Psychoneuroendocrinology (2010) **35**, 694—705

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

The beneficial effects of estradiol on attentional processes are dependent on timing of treatment initiation following ovariectomy in middle-aged rats

Johannes Bohacek [a,*], **Jill M. Daniel** [a,b]

[a] *Program in Neuroscience, Tulane University, New Orleans, LA 70118, USA*
[b] *Department of Psychology, Tulane University, New Orleans, LA 70118, USA*

Received 2 July 2009; received in revised form 23 August 2009; accepted 9 October 2009

KEYWORDS
Estrogen;
Ovariectomy;
Cognition;
Attention;
5-Choice serial reaction time task (5-CSRTT)

Summary The goal of the present study was to explore the effects of long-term hormone deprivation on the ability of subsequent estrogen treatment to affect attention performance on the 5-choice serial reaction time task (5-CSRTT). In an initial experiment to assess estradiol effects in young adults, 2-month-old rats were trained on the 5-CSRTT, then ovariectomized and immediately implanted with capsules containing cholesterol ($n = 10$) or estradiol ($n = 10$). Then rats were tested on the 5-CSRTT under baseline task parameters, under increased task difficulty (behavior challenge condition), and finally in muscarinic and nicotinic drug challenge conditions. In a second experiment, 10-month-old rats were trained on the 5-CSRTT and at 12 or 17 months of age rats were ovariectomized and treated with estradiol or cholesterol, so that one group received continuous cholesterol control treatment, two groups received estradiol treatment immediately following ovariectomy (either at 12 or 17 months), and one group received delayed estradiol treatment initiated 5 months following ovariectomies. At 17 months of age, rats were tested on the 5-CSRTT. Baseline performance was comparable between estradiol- and cholesterol-treated rats of both age groups. However, young estradiol-treated rats outperformed controls when behavior was challenged by shortening the intertrial interval (Short ITI). In the same Short ITI condition, middle-aged rats receiving immediate estradiol treatment beginning at the age of 17 months, but not 12 months, outperformed controls as well as animals receiving delayed estradiol treatment. No differences between groups were found in the cholinergic drug challenge conditions. These data indicate that chronic estradiol treatment for approximately 1 month but not 6 months is able to enhance attention performance, and that prolonged ovarian hormone deprivation attenuates these beneficial effects of subsequent estradiol treatment. These findings have implications for informing clinical research about the importance of timing and duration of hormone treatment.

* Corresponding author. Current address: Brain Research Institute, Medical Faculty of the University of Zurich and Department of Biology, Swiss Federal Institute of Technology, Winterthurerstrasse 190, 8057 Zurich, Switzerland. Tel.: +41 44 635 33 49; fax: +41 44 635 33 03.
E-mail address: bohacek@hifo.uzh.ch (J. Bohacek).

0306-4530/$ — see front matter
doi:10.1016/j.psyneuen.2009.10.010

1. Introduction

The steroid hormone estrogen has been purported to promote healthy cognitive aging in postmenopausal women (Sherwin, 2002). However, in a large clinical trial conducted by the National Institutes of Health, the Women's Health Initiative Memory Study, estrogen failed to benefit cognitive aging if administered to postmenopausal women on average about 20 years after menopause (Espeland et al., 2004; Shumaker et al., 2004). Following this unexpected outcome, the critical period hypothesis has been put forward, proposing the existence of a critical time period following loss of ovarian function during which estrogen treatment must be initiated in order to exert positive effects on cognitive function (Maki, 2006; Resnick and Henderson, 2002; Sherwin, 2007). Preliminary evidence in support of the critical period hypothesis in humans has been accumulated (Dumas et al., 2008; Henderson et al., 2005; MacLennan et al., 2006; Zandi et al., 2002). Studies using rodent models provide more experimental control over treatment regimens and have so far also provided support for the critical period hypothesis on a behavioral (Daniel et al., 2006; Gibbs, 2000) and molecular level (Bohacek et al., 2008; Bohacek and Daniel, 2009; McLaughlin et al., 2008), suggesting that enhancing effects of estrogen for cognitive function depend on early treatment initiation. To date, however, the behavior studies have focused exclusively on hippocampus-sensitive tasks, because a large body of work has identified mechanisms of estrogen action specifically in the hippocampus (for review, see Spencer et al., 2008). However, the effects of estrogen in other brain areas associated with cognition remain poorly understood. One prominent structure that is sensitive to the effects of estrogen is the prefrontal cortex, where estrogen can increase spine density (Luine et al., 2006; Morrison et al., 2006) and cholinergic activity (Gibbs and Gabor, 2003; Luine, 1985), similar to the effects of estrogen observed in the hippocampus (Spencer et al., 2008; Woolley, 1998). Because of the central role of the prefrontal cortex in higher order cognitive function, and given that this structure is sensitive to the effects of estrogen treatment, the goal of the present study was to test the validity of the critical period hypothesis as it applies to prefrontal cortex sensitive cognitive performance.

Attentional processes are an important cognitive domain mediated by the prefrontal cortex (Dalley et al., 2004). Therefore, in the present study we tested the effects of estrogen and the timing of treatment initiation using the 5-choice serial reaction time task (5-CSRTT), a well-established prefrontal cortex sensitive task (Dalley et al., 2004; Muir et al., 1996) that assesses a broad array of attention-related performance measures (Robbins, 2002). Although it has been shown that high-dose estradiol injections improve certain aspects of 5-CSRTT performance of ovariectomized young and aged rats (Barnes et al., 2006), constant supraphysiological levels of estrogen treatment have impairing effects on rats trained post-surgically on attention-related operant working memory and response timing tasks (Wang et al., 2008). Additionally, previous findings suggest that ovariectomized rats trained post-surgically outperform gonadally intact animals on a sustained attention task (McGaughy and Sarter, 1999). In order to establish the effects of our chronic estrogen treatment regimen using physiological levels of estradiol delivery, we first assessed 5-CSRTT performance in young adult ovariectomized rats trained before and tested after ovariectomy. Then, in a separate experiment, we tested the critical period hypothesis by training middle-aged rats on the task before ovariectomies, and then initiating chronic estradiol treatment either immediately or 5 months following ovariectomy. We hypothesized that the effects of delayed estrogen treatment would be different from the effects of immediate estrogen treatment.

The effects of estradiol on cognitive performance are, at least in part, mediated by the cholinergic system (Daniel and Dohanich, 2001; Lam and Leranth, 2003). In line with the prediction of the critical period hypothesis, we recently found that the ability of estradiol to affect protein levels of the cholinergic marker choline acetyltransferase (ChAT) and estrogen receptor alpha (ERα) are altered if treatment is initiated 5 months following ovariectomy (Bohacek et al., 2008; Bohacek and Daniel, 2009). Specifically, 5 months of ovarian hormone deprivation reduced the responsiveness of both ChAT and ERα to subsequent estradiol treatment in the hippocampus, but increased responsiveness in the prefrontal cortex. The observation that the effects of delayed estrogen treatment vary in these brain regions raises the possibility that different cognitive functions might differentially respond to delayed estrogen treatment. Further, based on the observation of region-specific alteration in ChAT levels following delayed estrogen treatment (Bohacek et al., 2008), we presented animals with systemic cholinergic drug challenges, the muscarinic receptor antagonist scopolamine, and the nicotinic antagonist mecamylamine, while performing the 5-CSRTT. We hypothesized that if the effects of estrogen on prefrontal cortex function are mediated by the cholinergic system, estrogen should be able to attenuate performance impairments induced by anti-cholinergic drugs as observed in hippocampus-sensitive memory tasks (e.g. Dohanich et al., 1994; Fader et al., 1998). Further, we predicted that delayed estrogen treatment would alter the responsiveness to cholinergic drug challenges compared to immediate estrogen treatment.

2. Methods

2.1. Experiment 1

2.1.1. Subjects

Twenty female Long-Evans hooded rats, approximately 2 months of age, were purchased from Harlan Sprague—Dawley (Indianapolis, IN). Rats were individually housed under a 12-h light/dark cycle, and tested during the light phase of the cycle. Rats were maintained on a balanced maintenance diet manufactured and fortified with nutrients specifically for rodents (Teklad Diet 2016, Harlan, IN). All animals were weighed daily following behavioral training and food was provided in their home cages to maintain their weights at 85% of their free-feeding weights.

