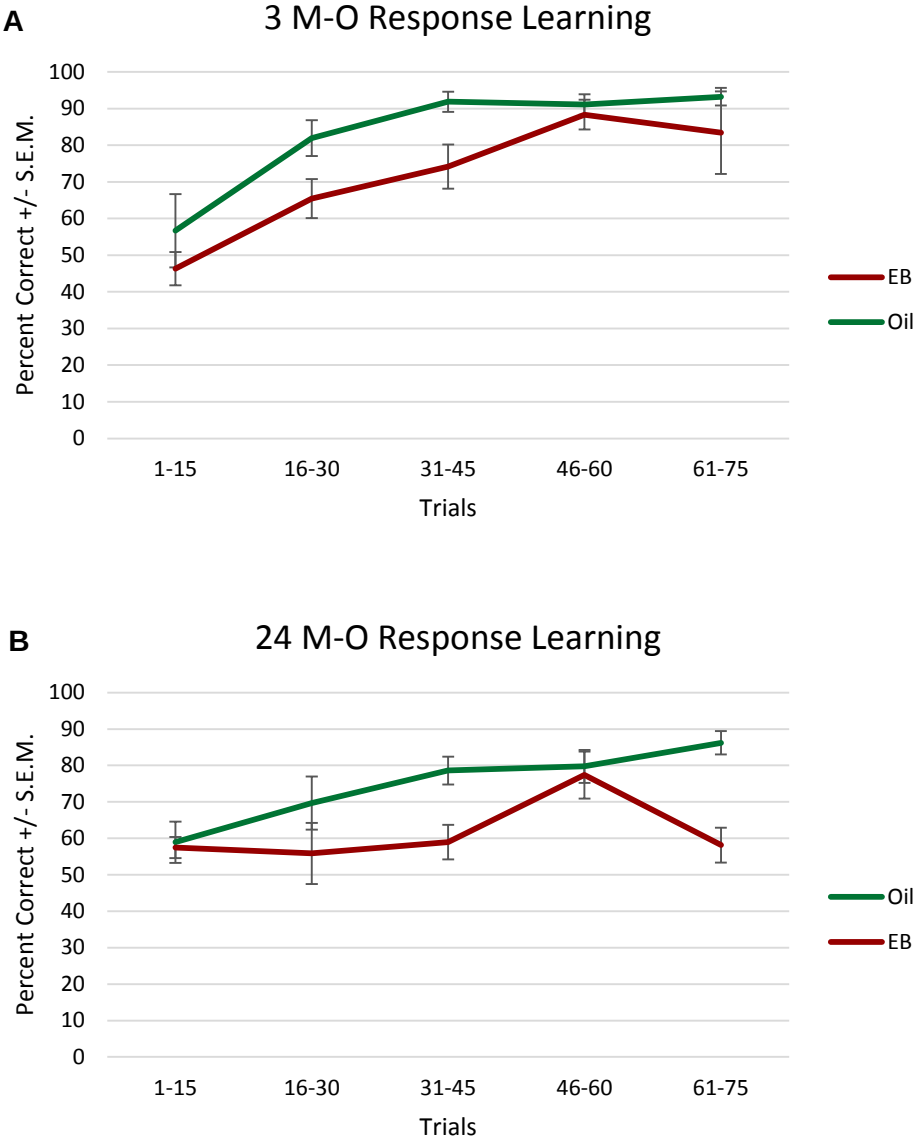


Figure 3. Changes in accuracy across trials for response training. Trials were grouped into 15-trial blocks. Old estradiol (E)-treated rats were slower to acquire the task than were oil-treated rats (B). Similarly in the young rats, those treated with estradiol were slower to acquire the task compared to those treated with oil (A). In addition, old rats took an overall longer time to learn the response task. Data are presented as percent correct ± standard errors.

