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Voluntary Exercise Impairs Initial Delayed Spatial Alternation Performance in Estradiol Treated Ovariectomized Middle-Aged Rats

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Abstract

Estrogens differentially modulate behavior in the adult female rodent. Voluntary exercise can also impact behavior, often reversing age associated decrements in memory processes. Our research group has published a series of papers reporting a deficit in the acquisition of an operant working memory task, delayed spatial alternation (DSA), following 17 β -estradiol treatment to middle-aged ovariectomized (OVX) rats. The current study examined if voluntary exercise could attenuate the 17 β -estradiol induced deficits on DSA performance. OVX 12-month old Long-Evans rats were implanted with a Silastic capsule containing 17 β -estradiol (10% in cholesterol: low physiological range) or with a blank capsule. A subset of the 17 β -estradiol and OVX untreated rats were given free access to a running wheel in their home cage. All rats were tested for 40 sessions on the DSA task. Surprisingly, we found running wheel access to impair initial acquisition of the DSA task in 17 β -estradiol treated rats, an effect not seen in OVX untreated rats given running wheel access. This deficit was driven by an increase in perseverative responding on a lever no longer associated with reinforcement. We also report for the first time a 17 β -estradiol induced impairment on the DSA task following a long intertrial delay (18-sec), an effect revealed following more extended testing than in our previous studies (15 additional sessions). Overall, running wheel access increased initial error rate on the DSA task in 17 β -estradiol treated middle-aged OVX rats, and failed to prevent the 17 β -estradiol induced deficits in performance of the operant DSA task in later testing sessions.

Keywords

working memory; voluntary wheel running; estradiol; DSA; middle-aged

Introduction

Specific cognitive processes appear to decline more rapidly in the aging female after menopause, including memory processes regulated by the prefrontal cortical system

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(Sherwin and Henry, 2008), but findings from research assessing the effects of hormone replacement therapies (HRT) on cognitive decline have been inconsistent (reviewed in Coker et al., 2010; Hogervorst and Bandelow, 2010; Lethaby et al., 2008; Sherwin and Henry, 2008). The use of HRT by menopausal women declined dramatically after the Women's Health Initiative's study found that these therapies can increase the risk of stroke, breast cancer and heart attack (Chlebowski et al., 2003; Rossouw et al., 2002; Wassertheil-Smoller et al., 2003). However, the number of women using HRT remains significant, with over 35 million women filling prescriptions for HRT in 2010 (North American Menopause Association). Therefore, determining the impact of HRT on cognitive processes in aging postmenopausal women remains important.

Studies in animal models have revealed that 17 β -estradiol differentially modulates performance on several distinct types of cognitive tasks (see Dohanich et al, 2009; Frick, 2009; Korol, 2004; Luine, 2008). In particular, 17 β -estradiol restores the performance of ovariectomized (OVX) female rats to pre-OVX levels on tasks that engage the hippocampus (Daniel et al., 1997; Daniel and Dohanich, 2001; Davis et al., 2005; Korol and Kolo, 2002; Zurkovsky et al., 2006, 2007), but can impair the performance of OVX female rats on tasks that engage other brain structures, including prefrontal cortical and striatal memory systems (Davis et al., 2005; Korol and Kolo, 2002; Neese et al., 2010a, 2013; Wang et al., 2008, 2009; Zurkovsky et al., 2007). These findings suggest that 17 β -estradiol can have task and brain region-specific effects on cognition. Of particular importance are tasks that tap the prefrontal cortex as chronic 17 β -estradiol treatment to OVX rodents can alter dopamine signaling, resulting in reduced prefrontal tissue dopamine content (Dupont et al., 1981; Luine et al., 1998; Pandaranandaka et al., 2006). Further, rodent research has found that alterations in prefrontal dopamine result in performance deficits on a variety of delayed response tasks (Bubser and Schmidt, 1990; Kozlov et al., 2001; Mizoguchi et al., 2009; Vijayraghavan et al., 2007; Zahrt et al., 1997), providing one mechanism through which chronic 17 β -estradiol treatment can negatively alter behaviors dependent on this brain region.