2.1.2. Apparatus

The test apparatus consists of four 25 cm $\times$ 25 cm aluminum chambers (Lafayette Instrument Co., Lafayette, IN). The rear wall of each chamber is concavely curved and contains five apertures, each 2.5 cm square, 4 cm deep, and set 2 cm above floor level. Each hole can be illuminated with a 3 W lightbulb located at the rear of the hole. Each hole has an

infrared photocell beam monitoring the entrance. The four conditioning chambers are individually housed in sound attenuating cabinets. Each chamber is illuminated by a 3 W house light, and each chamber is equipped with a speaker that can deliver bursts of white noise. The front wall can be opened to place in and remove the animal from the chamber. On the front wall, 25 cm from each nose poke hole, there is a food magazine where 45 mg food pellets (Test Diet, Richmond, IN) can be automatically dispensed. Each animal received one session of training per day throughout the experiment. House lights were on unless stated otherwise.

2.1.3. Pre-surgery training

First, animals were successively shaped to retrieve food rewards from the food tray and to poke any of the holes to receive food rewards. Then, each animal was trained daily for 30 min on the 5-choice serial reaction time task (5-CSRTT) by passing through several training stages of increasing difficulty. An animal was moved to the next training stage once it performed 100 trials per session on 2 consecutive days with an accuracy $>$80% and $<$20% omissions. Accuracy reflects the number of correct responses divided by the number of incorrect responses, whereas omissions are the failure to respond to the stimulus (misses). Each rat was always trained in the same conditioning chamber and the same four rats were always trained together (balanced across groups) at approximately the same time of the light phase of the day.

For the initial training stage, the animal was placed in the chamber and initiated the trial by retrieving a single food pellet from the food tray. After a fixed 5 s intertrial interval (ITI), one of the five horizontal lights was illuminated for a maximum of 60 s (cue duration) or until a response had been made. After the light first turned on, the animal had 60 s (limited hold period) to respond by making a nose poke into the previously lit aperture. Correct responses were immediately rewarded with delivery of a food pellet into the food magazine, and retrieval of the food restarted the new trial after a 5 s ITI. Several types of errors were recorded: (i) repeated nose pokes into the correct aperture were recorded as perseverative responses; (ii) nose pokes into a non-lit aperture were recorded as incorrect responses; (iii) nose pokes during the ITI were recorded as premature responses; (iv) failures to respond within the limited hold period were recorded as an omission. All errors were punished by switching off the house light for a 5 s time-out period and no food was delivered. Responses to holes during this period would restart the time-out period. Each day a testing session was terminated after 100 trials had been completed or 30 min had expired, whichever occurred first.

For subsequent training stages all parameters remained the same, but the stimulus duration was successively decreased from 60 to 0.6 s, and the limited hold period was successively decreased from 60 to 5 s. For the final training stage (baseline training), the cue duration was further reduced to 0.5 s. Training with this protocol continued until all animals performed at a criterion of $>$70% accuracy with $<$20% omissions for 5 consecutive days.

2.1.4. Ovariectomies and hormone treatment

After all animals had reached criterion performance, rats were ovariectomized while under anesthesia induced by injections of ketamine (100 mg/kg i.p., Bristol Laboratories, Syracuse, NY) and xylazine (7 mg/kg i.p., Miles Laboratories, Shawnee, KS). Rats were divided into two groups, matched in terms of their pre-surgery baseline performance (accuracy, omissions, and percent correct). During surgeries, animals were implanted with 5 mm SILASTIC brand capsules (0.58-in. i.d. and 0.077-in. o.d.) containing either 100% cholesterol, a control substance (Cholesterol, $n = 10$) or 25% 17β-estradiol diluted with cholesterol (Immediate E, $n = 10$). Following surgeries, animals were given 1 week to recover before testing continued, and on post-surgical day 5 they were again food deprived.

2.1.5. Post-operative retraining and testing

One week following ovariectomies, all animals were retrained on the baseline procedure for 14 sessions (1 session daily) with standard baseline parameters. Then, the following series of manipulations to challenge performance were introduced for one daily session each, separated from each other by 1 day of baseline training.

Short stimulus. At standard baseline parameters, stimulus duration was reduced so that the stimulus light was illuminated for only 0.25 s.

Unpredictable short ITIs. Various short ITIs (1.5, 2.0, 3.0, and 4.5 s) were presented in even numbers pseudorandomly distributed across the 100 trials.

Unpredictable long ITIs. Various long ITIs (4.5, 5.5, 6.5, 7.5 s) were presented in even numbers pseudorandomly distributed across the 100 trials.

Distracting noise. Bursts of white noise (0.5 s, 85 dB, 800 Hz) were presented at various time points during the 5-s ITI (0.5, 2.5, 3.5, or 4.5 s after start of the ITI). In 20% of the trials, no noise bursts were presented.

2.1.6. Scopolamine drug challenge

After completion of testing under behavior challenge conditions, scopolamine dose—effect curves were established across multiple sessions of baseline testing. Scopolamine hydrobromide (Sigma, St. Louis, MO) was dissolved in sterile saline (0.9%) and delivered i.p. in 0.1 ml/100 g bodyweight 20 min prior to the start of testing. Drug doses, administered in half-log increments, ranged from 0.01 to 1.0 mg/kg. Doses were administered interspersed by 1 day of baseline training without injections, and 1 day of baseline training with saline injections. Therefore, to avoid the development of tolerance, each scopolamine injection was separated from the next scopolamine injection by a 72-h period, and the highest dose was given on the last day.

2.1.7. Mecamylamine drug challenge

After completion of scopolamine testing, mecamylamine dose—effect curves were established across multiple sessions. Doses of mecamylamine hydrobromide (Sigma) were dissolved in sterile saline (0.9%) and delivered i.p. in 0.1 ml/100 g bodyweight 20 min prior to the start of testing. Drug doses ranged from 0.32 to 3.2 mg/kg. The injection schedule was as described for scopolamine.

2.1.8. Behavior measures

Throughout testing, the following set of behavioral measures was recorded by automated computer software (ABET II, Lafayette Instruments) on a PC connected to the conditioning chambers.

Percent correct. This cumulative measure reflects the total number of correct responses relative to the total number of trials completed. This measure indicates overall performance in the attention task where attention has to be sustained and divided across several spatial locations.

Percent omissions. An omission is a failure to respond during the limited hold period. This can reflect a failure to detect the stimulus due to attentional deficits, or alternatively, a failure to respond can indicate motivational or motor deficits. This distinction can be interpreted more conclusively together with the speed measures.

Speed. Two measures of speed are collected. First, the time between the onset of the stimulus and the time when the nose poke breaks the light beam in the aperture is measured as correct response latency. Further, latency to retrieve the food reward (reward latency) is defined as time between performance of a correct nose poke and the opening of the food magazine. If latency to respond is affected independently of reward latency, this indicates possible changes in decisional mechanisms, whereas decreases in reward latency might reflect incentive motivational factors. If both measures are affected, motivational and/or motor functions could be affected (Muir et al., 1996; Robbins, 2002).

Premature responses. Premature responses are defined as the number of responses in the apertures during the ITI. This reflects deficits in inhibitory mechanisms of response preparation.

Perseverative responses. Perseverative responses are defined as additional responding in the apertures following either a correct or an incorrect response. This reflects inhibitory processes of response control, but compared to premature responses, this measure is less related to response preparation.

2.1.9. Hormone treatment efficacy

Following the conclusion of all testing procedures on the 5-CSRTT, vaginal smears of all animals were collected across 4 consecutive days to confirm treatment efficacy. After maintaining all rats for several weeks on a free-feeding schedule, animals were weighed, blood was collected from the jugular vein while under anesthesia induced by ketamine (100 mg/kg) and xylazine (7 mg/kg) and then animals were sacrificed by decapitation. Uteri were removed and weighed to assess treatment efficacy. Blood was allowed to clot at RT for 90 min, and after centrifugation at 2000 $\times$ *g* for 15 min at RT serum was collected. To confirm the dosage range of circulating estradiol levels produced by the Silastic implants, a subset of serum samples from each group was randomly selected and commercially assayed using RIA (UVA Center for Research in Reproduction Ligand Assay and Analysis Core, Charlottesville, VA). The reportable assay range was 10—900 pg/ml, with a sensitivity of 10 pg/ml.