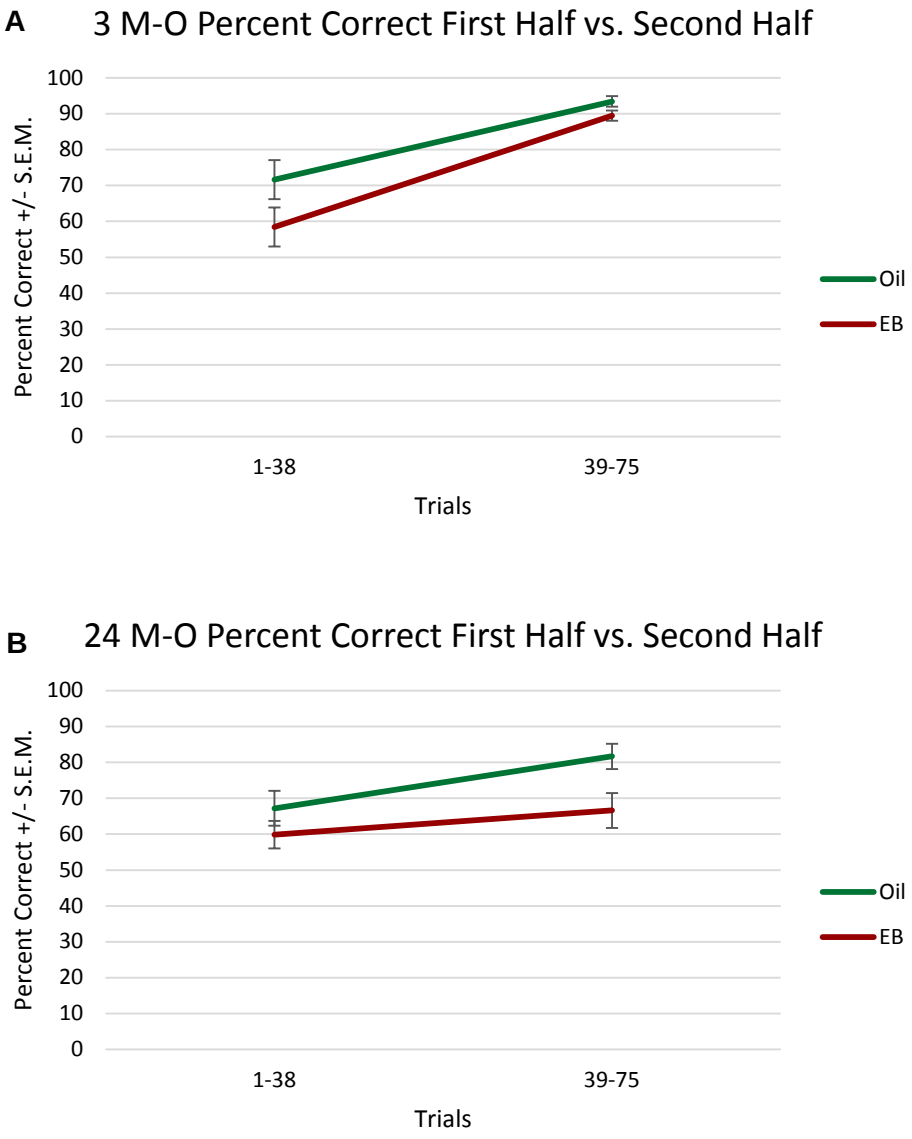


Figure 4. Changes in accuracy during the first and second half of the response task. While all groups display improved performance, the slope of the curve for the young estradiol (E)-treated rats (A) is steeper than that of the old estradiol (E)-treated rats (B). Overall, the old rats learned more quickly between the first and second half of the task. Data are presented as percent correct ± standard error.