Our research group has published a series of studies showing that 17 β -estradiol treatment impairs the performance of young, middle-aged, and old OVX rats on an operant test of working memory, the delayed spatial alternation (DSA) task (Neese et al., 2010a, 2013; Wang et al., 2008, 2009). 17 β -estradiol typically improves the performance of OVX rodents on maze-based working memory tasks, but these tasks often use long intertrial delays (see Aggleton et al., 1995; Pontecorvo et al., 1996; Van Hest and Steckler, 1996), and have been shown to rely heavily on the hippocampus for accurate performance (D'Hooge and DeDeyn, 2001; Floresco et al., 1997; Goldman-Rakic, 1995; Jones, 2002). Conversely, by utilizing short intertrial delays with automated operant testing the prefrontal cortex is selectively engaged. Specifically, lesioning or pharmacological inactivation of the prefrontal cortex results in clear deficits on operant working memory tasks with intertrial delays as short as 1–3 sec, whereas hippocampal inactivation does not alter performance at these short delays (Chudasama and Muir, 1997; Harrison and Mair, 1996; Mair et al., 1998; Sloan et al., 2006; Van Haaren et al., 1985, 1988; Young et al., 1996). With longer intertrial delays (>10–15 sec), hippocampal disruption begins to impair performance accuracy (Chudasama and Muir, 1997; Dunnett, 1985; Kirkby and Higgins, 1998; Izaki et al., 2008; Maruki et al., 2001). Therefore, operant working memory tasks that include short intertrial delays can specifically tap the prefrontal cortical system.

Voluntary exercise (i.e. running wheel access) has been studied as an intervention to reduce age associated cognitive decline in rodents (see van Praag, 2008). Voluntary exercise can improve the performance of gonadally-intact young, middle-aged and old male and female rodents on a variety of cognitive tasks, including maze-based reference and working

memory tasks (Leggio et al., 2005; Marlatt et al., 2012; Van der Borgh et al., 2007; van Praag et al., 1999, 2005), tests of recognition memory (Hopkins et al., 2011), and passive avoidance tasks (Samorajski et al., 1985). Of note, treadmill exercise for 20 mins three times per week reverses deficits in reference and working memory in the Morris water maze in OVX female rats but fails to improve memory in gonadally intact female rats (Ben et al., 2010). However, little is known about the effects of exercise on memory processes in aging (>12 month old) OVX rats.

Human research also suggests a link between exercise and cognitive health during aging (see Plassman et al., 2010; van Uffelen et al., 2008), and one study found that fitness levels measured at the time of testing and duration of HRT taken previously can interact to differentially affect cognition in aging women (Erickson et al., 2007). Specifically, this study found that long term HRT (over 11 years) resulted in more perseverative errors on the Wisconsin Card Sorting Task in women with a lower fitness level, relative to women on long term HRT that were more physically fit. These study results suggest that long term HRT may impair some aspects of cognition mediated by the prefrontal cortex (see also Coker et al., 2010; Hogervorst and Bandelow, 2010; Lethaby et al., 2008), and that physical activity may protect against these effects in aging women undergoing HRT.

The current study was conducted to determine if the detrimental effects of 17 β -estradiol treatment on a prefrontal-cortex sensitive operant DSA task could be prevented by voluntary daily exercise. Middle-aged rats were OVX, and treated with 17 β -estradiol or left untreated. A subset of the 17 β -estradiol-treated and untreated OVX rats were given free access to a running wheel in their home cage. Thus, the goal of this study was to determine if the 17 β -estradiol-induced deficit in operant DSA performance could be attenuated by voluntary running wheel activity. All rats were subject to 40 sessions of DSA testing.

Methods

Animals and Exposure

Twenty-nine 12-month old female Long-Evans retired breeder rats were obtained from Harlan (Indianapolis, IN) in 2 cohorts (see below) and maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). Rats were housed in a temperature and humidity controlled room (22°C, 40–55% humidity) on a 12-hour reverse light-dark cycle (lights off at 8:30 am). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Illinois at Urbana-Champaign and were in accordance with the guidelines of the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (National Institutes of Health, 2002) and the *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (National Research Council, 2003).