2.2. Experiment 2

2.2.1. Subjects, apparatus, and pre-surgery training

Female Long-Evans hooded rats, retired breeders, were purchased from Harlan Sprague—Dawley at the age of 10 months. One week after arrival, animals were food restricted and maintained at 85% of their free-feeding weight. The apparatus and 5-CSRTT training were as described in Experiment 1.

2.2.2. Ovariectomies and hormone treatment

At 12 months of age, during the final 10 days of 5-CSRTT training, daily vaginal smears were collected by lavage to confirm normal estrous cycles. After all animals had reached criterion performance (accuracy >70%, omissions <20%), animals were divided into four groups ($n = 10$), matched in terms of their pre-surgery baseline performance. Animals then underwent ovariectomies ($n = 30$) or sham surgeries ($n = 10$) and were then maintained in their homecages for a 5-month period. Once per week during this period, animals were weighed and their implanted capsules manipulated by hand to avoid tissue buildup. Additionally, daily vaginal smears were collected from each of the animals during the final 2 weeks of the 5-month period to confirm endocrine status.

After 5 months, at 17 months of age, all rats underwent a second set of surgeries. Animals that had previously been ovariectomized underwent sham surgeries and were implanted either with a cholesterol control capsule to replace the previous cholesterol capsule (Cholesterol, $n = 10$), received a new 25% estradiol capsule to replace the old estradiol capsule (Immediate E-12, $n = 10$), or their cholesterol capsule was replaced by a 25% estradiol capsule (Delayed E, $n = 10$). Animals that had previously received sham surgeries were ovariectomized and implanted with a 25% estradiol capsule (Immediate E-17, $n = 10$).

2.2.3. Post-operative retraining and testing

Throughout the 5-month period following the initial surgeries, animals received 1—2 baseline training sessions per week to maintain familiarity with the task contingencies until the second set of surgeries (Barnes et al., 2006; Muir et al., 1999). Beginning 1 week following the second set of surgeries, animals were retrained on the baseline procedure for 14 consecutive days with standard baseline parameters. Then, the same series of manipulations as described in Experiment 1 were introduced for one daily session each, to challenge performance. Finally, scopolamine and mecamylamine dose—effect curves were established as described in Experiment 1. The same measures as described for baseline training were recorded.

Following behavior testing animals were put on free-feeding for 2 weeks before being sacrificed. As described in Experiment 1, body weight and uterine weight were measured to confirm treatment efficacy, and randomly selected serum samples were analyzed for circulating estradiol levels.

2.2.4. Data analyses

For each experiment, baseline performance on all dependent variables (percent correct, percent omissions, correct response latency, reward latency, premature responses, and perseverative responses) was compared across the last 5 days of the 14-day retraining period, using repeated measures ANOVAs, with test day as within-subjects factor, and hormone treatment group as between-subjects factor. Similarly, performance across all behavior challenge conditions (Baseline, Short Stimulus, Short ITI, Long ITI, Noise) was analyzed for all dependent variables using overall repeated measures ANOVAs with behavior challenge condition as within-subjects factor, and with hormone group as between-subjects factor. For scopolamine and mecamyla-

mine drug challenge trials, performance was analyzed with ANOVAs as described for baseline testing, but with repeated measures on drug dose. When appropriate, Duncan's multiple range *post hoc* tests ($p < 0.05$) were employed.

3. Results

3.1. Experiment 1

One animal in the Immediate E group died during the course of the experiment and was excluded from all analyses, resulting in the following final group numbers: Cholesterol ($n = 10$), Immediate E ($n = 9$). All animals successfully acquired the task as indicated by criterion level performance within 60 training sessions before undergoing ovariectomies at the age of 4 months.

3.1.1. Baseline performance

After recovery from surgery, all animals were able to reacquire the task within 14 consecutive training sessions with no differences between groups, and performance remained stable across the last 5 days of retraining. There were no significant differences between hormone groups on any measure of performance under baseline conditions over the last 5 days of retraining (see Table 1), indicating that ovariectomy and subsequent estradiol or cholesterol administration did not affect motor function, sensory function, motivational factors, or the overall ability of the animals to perform the task (Robbins, 2002).

3.1.2. Behavior challenges

On the primary measure for task performance, percent correct, there was no significant main effect of group, but a significant main effect of behavior challenge condition ($F(4,68) = 37.94$, $p < 0.001$) and a significant interaction between behavior challenge condition and hormone group ($F(4,68) = 3.40$, $p < 0.05$). *Post hoc* analyses revealed that performance was impaired compared to baseline performance when a short stimulus or a short intertrial interval (ITI) was presented (see Fig. 1A). As follow up on the significant interaction between hormone group and behavior challenge condition, *t*-test revealed that under the Short ITI condition, the Cholesterol group was more impaired than the Immediate E group ($t(17) = 2.48$, $p < 0.05$). Further, the difference in performance under the Short ITI condition was not dependent on ITI duration, because an ANOVA across ITI durations revealed a significant effect of hormone group ($F(1,17) = 6.143$, $p < 0.05$), but no significant effect of ITI duration and no significant interaction (Fig. 2A). None of the other measures were significantly affected during the Short ITI condition.

There were no significant main effects of group and no significant interactions between hormone group and condition for percent omissions (Fig. 3A) or for any other measures across baseline challenge conditions. As expected, there were several significant main effects of behavior challenge condition, indicating that manipulating the task parameters taxed different aspects of task performance. First, there was a main effect for percent omissions ($F(4,68) = 45.27$, $p < 0.001$), and *post hoc* tests revealed that omissions increased in the Short ITI condition relative to the baseline condition. Second, there was a main effect on correct response latency ($F(4,68) = 8.09$; $p < 0.001$), and *post hoc* tests revealed decreased response latency in the Long ITI condition compared to baseline. Third, there was a main effect of premature responses ($F(4,68) = 27.38$, $p < 0.001$), and *post hoc* tests revealed that more premature responses were committed in the Long ITI condition, and fewer premature responses occurred in the Short ITI condition relative to baseline. Finally, there was a main effect on perseverative responding ($F(4,68) = 15.47$, $p < 0.001$), and *post hoc* tests revealed that the number of perseverative responses increased in the Long ITI condition compared to baseline. Because no hormone treatment effects were found in any of these measures, these data are not shown.

3.1.3. Scopolamine challenge

Across scopolamine doses, there were no effects of hormone group and no interactions on any measure. However, with increasing drug dose, the percentage of correct responses was significantly reduced ($F(4,68) = 12.92$, $p < 0.001$, see Fig. 4A). Further, scopolamine increased the percentage of omissions ($F(4,68) = 11.12$; $p < 0.001$), lowered reward latency ($F(4,68) = 4.17$; $p < 0.01$), and increased the number of premature responses ($F(4,68) = 5.95$; $p < 0.001$) in a dose-dependent fashion. Because no group differences emerged, data are not shown for these measures.

3.1.4. Mecamylamine challenge

Across mecamylamine doses, there were no effects of hormone group and no interactions on any measure. However,

Table 1 5-CSRTT performance across the last 5 days of post-ovariectomy retraining under baseline conditions.