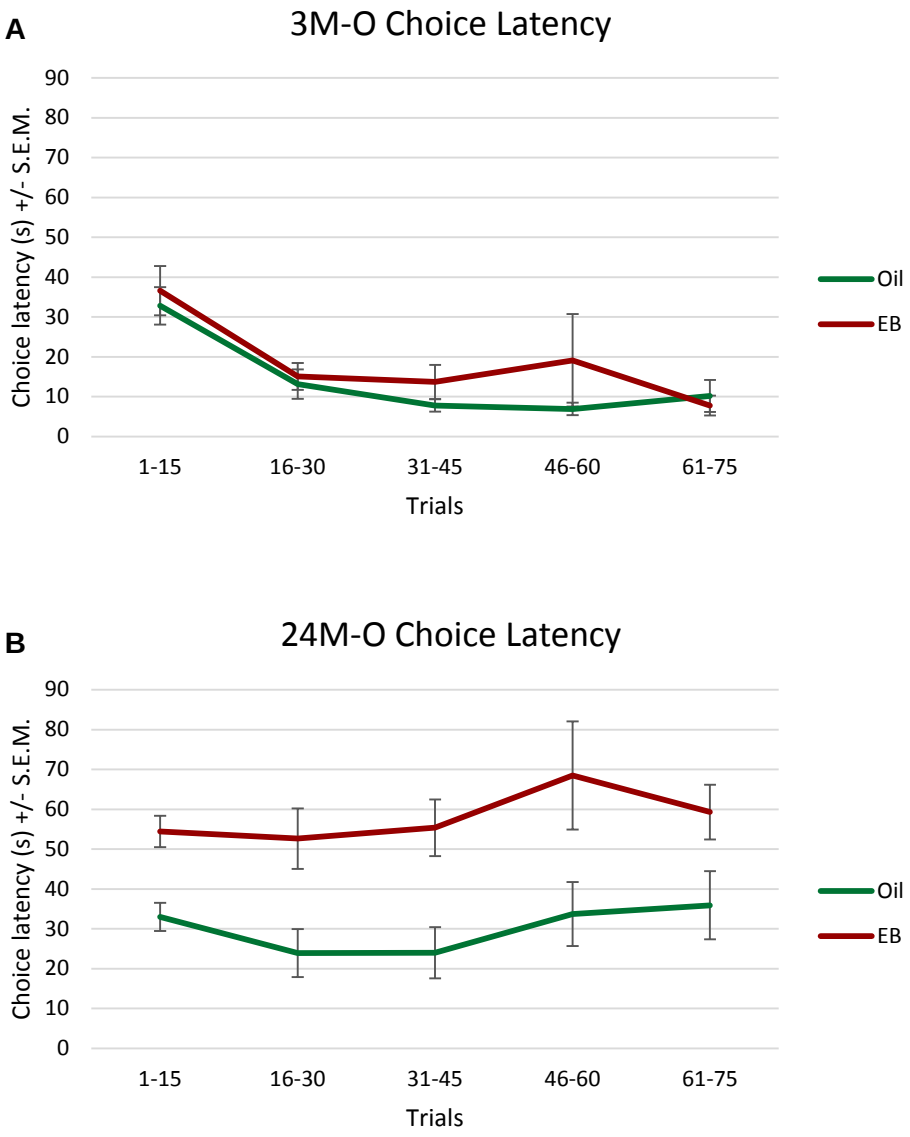


Figure 5. Changes in choice latency across training for young (A) and old (B) rats. Choice time were similar for the young age group treated with either estradiol or oil. However in the old age group, estradiol-(E) treated rats had an overall higher choice latency compared to oil-treated rats.