	Percent correct		Percent omissions		Reward latency (s)		Correct latency (s)		Premature responses		Perseverative responses	
	M	S.E.M.	M	S.E.M.	M	S.E.M.	M	S.E.M.	M	S.E.M.	M	S.E.M.
Young adult												
Cholesterol	71.88	2.83	7.74	1.46	1.01	0.04	0.65	0.02	6.08	2.06	11.64	2.38
Immediate E	70.91	2.99	8.84	1.54	0.92	0.04	0.63	0.02	8.91	2.17	16.78	2.51
Middle aged												
Cholesterol	68.23	3.22	8.64	1.98	1.04	0.06	0.69	0.04	10.82	2.50	14.26	2.19
Immediate E-12	65.46	1.67	8.88	1.56	0.99	0.04	0 70	0.03	8.10	1.52	19.46	2.83
Immediate E-17	68.15	2.17	10.84	2.91	0.97	0.03	0.65	0.02	10.51	2.62	15.48	2.10
Delayed E	66.15	1.69	11.13	1.28	1.06	0.05	0.69	0.04	9.60	1.64	13.80	1.26

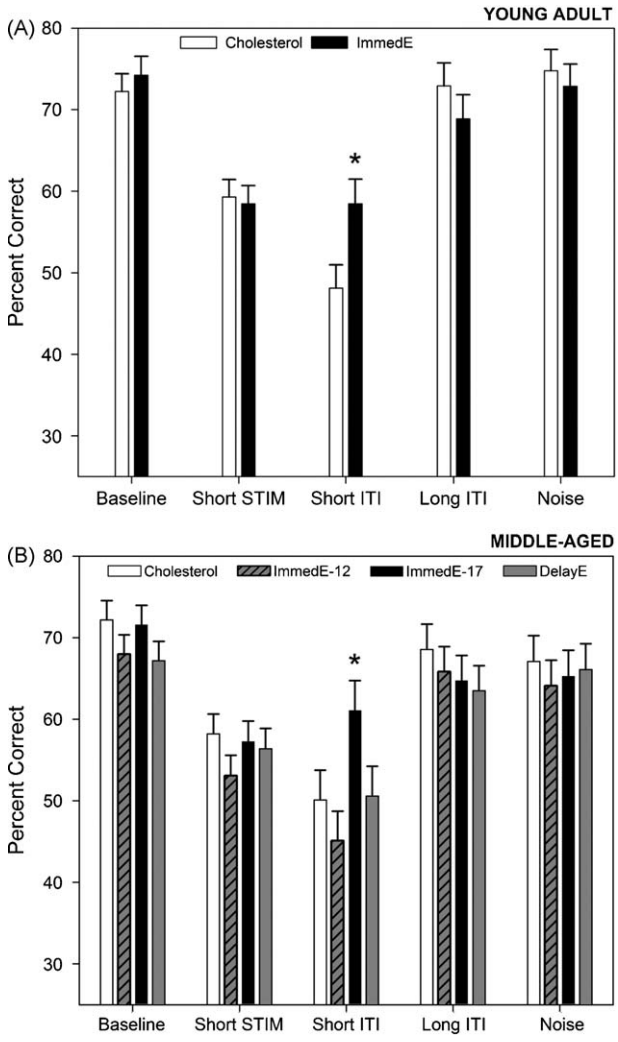

Figure 1 Effect of estradiol treatment on 5-CSRTT task performance under behavior challenge conditions. Percent correct choices (+S.E.M.) across all behavior challenge conditions are shown for young adult rats (A) and middle-aged rats (B). *$p < 0.05$ ImmedE versus Cholesterol (A). *$p < 0.05$ ImmedE-17 versus all other groups (B).

with increasing drug dose, the percentage of correct responses was significantly reduced ($F(2,34) = 106.08$, $p < 0.001$, see Fig. 4C). Further, mecamylamine increased the percentage of omissions ($F(2,34) = 125.99$, $p < 0.001$), and increased the correct response latency ($F(2,34) = 18.77$, $p < 0.001$) in a dose-dependent fashion. Because no group differences emerged, data are not shown for these measures.

3.1.5. Hormone treatment efficacy

There was a significant difference in body weight between hormone groups, $t(17) = 2.95$, $p < 0.01$. Estradiol-treated rats weighed less (mean $\pm$ S.E.M.; 245.11 ± 7.16 g) than cholesterol-treated rats (276.10 ± 7.58 g). Analyses also revealed a significant difference in uterine weight between groups, $t(17) = 9.37$, $p < 0.001$, with the uteri of estradiol-treated rats weighing more (75.21 ± 7.23 mg) than the uteri of cholesterol-treated animals (10.71 ± 0.72 mg).

Light microscopic examination of vaginal smears collected across 4 consecutive days following behavior testing revealed that hormone treatments were effective. Smears of all ovariectomized rats receiving cholesterol control treatment were characterized by a predominance of leucocytes. In contrast, smears of all ovariectomized rats receiving estradiol replacement were characterized by predominantly cornified cells with some nucleated epithelial cells. In agreement with previous work from our lab (Bohacek and Daniel, 2007), Silastic implants containing 25% estradiol produced estrogen levels in the physiological range (mean $\pm$ S.E.M.; 36.86 ± 5.35 pg/ml) whereas control implants produced estradiol levels that were below detection limit of the assay (8.70 ± 2.77 pg/ml).

3.2. Experiment 2

During scopolamine injections, one animal of the Immediate E-12 group died, and during mecamylamine injections, one animal from the Delay E group died. Thus, the total number of rats was reduced to 39 for analyses of scopolamine data, and further reduced to 38 rats for analyses of mecamylamine data.

3.2.1. Baseline performance

After recovery from surgery, all animals were able to reacquire the task within 14 consecutive training sessions with no differences between groups, and performance remained stable across the last 5 days of retraining. There were no significant differences between hormone groups on any measure of performance under baseline conditions over the last 5 days of retraining (see Table 1), indicating that ovariectomy and subsequent estradiol or cholesterol administration did not affect motor function, sensory function, motivational factors, or the overall ability of the animals to perform the task (Robbins, 2002).

3.2.2. Behavior challenges

On the primary measure for task performance, percent correct, there was no significant main effect of hormone group, but a significant main effect of behavior challenge condition ($F(4,144) = 44.83$, $p < 0.001$) and a significant interaction between behavior challenge condition and hormone group ($F(12,144) = 1.91$, $p < 0.05$). *Post hoc* analyses revealed that performance was impaired compared to baseline performance under all behavior challenge conditions (Fig. 1B). As follow up on the significant interaction between group and condition, a one-way ANOVA revealed a significant effect of group under the Short ITI condition ($F(3,39) = 3.41$, $p < 0.05$). *Post hoc* analyses revealed that the Immediate E-17 group performed significantly better than all other groups, which did not differ from each other. Further, the difference in performance under the Short ITI condition was not dependent on ITI duration, because an ANOVA across ITI durations revealed a significant effect of group ($F(3,39) = 3.41$, $p < 0.05$), but no significant effect of ITI duration and no significant interaction (Fig. 2B). None of the other performance measures were significantly affected during the Short ITI condition.

In addition, for percent omissions there was a significant main effect of behavior challenge condition ($F(4,144) = 69.29$, $p < 0.001$), and a significant interaction between hormone groups and behavior challenge conditions ($F(12,144) = 2.18$, $p < 0.05$). *Post hoc* tests revealed that

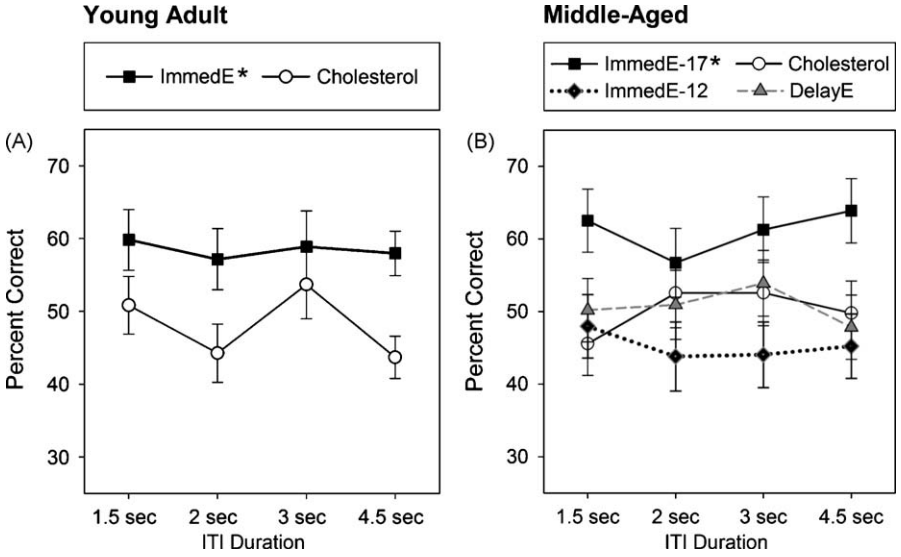

Figure 2 Effect of estradiol treatment on 5-CSRTT task performance across ITI durations in the Short ITI condition in young adult and middle-aged rats. Percent correct choices (±S.E.M.) across different ITI delays in the Short ITI condition are shown for young (A) and middle-aged (B) rats. *$p < 0.05$ ImmedE versus Cholesterol (A). *$p < 0.05$ ImmedE-17 versus all other groups (B).

the number of omissions significantly increased during Short ITI condition and during the noise distractor condition, compared to baseline performance. To follow up on the significant interaction, ANOVAs revealed a significant main effect of group only during the Long ITI condition. *Post hoc* tests indicated that the Delayed E group showed significantly more omissions than the Cholesterol and Immediate E-12 groups, whereas the Immediate E-17 group did not differ statistically from any of the other groups (Fig. 3B).

There were no significant main effects of hormone group and no significant interactions between hormone group and behavior challenge condition for all other measures across baseline challenge conditions. As expected, similar to the data obtained in young adult rats (see Experiment 1), there were several significant main effects of behavior challenge condition, indicating that manipulating the task parameters taxed different aspects of task performance. First, there was a significant main effect on correct response latency ($F(4,144) = 15.23$; $p < 0.001$), and *post hoc* tests revealed that correct response latency was increased in the Short ITI condition compared to baseline, but decreased in the Long ITI condition. Second, there was a significant effect on premature responses ($F(4,144) = 97.59$, $p < 0.001$), and *post hoc* tests revealed that compared to baseline performance, more premature responses were committed in the Short Stimulus and Long ITI conditions, and fewer premature responses occurred in the Short ITI condition. Finally, there was a significant effect on perseverative responding ($F(4,144) = 13.40$, $p < 0.001$), and *post hoc* tests revealed that perseverative responses increased in the Short Stimulus condition compared to baseline. Because no treatment effects were found in any of these measures, these data are not shown.

3.2.3. Scopolamine challenge

Across scopolamine doses, there were no effects of hormone group and no interactions on any measure. However, with increasing drug dose, the percentage of correct responses was significantly reduced ($F(3,105) = 33.05$, $p < 0.001$, Fig. 4B). Further, scopolamine increased the percentage of omissions ($F(3,105) = 30.03$; $p < 0.001$), increased reward latency ($F(3,105) = 7.00$; $p < 0.001$), increased correct response latency ($F(3,105) = 8.06$, $p < 0.001$), and increased the number of premature responses ($F(3,105) = 15.81$; $p < 0.001$) in a dose-dependent fashion. Because no group differences emerged, these data are not shown.

3.2.4. Mecamylamine challenge

Across mecamylamine doses, there were no effects of hormone group and no interactions on any measure. However, with increasing drug dose, the percentage of correct responses was significantly reduced ($F(2,68) = 24.97$, $p < 0.001$, Fig. 4D). Further, mecamylamine increased the percentage of omissions ($F(2,68) = 22.14$, $p < 0.001$) and increased correct response latency ($F(2,68) = 6.43$, $p < 0.01$) in a dose-dependent fashion. Because no group differences emerged, these data are not shown.

3.2.5. Hormone treatment efficacy

There was a significant main effect of hormone group for body weight ($F(3,37) = 3.55$, $p < 0.05$). *Post hoc* analyses revealed that animals in the Cholesterol group weighed significantly more (mean ± S.E.M.; 341.20 ± 5.36 g) than rats in all other groups: Immediate E-12 (314.56 ± 6.04 g), Immediate E-17 (320.70 ± 5.90 g), and Delayed E (322.22 ± 7.43 g). There was also a significant main effect of hormone group for uterine weight ($F(3,37) = 31.44$, $p < 0.001$). *Post hoc* analyses revealed that uteri of the Cholesterol group (13.61 ± 2.20 mg) weighed significantly less than uteri of the Immediate E-12 group (76.815 ± 16.05 mg), the Immediate E-17 group (81.64 ± 26.10 mg) and the Delayed E group (65.19 ± 17.29 mg).

Light microscopic examination of vaginal smears collected before the initial surgeries at the age of 12 months confirmed that all rats were still cycling. Smears showed changes in vaginal cytology across days, although there was evidence of

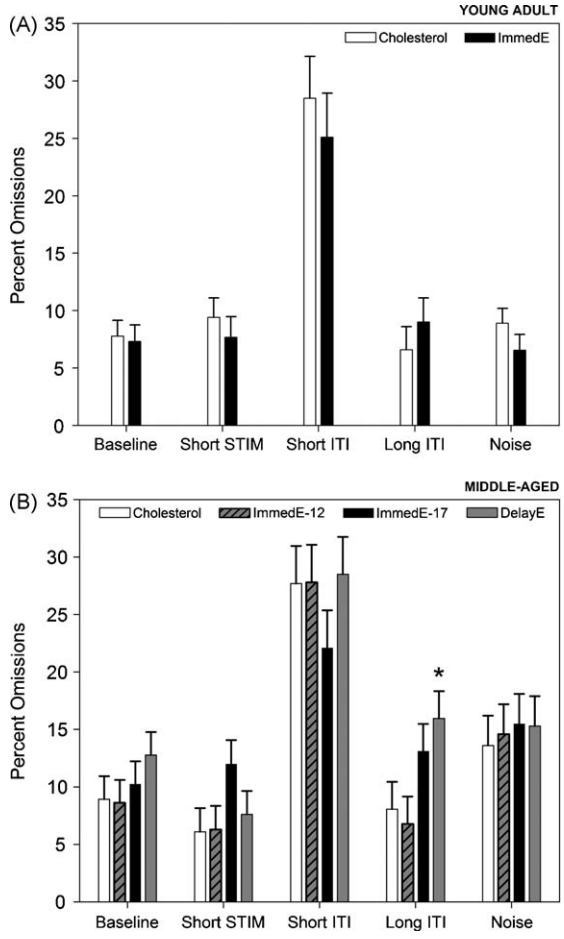

Figure 3 Effect of estradiol treatment on percent omissions on 5-CSRTT under behavior challenge conditions in young adult and middle-aged rats. Percent omissions (+S.E.M.) across all behavior challenge conditions are shown for young adult rats (A) and middle-aged rats (B). $^{*}p < 0.05$ DelayE versus Cholesterol and ImmedE-12.

disruptions in the cycles. The length of the cycles, as determined by the number of days between consecutive estrous stages, ranged from 4 to 7 days.

Smears collected during the final 2 weeks of the 5 months following the initial surgeries indicated that hormone treatments were effective. Smears of ovariectomized rats that had estradiol implants were characterized by a predominance of cornified cells with some nucleated epithelial cells. Smears of ovariectomized rats that had cholesterol implants were characterized by a predominance of leukocytes. Smears of the gonadally intact rats showed that two animals were acyclic and had entered constant estrus, during which vaginal cytology was characterized by a large number of cornified epithelial cells. The remaining 8 rats in the gonadally intact group were still cycling, although there were clear irregularities in their cycles. The length of the cycles, as determined by the number of days between two consecutive estrous stages, ranged from 5 to 8 days. Finally, examination of smears taken across 4 days after behavior testing had been completed showed effectiveness of estradiol and cholesterol treatments as described above.

Similar to the results obtained for young adult animals in Experiment 1, Silastic implants containing 25% estradiol produced estradiol levels in the physiological range in the Immediate E-12 group (mean $\pm$ S.E.M.; 30.14 ± 12.62 pg/ml), the Immediate E-17 group (33.28 ± 7.35 pg/ml), and the Delayed E group (36.32 ± 12.63 pg/ml), whereas control implants produced estradiol levels that were below detection limit of the assay (9.43 ± 2.12 pg/ml).

4. Discussion

The current work demonstrates for the first time that chronic estradiol treatment can enhance performance of the prefrontal cortex sensitive 5-choice serial reaction time task (5-CSRTT) when task difficulty is increased by unpredictably shortening the intertrial interval (Short ITI). Most importantly, this estradiol-induced effect is dependent on the timing of treatment initiation following ovariectomy, such that estradiol treatment initiated following a 5-month period of ovarian hormone deprivation fails to enhance task performance. Hence, these findings support the critical period hypothesis suggesting that there is a critical time period following loss of ovarian function during which estradiol treatment must be initiated in order to produce beneficial effects on prefrontal cortex sensitive task performance.