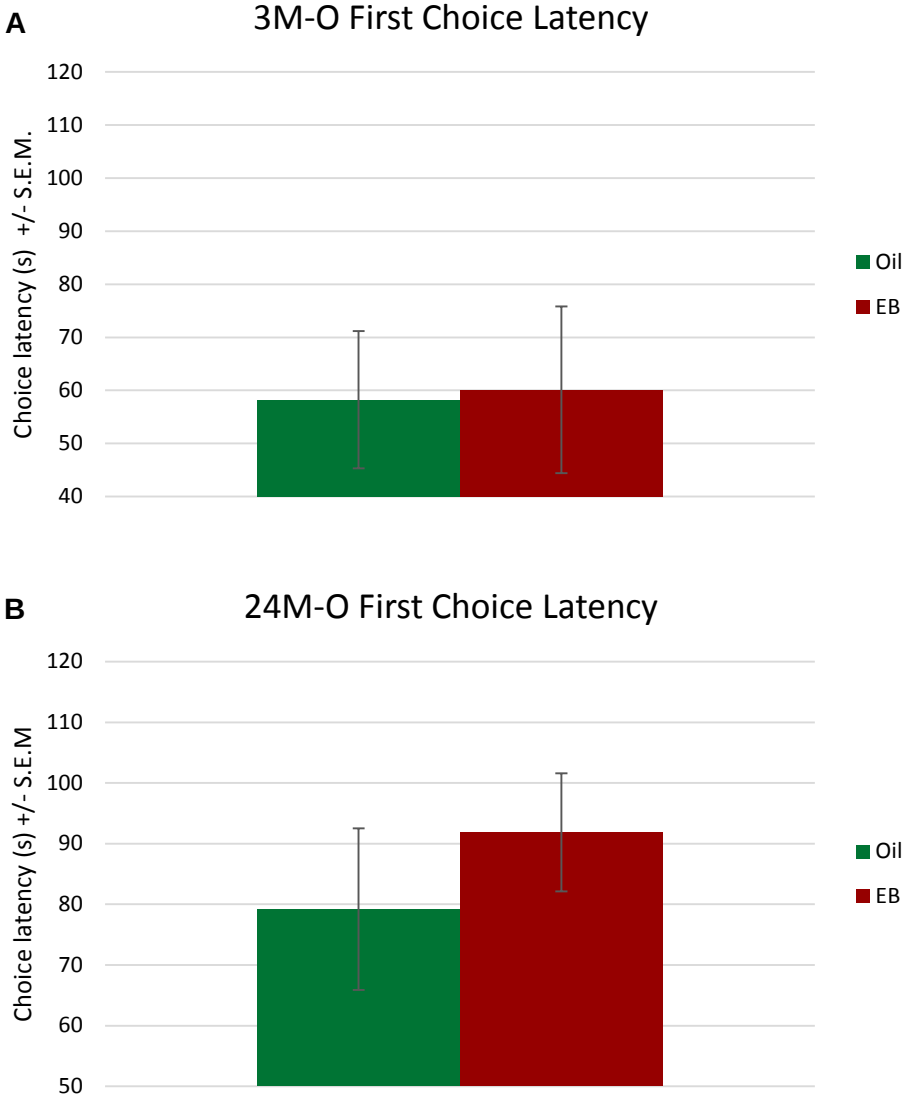


Figure 6. Choice latency for the first trial made in young (A) and old (B) rats. Data show that there is no significant difference between the time spent before making their first choice in both within treatments. Old rats did, however, require more time compared to young rats.

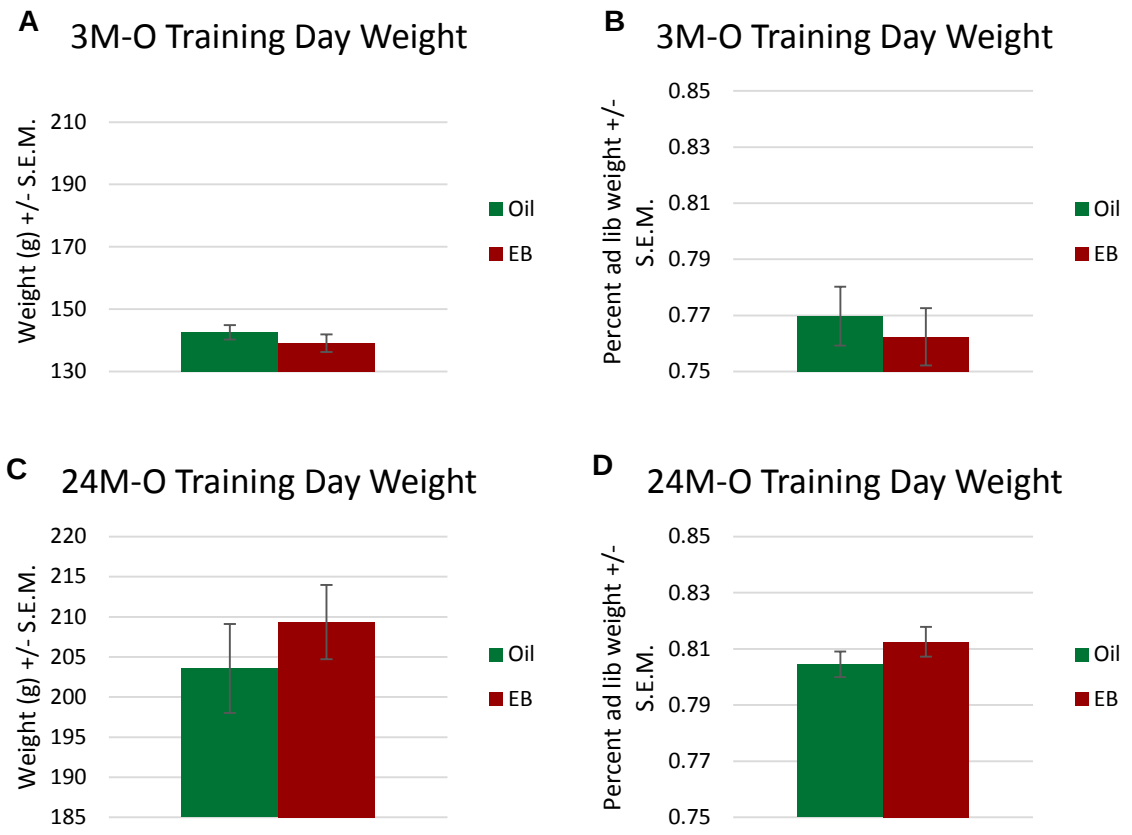


Figure 7. Body weights on training day for young (A and B) and old (C and D) rats. (A and C) And (B and D) depict training day weight in grams and in percent weight from *ad lib.* weight, respectively. There was no significant difference in the total weight and percent weight form *ad lib*. weight.

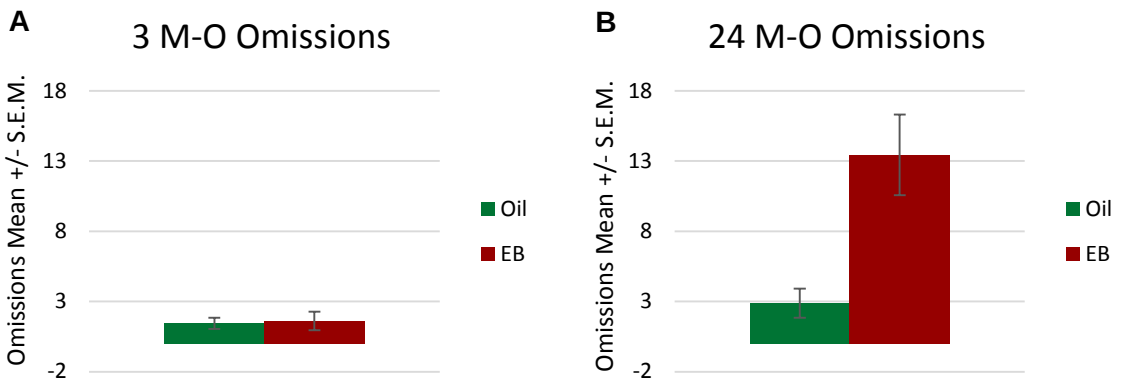


Figure 8. The average number of omissions for each age and treatment group. Omissions were considered as reaching maximum latency or falling off the maze. (A) shows the average number of omissions for young rats. (B) shows the average number of omissions for old rats. There was a significant difference between average and total number of omissions for old animals, but no significant difference in young rats. Data is presented as mean and total + standard error.