4.1. Estrogen and attention

A recent study by Barnes et al. (2006) has to date been the only report assessing the effects of estradiol treatment on 5-CSRTT performance in rodents. Although their study generally agrees with our findings that estradiol treatment enhances certain aspects of attention only under behavior challenge conditions, these authors found effects on different task manipulations. Several methodological differences could account for these minor differences. First, different rat strains were used. Second, we initiated hormone treatment during ovariectomies, while Barnes and colleagues initiated treatment 5 weeks after ovariectomies. Finally, they used daily 10 μg estradiol injections that produce high, supraphysiological estradiol levels in the range of 130–200 pg/ml (Barnes et al., 2006; Woolley and McEwen, 1994), whereas we used chronic estradiol delivery via Silastic implants which produced physiological levels of circulating estradiol of around 35 pg/ml. Notably, a previous study found that chronic estrogen treatment was only effective at enhancing memory performance in aged female rats when it was primed with high-dose estrogen injections (Markowska and Savonenko, 2002). Further research is needed to determine whether different estradiol treatment regimens can differently impact 5-CSRTT performance.

However, the results of two previous studies using prefrontal cortex sensitive attention tasks contrast with our findings. Both studies used different types of attention tasks involving lever-press operant conditioning. One study found that ovariectomy beneficially affected performance compared to ovarian intact rats (McGaughy and Sarter, 1999), and the other report showed that estrogen treatment had impairing rather than enhancing effects on performance (Wang et al., 2008). Apart from the overall differences in task requirements, a major methodological difference is that

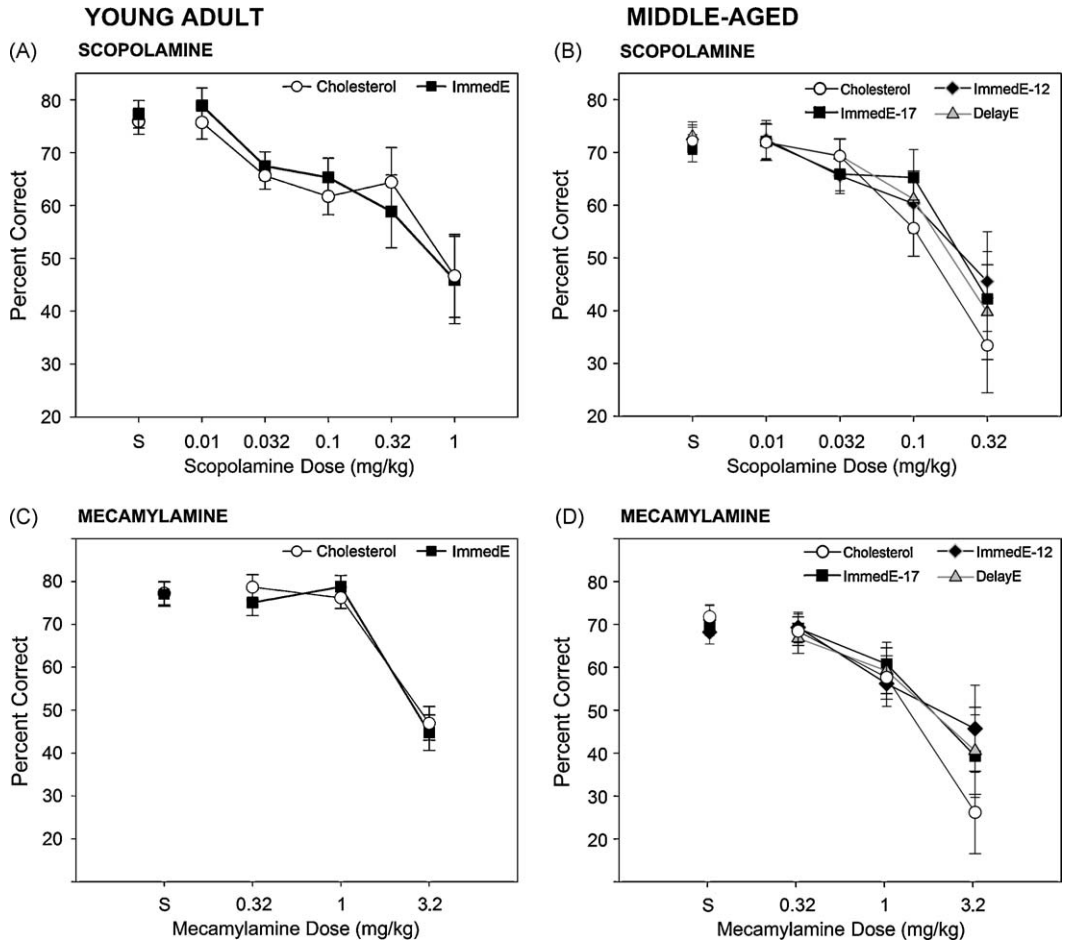

Figure 4 Effect of systemic scopolamine and mecamylamine injections on 5-CSRTT baseline performance of young adult and middle-aged rats. Rats were injected with scopolamine (A and B) or mecamylamine (C and D) 20 min before testing. Percent correct (+S.E.M.) are shown for young adult rats (left) and middle-aged rats (right), S = saline.

in our experiments rats were trained under intact ovarian conditions, but tested following manipulation of ovarian hormone levels, very similar to the study by Barnes et al. (2006). The two studies that found estrogen-induced impairments both trained their animals after hormone levels had been altered (McGaughy and Sarter, 1999; Wang et al., 2008), thus possibly allowing the animals to adopt different learning strategies that might have favored the ovariectomized control group (Korol, 2004). While this possibility needs to be investigated for prefrontal cortex sensitive tasks, training rats on hippocampus-sensitive tasks after ovariectomy did not interfere with the ability of estrogen to enhance performance (Daniel et al., 2006; Gibbs, 2000). This suggests that either different mechanisms are engaged in these two brain systems, or that other factors such as task requirements or hormone replacement regimens account for the differences between studies.

4.2. Immediate versus delayed estrogen treatment

In the present study, young as well as middle-aged female rats were able to reach comparable levels of 5-CSRTT performance independent of ovarian hormone condition. This is in agreement with a previous study (Barnes et al., 2006) and indicates that neither ovariectomy nor estradiol administration led to gross impairments in overall attention performance, nor did it affect motivational or sensorimotor processes (Robbins, 2002). However, when the intertrial interval was shortened in an unpredictable manner (Short ITI), group differences emerged. In young adult ovariectomized rats, estradiol-treated animals reached a significantly higher percentage of correct responses compared to cholesterol-treated rats. The Short ITI condition requires animals to continuously monitor their readiness to respond, rather than being able to rely on automatic processing to focus attention on the potential stimulus locations at a particular time as it can be done under baseline conditions (Robbins, 2002). Further, it increases attentional load by presenting the stimulus targets at a higher event rate. Impairments in performance likely represent a deficit in the control of the orienting response to target stimuli (Carli et al., 1983; Robbins et al., 1998). Analysis of performance across ITIs revealed that there was no difference in performance between different ITI durations. This suggests that the impaired performance was due to the unpredictability of the stimuli or the change in task contingencies rather than the decreased ITI duration *per se*.

Importantly, the same estrogen-induced enhancement of performance under the Short ITI condition described in young adult rats was also observed in middle-aged animals, such that rats receiving immediate estrogen treatment following ovariectomy at 17 months of age outperformed all other groups. First, this corroborates the role of chronic estradiol treatment to enhance performance under the Short ITI condition and demonstrates task reliability. Second, it indicates that estradiol treatment initiated immediately following ovariectomy at the age of 17 months has a positive effect on performance, but when treatment is delayed for 5 months, no behavior benefits are observed. This is in agreement with the critical period hypothesis and with previous findings from hippocampus-sensitive tasks suggesting that immediate but not delayed estrogen treatment has positive effects on cognitive function (Daniel et al., 2006; Gibbs, 2000). Unexpectedly, however, long-term chronic estradiol treatment initiated at the age of 12 months immediately following ovariectomy failed to enhance cognitive performance when tested in the Short ITI condition approximately 6 months later. This contrasts with our previous report where both groups, Immediate E-12 and Immediate E-17, outperformed the control group on a hippocampus-sensitive working memory task (Daniel et al., 2006). These results suggest that chronic estradiol treatment positively affects attention performance in rats, but that after prolonged chronic estradiol treatment this beneficial effect is lost. In women, a similar finding has been reported, where hormone therapy for shorter than 10 years was found to enhance prefrontal cortex sensitive performance on the Wisconsin Card Sorting Test and concomitantly increased prefrontal cortex volume as measured by MRI, whereas hormone treatment for longer than 10 years was associated with impairments on both measures (Erickson et al., 2007).