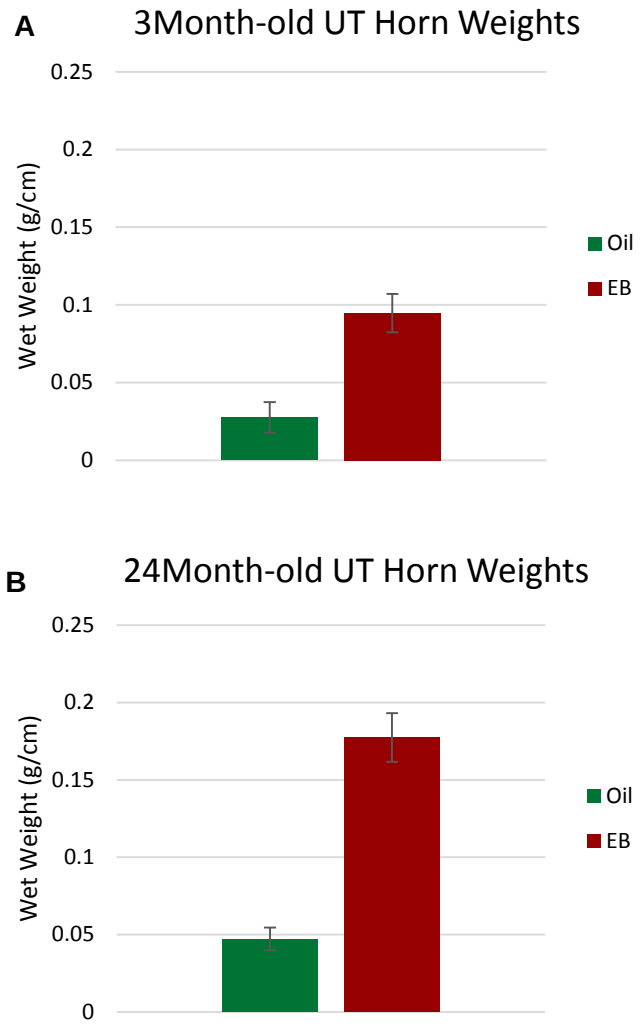
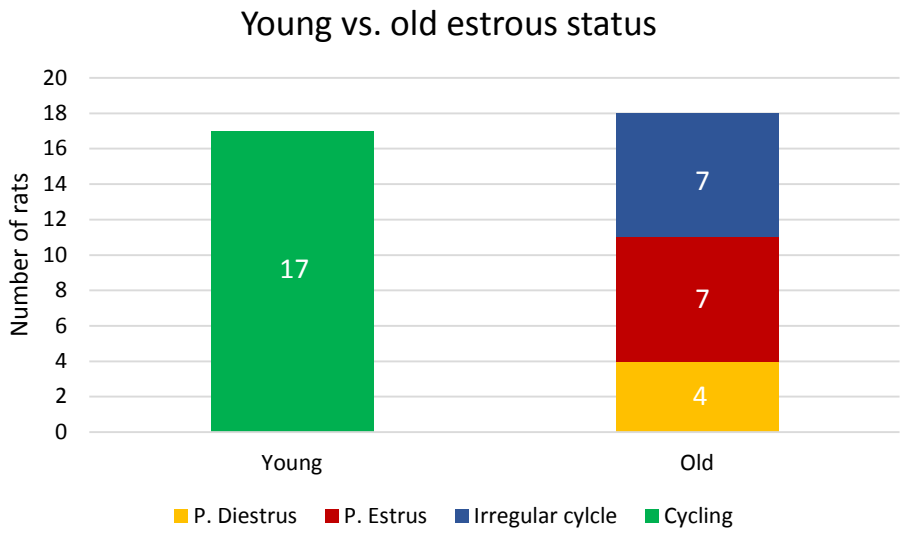


Figure 9. Wet uterine horn weight for young (A) and old (B) rats. Together, those treated with estradiol had significantly heavier uterine horn weights compared to those treated with oil and overall, old rats had heavier wet weights.



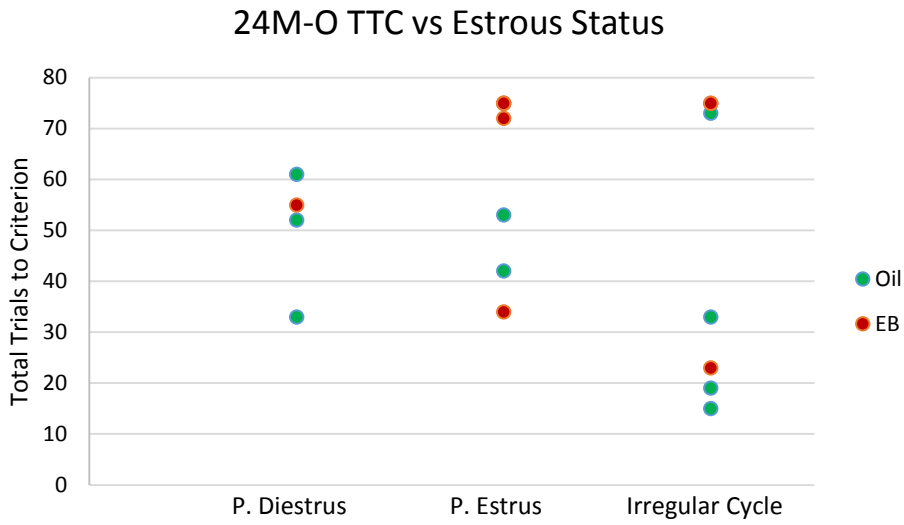


Figure 10. Comparison of young and old estrous status prior to ovariectomy. All young rats displayed a regular 4-6 day estrous cycle. Instead of a regular cycle, old rats displayed characteristics of persistent diestrus, persistent estrus, or irregular cycling. There was no correlation between estrus status in old rats and performance on the response task in terms of trials to criterion.

References

Adams, M.M., Morrison, J.H. (2003). Estrogen and the Aging Hippocampal Synapse. Cerebral Cortex. 13:1271-1275.

Adams, M.M., Fink, S.E., Shah, R.A., Janssen, W.G., Hayashi S., Milner, T.A., McEwen, B.S., Morrison, J.H. (2002). Estrogen and aging affect the subcellular distribution of estrogen receptor-alpha in the hippocampus of female rats. *Exp Neruol*, 170:345-356.

Barnes, C.A., (1991). Memory changes with age: Neurobiological correlates. In J. L. Martinez, Jr. and R.P. Kesner (Eds.), Learning and memory: A biological view (pp.259-296). New York: Academic Press.

Barnes, C.A., Nadel, L., Honig, W.K. (1980). Spatial memory deficits in senescent rats. Canad. J. Psychol./Rev. canad. Psychol., 34(1)

Boulware, M.I., Weik, J.P., Becklund, B.R., Kuo, S.P., Groth, R.D., Mermelstein, P.G. (2005). Estradiol activates group I and II metabotropic glutamate receptor signaling, leading to opposing influence on cAMP response element-binding protein. *Journal of Neuroscience*, 25(20):5066-5078.

Boulware, M.I., Kordasiewiz, H., Mermelstein, P.G. (2007), Caveolin proteins are essential for distinct effects of membrane estrogen receptors in neurons. *The Journal of Neuroscience*. 27(37):9941-9950

Chang, Q., and Gold, P. E. (1999). Effects of intra-hippocampal morphine injections on place and response learning [Abstract]. *Society for Neuroscience Abstracts*, 25, 77.5.

Chang, Q., and Gold, P.E. (2001). Inactivation of dorsal striatum impairs acquisition of response learning only in cue-deficient environments [Abstract]. *Society for Neuroscience Abstracts*, 27, 77.5.

Cyr, M., Othman, G., Thibault, C., Morissette, M., Landry, M., di Paolo, T.D. (2001). Ovarian steroids and selective estrogen receptor modulators activity on rat brain NMDA and AMPA receptors. *Brain Research Reviews*. 37: 153-161.

Daniel, J.M. (2013). Estrogens, estrogen receptors and female cognitive aging: Impact of timing. Invited review in Special Issue, Hormones & Neurotrauma, *Hormones and Behavior*, 63: 231-237.

Francesconi, A., Kumari, R., Zukin, R.S. (2009). Regulation of group I metabotropic glutamate receptor trafficking and signaling by the caveolar/lipid raft pathway. *The Journal of Neuroscience*. 29(11):3590-3602.

Frick, K.M. (2009). Estrogens and age-related memory declines in rodents: what have we learned and where do we go from here? *Horm. Behavior*. 55, 2-23.

Frye, C.H. (1995). Estrus-associated decrement in a water maze tsk are limited to acquisition. *Physiology and Behavior*. 57, 5-14.

Hallagher, M., Rap, P.R. (1997). The use of animal models to study the effects of aging on cognition. Annu. Rev. Psychol. 48, 339-370.

Gibbs, R.B., (2000). Long-term treatment with estrogen and progesterone enhances acquisition of a spatial memory task by ovariectomized aged rats. *Neurobiology of Aging*. 21, 107-116.

Gold, P.E., Korol, D.L. (2014). Forgetfulness during aging: An integrated biology. *Neurobiology of Learning and Memory*. 112(2014) 130-138.

Hall, J.M., Couse, J.F., Korach, K.S. (2001). The multifaced mechanisms of estradiol and estrogen receptor signaling. *Journal of Biological Chemistry*. 276:40 pp. 36869-36872.