An alternative interpretation of our pattern of group effects could be that long-term ovariectomy for a 5-month period has detrimental effects on performance, independent of the presence or absence of estradiol. However, the observation that young, short-term (1 month) ovariectomized rats show almost the identical performance deficit in the Short ITI condition as middle-aged long-term (6 months) ovariectomized rats argues against this possibility, showing that the increased length of time without ovaries does not seem to lead to more substantial performance deficits (see Fig. 1). Importantly though, a comparison between young virgin and middle-aged retired breeder rats must be considered with caution because cognitive differences dependent on age and reproductive experience have been described previously (Macbeth et al., 2008; Pawluski et al., 2006).

A similarly selective impairment of performance only under the Short ITI condition as observed in cholesterol-treated rats has previously been reported following manipulations that dramatically reduce norepinephrine levels in the prefrontal cortex (Carli et al., 1983; Robbins et al., 1998). Estrogen modulates norepinephrine levels in the brain (for review, see Herbison et al., 2000) and can increase stimulation-induced norepinephrine release in the frontal cortex (Karkanias et al., 1998). Hence, it is possible that the effects of estradiol treatment on 5-CSRTT performance during the Short ITI condition might reflect a role for estradiol in modulating the noradrenergic system in the prefrontal cortex. Further studies are needed to address this possibility.

An additional effect of estradiol treatment was observed in the Long ITI condition, when omissions significantly increased only in the delayed estradiol group in middle-aged animals. This effect is difficult to interpret, because a selective increase in omissions can either represent a decrease in motivation or a failure to attend to the stimuli (Robbins, 2002). Also, the purpose of the Long ITI condition is mainly to challenge behavior inhibition and response control by increasing the time during which the animal has to wait for the stimulus onset (Dalley et al., 2004), and we did not observe differences in premature responses between groups.

4.3. Scopolamine and mecamylamine challenges

Cholinergic neurotransmission in the prefrontal cortex plays a central role in attention processes (Parikh and Sarter, 2008; for reviews, see Robbins, 2002) and is crucial for 5-CSRTT performance (Dalley et al., 2001; McGaughy et al., 2002; Muir et al., 1995; Robbins et al., 1998). Accordingly, our data show a dose-dependent decrease in overall task performance following systemic injections of scopolamine or mecamylamine. Similar to previous findings in males (Jones et al., 1995), middle-aged female rats were more sensitive to the performance impairing effects of scopolamine, as evidenced by an approximately half-log shift to the left in their dose—response curves as compared to the curves of the young adult rats (see Fig. 4A and B), and more sensitive to the impairing effects of mecamylamine (Fig. 4C and D). However, contrary to our hypothesis, we did not observe group differences between estradiol- and cholesterol-treated ovariectomized rats in young adult or in middle-aged rats in response to cholinergic drug challenges. This is specifically surprising in the delayed estradiol group in middle-aged animals, because we have previously observed that delayed estrogen treatment results in high levels of ChAT protein expression in the prefrontal cortex as compared to immediate treatment (Bohacek et al., 2008). Further, this finding differs from previous reports showing that estradiol treatment can protect against the impairing effects of systemically or centrally administered scopolamine on performance of hippocampus-sensitive learning and memory tasks (Dohanich et al., 1994; Fader et al., 1998). These results suggest a dissociation between the effects of estradiol on hippocampus and prefrontal cortex sensitive performance, with the former being more dependent on the cholinergic system. Further, our findings indicate that the increased levels of ChAT and estrogen receptor alpha that we have previously found in the prefrontal cortex in response to delayed estrogen treatment (Bohacek et al., 2008; Bohacek and Daniel, 2009) seem to be associated with behavioral deficits rather than benefits.

4.4. Conclusion

The current work presents evidence that chronic estrogen administration can enhance certain aspects of prefrontal cortex sensitive attention performance in young and middle-aged ovariectomized rats. Because the Women's Health Initiative Memory Study has failed to find protective effects of hormone therapy for cognitive aging if administered to older postmenopausal women, the current findings support the suggestion of the critical period hypothesis that treat-

ment initiated many years following menopause might lose its beneficial effects on cognitive function (Maki, 2006; Resnick and Henderson, 2002; Sherwin, 2007). These findings are the first to extend the validity of the critical period hypothesis across cognitive domains to prefrontal cortex dependent functions in rodent models, as so far only hippocampus-sensitive memory performance was shown to be sensitive to the timing of treatment initiation (Daniel et al., 2006; Gibbs, 2000). Further, the current findings suggest that continuous estradiol administration only benefits attention performance for a certain period of time. Benefits may be lost if treatment is continued beyond that period. Despite mounting evidence that estradiol can affect the prefrontal cortex on a molecular as well as on a behavioral level, a clear understanding of the underlying mechanisms is still lacking. Future studies will need to address mechanisms by which estrogen exerts its enhancing effects on 5-CSRTT performance, and establish how these mechanisms become dysregulated by a prolonged period of ovarian hormone deprivation, and by prolonged exposure to continuous estrogen treatment.

Role of funding source

Funding for this study was provided by National Science Foundation Grant 0715725 (J.M.D.). The NSF had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of interest

All authors declare that they have no conflicts of interest.

Acknowledgements

We gratefully acknowledge Brandon Parker, Nicole Ulrich, and Richard Silbert for their help in animal training and testing. We thank Drs. Gary Dohanich, Laura Schrader, and Paul Colombo for helpful discussions throughout the course of the study.

References

Barnes, P., Staal, V., Muir, J., Good, M.A., 2006. 17-Beta estradiol administration attenuates deficits in sustained and divided attention in young ovariectomized rats and aged acyclic female rats. Behav. Neurosci. 120, 1225—1234.

Bohacek, J., Bearl, A.M., Daniel, J.M., 2008. Long-term ovarian hormone deprivation alters the ability of subsequent oestradiol replacement to regulate choline acetyltransferase protein levels in the hippocampus and prefrontal cortex of middle-aged rats. J. Neuroendocrinol. 20, 1023—1027.

Bohacek, J., Daniel, J.M., 2007. Increased daily handling of ovariectomized rats enhances performance on a radial-maze task and obscures effects of estradiol replacement. Horm. Behav. 52, 237—243.

Bohacek, J., Daniel, J.M., 2009. The ability of oestradiol administration to regulate protein levels of oestrogen receptor alpha in the hippocampus and prefrontal cortex of middle-aged rats is altered following long-term ovarian hormone deprivation. J. Neuroendocrinol. 21, 640—647.

Carli, M., Robbins, T.W., Evenden, J.L., Everitt, B.J., 1983. Effects of lesions to ascending noradrenergic neurones on performance of a, 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. Behav. Brain Res. 9, 361—380.

Dalley, J.W., Cardinal, R.N., Robbins, T.W., 2004. Prefrontal executive functions in rodents: neural and neurochemical substrates. Neurosci. Biobehav. Rev. 28, 771—784.

Dalley, J.W., McGaughy, J., O'Connell, M.T., Cardinal, R.N., Levita, L., Robbins, T.W., 2001. Distinct changes in cortical acetylcholine and noradrenaline efflux during contingent and noncontingent performance of a visual attentional task. J. Neurosci. 21, 4908—4914.

Daniel, J.M., Dohanich, G.P., 2001. Acetylcholine mediates the estrogen-induced increase in NMDA receptor binding in CA1 of the hippocampus and the associated improvement in working memory. J. Neurosci. 21, 6949—6956.

Daniel, J.M., Hulst, J.L., Berbling, J.L., 2006. Estradiol replacement enhances working memory in middle-aged rats when initiated immediately after ovariectomy but not after a long-term period of ovarian hormone deprivation. Endocrinology 147, 607—614.

Dohanich, G.P., Fader, A.J., Javorsky, D.J., 1994. Estrogen and estrogen—progesterone treatments counteract the effect of scopolamine on reinforced T-maze alternation in female rats. Behav. Neurosci. 108, 988—992.

Dumas, J., Hancur-Bucci, C., Naylor, M., Sites, C., Newhouse, P., 2008. Estradiol interacts with the cholinergic system to affect verbal memory in postmenopausal women: evidence for the critical period hypothesis. Horm. Behav. 53, 159—169.

Erickson, K.I., Colcombe, S.J., Elavsky, S., McAuley, E., Korol, D.L., Scalf, P.E., Kramer, A.F., 2007. Interactive effects of fitness and hormone treatment on brain health in postmenopausal women. Neurobiol. Aging 28, 179—185.