Korol D.L., and Kolo L.L., (2002). Estrogen-induced changes in place and response learning

in young adult female rats. *Behavioral Neuroscience*, 116(3), 411-420.
Korol, D.L., (2002). Enhancing cognitive function across the life span. *Annal of the New York Academy of Sciences*, 959, 167-179.
Korol D.L., Malin E.L., Borden K.A., Busby R.A., Couper-Leo J., (2004). Shifts in preferred learning strategy across the estrous cycle in female rats. *Hormones and Behavior*, 45(2004) 330-338.
Korol, D.L., Newman, L.A., and Gold, P.E. (2014). Senile or sage? Improved memory and sensitivity to cognitive priming accompany aging in male rats. [Abstract]. *Society for Neuroscience Abstracts*. Program No. 653.09.2014
Markowska, A.L., Sex Dimorphisms in the rate of age-related decline in spatial memory: Relevance to alteration in the estrous cycle. *Journal of Neuroscience*. 19(18):8122-8133.
Markus, E., Zecevic, M. (1997). Se differences and estrous cycle changes in hippocampus-dependent fear conditioning. *Psychobioloy*. 25, 246-252.
Mehra, R.D., Sharma, K., Nyakas, C., Vij, U. (2005). Estrogen receptors alpha and beta immunoreactive neurons in normal adult and aged female rate hippocampus: A qualitative and quantitative study. *Brain Res*. 1056:22-35.
Mermelstein PG, Becker JB, Surmeier DJ. Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. *J Neurosci*. 1996;16:595–604.
Milner, T.A., Ayoola, K., Drake, C.T., Herrick, S.P., Tabori, N.E., McEwen, B.S., Warrier, S., Alves, S.E. (2005), Ultrastructural localization of estrogen receptor beta immunoreactivity in the rat hippocampus formation. *J Comp Neurol*. 491:81-95.
Patel, H.H., Murray, F., Insel, P.A. (2008) Caveolae as organizers of pharmacologically relevant signal transduction molecules. *Annu Rev Pharmacol Toxicol*. 48:359-391.
Peters, R. (2006). Ageing and the brain. *Postgrad Med J*. 82:84-88
Remage-Healey L, London SE, Schlinger BA. (2009). Birdsong and the neural production of steroids. J *Chem Neuroanat*. 39:72–81.
Rodgers, S.P., Bohacek, J., Daniel, J.M. (2010). Transient estradiol exposure during middle age in ovariectomized rats exert lasting effects on cognitive function and the hippocampus. *Endocrinology*. 151:1194-1203
Savonenko A.V., Markowska A.L., (2003). The cognitive effects of ovariectomy and estrogen replacement are modulated by aging. *Neuroscience*. 119(3):821-30
Sherwin, B.B., Henr, J.F. (2008). Brain aging modulates the neuroprotective effects of estrogen on selective aspects of f cognition in women: a critical review. Front. *Neuroendocrinol*. 29, 88-113.
Shughrue, PJ., Lane, M.V., Merchenthaler, I. (1997). Comparative distribution of estrogen receptor-alpha and –beta mRNA in the rat central nervous system. *J Comp Neurol* 388:507-525.
Shors, T.J., Lewczk, C., Pacynski, M., Mathew, P.R., Pickett, J. (1998). Stages of estrous mediate the stress-induced impairment of associative learning in the female rat. *NeuroReport* , 9, 419-423.
Stern, C.M., Mermelstein, P.G. (2010). Caveolin regulation of neuronal intracellular signaling. *Cell*. 67:3785-3795.
Warwick, H.K., Nahorski, S.R., Challiss, R.A. (2005). Group I metabotropic glutamate receptors, mGlu1a and mGlu5a couple to cyclic AMP response elements binding protein (CREB) through common Ca^{2+} - and protein kinase C-dependent pathway. *Journal of Neurochemistry*, 93, 232-245.

Weiland, N.G. (1992). Estradiol selectively regulates agonist binding sites on the N-methyl-D-aspartate receptor complex in the CA1 region of the hippocampus. *Endocrinology* 131:662-668.

Woolley, C.S., McEwen, B.S. (1992). Estradiol mediates fluctuations in hippocampal synaptic density during the estrous cycle in the adult rat. *J Neuroscience. 12:2549-2554*

Woolley, C.S, McEwen, B.S. (1993). Roles of estradiol and progestrone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. *J comp Neurol* 336:293-306.

Woolley, C.S., McEwen, B.S. (1994). Estradiol regulates hippocampal dendritic spinde densidt via an *N*-methyl-*D*-aspartate receptor dependent mechanism. *J Neuroscience*. 14:7680-7687.