Espeland, M.A., Rapp, S.R., Shumaker, S.A., Brunner, R., Manson, J.E., Sherwin, B.B., Hsia, J., Margolis, K.L., Hogan, P.E., Wallace, R., Dailey, M., Freeman, R., Hays, J., 2004. Conjugated equine estrogens and global cognitive function in postmenopausal women: women's health initiative memory study. JAMA 291, 2959—2968.

Fader, A.J., Hendricson, A.W., Dohanich, G.P., 1998. Estrogen improves performance of reinforced T-maze alternation and prevents the amnestic effects of scopolamine administered systemically or intrahippocampally. Neurobiol. Learn. Mem. 69, 225—240.

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

Gibbs, R.B., Gabor, R., 2003. Estrogen and cognition: applying preclinical findings to clinical perspectives. J. Neurosci. Res. 74, 637—643.

Henderson, V.W., Benke, K.S., Green, R.C., Cupples, L.A., Farrer, L.A., 2005. Postmenopausal hormone therapy and Alzheimer's disease risk: interaction with age. J. Neurol. Neurosurg. Psychiatry 76, 103—105.

Herbison, A.E., Simonian, S.X., Thanky, N.R., Bicknell, R.J., 2000. Oestrogen modulation of noradrenaline neurotransmission. Novartis Found. Symp. 230, 74—85.

Jones, D.N., Barnes, J.C., Kirkby, D.L., Higgins, G.A., 1995. Age-associated impairments in a test of attention: evidence for involvement of cholinergic systems. J. Neurosci. 15, 7282—7292.

Karkanias, G.B., Morales, J.C., Etgen, A.M., 1998. Effects of diabetes and estradiol on norepinephrine release in female rat hypothalamus preoptic area cortex. Neuroendocrinology 68, 30—36.

Korol, D.L., 2004. Role of estrogen in balancing contributions from multiple memory systems. Neurobiol. Learn. Mem. 82, 309—323.

Lam, T.T., Leranth, C., 2003. Role of the medial septum diagonal band of Broca cholinergic neurons in oestrogen-induced spine

synapse formation on hippocampal CA1 pyramidal cells of female rats. Eur. J. Neurosci. 17, 1997—2005.

Luine, V., Attalla, S., Mohan, G., Costa, A., Frankfurt, M., 2006. Dietary phytoestrogens enhance spatial memory and spine density in the hippocampus and prefrontal cortex of ovariectomized rats. Brain Res. 1126, 183—187.

Luine, V.N., 1985. Estradiol increases choline acetyltransferase activity in specific basal forebrain nuclei and projection areas of female rats. Exp. Neurol. 89, 484—490.

Macbeth, A.H., Scharfman, H.E., MacLusky, N.J., Gautreaux, C., Luine, V.N., 2008. Effects of multiparity on recognition memory, monoaminergic neurotransmitters, and brain-derived neurotrophic factor (BDNF). Horm. Behav. 54, 7—17.

MacLennan, A.H.M., Henderson, V.W.M., Paine, B.J.B., Mathias, J.P., Ramsay, E.N.B., Ryan PMBBF, Stocks, N.P.M., Taylor, A.W.M., 2006. Hormone therapy, timing of initiation, and cognition in women aged older than, 60 years: the REMEMBER pilot study [Article]. Menopause 13, 28—36.

Maki, P.M., 2006. Hormone therapy and cognitive function: is there a critical period for benefit? Neuroscience 138, 1027—1030.

Markowska, A.L., Savonenko, A.V., 2002. Effectiveness of estrogen replacement in restoration of cognitive function after long-term estrogen withdrawal in aging rats. J. Neurosci. 22, 10985—10995.

McGaughy, J., Dalley, J.W., Morrison, C.H., Everitt, B.J., Robbins, T.W., 2002. Selective behavioral and neurochemical effects of cholinergic lesions produced by intrabasalis infusions of, 192 IgG-saporin on attentional performance in a five-choice serial reaction time task. J. Neurosci. 22, 1905—1913.

McGaughy, J., Sarter, M., 1999. Effects of ovariectomy, 192 IgG-saporin-induced cortical cholinergic deafferentation, and administration of estradiol on sustained attention performance in rats. Behav. Neurosci. 113, 1216—1232.

McLaughlin, K.J., Bimonte-Nelson, H., Neisewander, J.L., Conrad, C.D., 2008. Assessment of estradiol influence on spatial tasks and hippocampal CA1 spines: evidence that the duration of hormone deprivation after ovariectomy compromises, 17beta-estradiol effectiveness in altering CA1 spines. Horm. Behav. 54, 386—395.

Morrison, J.H., Brinton, R.D., Schmidt, P.J., Gore, A.C., 2006. Estrogen, menopause, and the aging brain: how basic neuroscience can inform hormone therapy in women. J. Neurosci. 26, 10332—10348.

Muir, J.L., Fischer, W., Bjorklund, A., 1999. Decline in visual attention and spatial memory in aged rats. Neurobiol. Aging 20, 605—615.

Muir, J., Everitt, B., Robbins, T., 1995. Reversal of visual attentional dysfunction following lesions of the cholinergic basal forebrain by physostigmine and nicotine but not by the, 5-HT3 receptor antagonist, ondansetron. Psychopharmacology 118, 82—92.

Muir, J.L., Everitt, B.J., Robbins, T.W., 1996. The cerebral cortex of the rat and visual attentional function: dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task. Cereb. Cortex 6, 470—481.

Parikh, V., Sarter, M., 2008. Cholinergic mediation of attention: contributions of phasic and tonic increases in prefrontal cholinergic activity. Ann. N. Y. Acad. Sci. 1129, 225—235.

Pawluski, J.L., Vanderbyl, B.L., Ragan, K., Galea, L.A., 2006. First reproductive experience persistently affects spatial reference and working memory in the mother and these effects are not due to pregnancy or 'mothering' alone. Behav. Brain Res. 175, 157—165.

Resnick, S.M., Henderson, V.W., 2002. Hormone therapy and risk of Alzheimer disease: a critical time. JAMA 288, 2170—2172.

Robbins, T., 2002. The, 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. Psychopharmacology 163, 362—380.

Robbins, T.W., Granon, S., Muir, J.L., Durantou, F., Harrison, A., Everitt, B.J., 1998. Neural systems underlying arousal and attention, Implications for drug abuse. Ann. N. Y. Acad. Sci. 846, 222—237.

Sherwin, B.B., 2007. The critical period hypothesis: can it explain discrepancies in the oestrogen-cognition literature? J. Neuroendocrinol. 19, 77—81.

Sherwin, B.B., 2002. Estrogen and cognitive aging in women. Trends Pharmacol. Sci. 23, 527—534.

Shumaker, S.A., Legault, C., Kuller, L., Rapp, S.R., Thal, L., Lane, D.S., Fillit, H., Stefanick, M.L., Hendrix, S.L., Lewis, C.E., Masaki, K., Coker, L.H., for the Women's Health Initiative Memory Study, 2004. Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: women's health initiative memory study. JAMA 291, 2947—2958.

Spencer, J.L., Waters, E.M., Romeo, R.D., Wood, G.E., Milner, T.A., McEwen, B.S., 2008. Uncovering the mechanisms of estrogen effects on hippocampal function. Front. Neuroendocrinol. 29, 219—237.

Wang, V.C., Sable, H.J.K., Ju, Y.H., Allred, C.D., Helferich, W.G., Korol, D.L., Schantz, S.L., 2008. Effects of chronic estradiol treatment on delayed spatial alternation and differential reinforcement of low rates of responding. Behav. Neurosci. 122, 794—804.

Woolley, C.S., McEwen, B.S., 1994. Estradiol regulates hippocampal dendritic spine density via an N-methyl-D-aspartate receptor-dependent mechanism. J. Neurosci. 14, 7680—7687.

Woolley, C.S., 1998. Estrogen-mediated structural and functional synaptic plasticity in the female rat hippocampus. Horm. Behav. 34, 140—148.

Zandi, P.P., Carlson, M.C., Plassman, B.L., Welsh-Bohmer, K.A., Mayer, L.S., Steffens, D.C., Breitner, J.C., 2002. Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study. JAMA 288, 2123—2129.