Rats were single-housed in plastic cages (22.9 × 21.6 × 20.3 inch) with woodchip bedding. All rats were subject to isoflurane gas anesthesia prior to OVX and the implant of a Silastic capsule that was either left blank (n=13) or contained 17 β -estradiol (n=16). The Silastic capsules were 1 cm in length (1.5 mm i.d., 1.96 mm o.d.) and were plugged with silicone and dried overnight before packing with either a 10% 17 β -estradiol/cholesterol mixture (Sigma, St. Louis, MO) or left blank, after which the other end was plugged with silicone. Capsules were soaked in sterile saline at 37°C overnight before insertion during surgery. Previous research in this lab (Neese et al., 2010a) has shown that these 17 β -estradiol implants produce stable serum estradiol concentrations of about 20–30 pg/ml for at least 10 weeks.

Beginning one week after OVX surgery, rats were weighed daily and food was restricted to maintain them at 85% of their free-feeding body weights. A subset of the 17 β -estradiol implanted rats (estradiol, n=8) and OVX rats given a blank implant (OVX, n=5) were placed into cages with a running wheel freely available. The running wheel was 34.3 inches in diameter with a 10.2 inch lane width. Wheel rotations were recorded once daily. Final experimental groups were as follows: OVX with 17 β -estradiol implant and running wheel access (estradiol-wheel, n=8), OVX with 17 β -estradiol implant and no running wheel access (estradiol-no wheel, n=8), OVX with a blank implant and running wheel access (OVX-wheel, n=5), and OVX with blank implant and no running wheel access (OVX-no wheel, n=8). Due to a limited number of wheels, the OVX-wheel group was included in a separate testing cohort.

Soy phytoestrogens are present in standard rodent diets and can influence behavior (see Brown and Setchell, 2001; Lee et al., 2009; Lund et al., 2001; Neese et al., 2010b, 2012; Pisani et al., 2012; Thigpen et al., 2004, 2007). Therefore, to avoid exposure to additional estrogens, the rats in this study were maintained on a low-soy diet (Harlan diet 2016, Madison, WI). Water was available *ad libitum*. During behavioral testing, rats were fed one hour after the daily test session was completed. Operant training began two weeks following OVX and occurred once daily, six days/week during the dark phase of the light cycle.

Operant Testing

Behavioral testing was conducted in standard automated operant chambers (Med Associates Inc., St. Albans, VT) housed in sound-attenuated wooden boxes. All of the test chambers had the same features and dimensions: 21.6 cm high with a 29.2 cm $\times$ 24.8 cm stainless-steel grid floor that rested just above a tray filled with woodchip bedding. Soy-free 45-mg food pellets (5TUL, Test diet, Richmond, IN) were dispensed through a pellet dispenser centered 2.5 cm above the floor on the operant panel. A pair of retractable response levers and a pair of stimulus cue lamps, one above each lever, were positioned symmetrically on both sides of the pellet dispenser. The levers were 5.7 cm from midline and 7.0 cm above the floor and the cue lights were located 5.7 cm above the levers. Each chamber also contained a Sonalert tone generator, a white noise generator, and a house light located on the back wall. Experimental contingencies were programmed using the Med-State behavioral programming language (Med-Associates, Vermont).

Response shaping and lever press training

Rats were trained to press the response levers by using an autoshaping program (Neese et al., 2010a, 2010b; Wang et al., 2008, 2009; Widholm et al., 2001, 2003). Autoshaping test sessions were terminated after 60 mins elapsed or 100 reinforcers were delivered. Criterion for this condition was set at 100 lever presses in two consecutive sessions. Following autoshaping, the rats were exposed to a continuous reinforcement schedule in which the lever associated with reinforcement alternated following delivery of every 5th reinforcer in order to prevent the rats from developing a lever or side preference. This cycle of alternating levers terminated after 100 reinforcers were received or 60 mins had elapsed. A performance criterion of 100 reinforcers earned for three consecutive sessions was established for this condition.

Training Phases Prior to DSA

After lever press training, the rats were trained to alternate their responses prior to beginning testing on the DSA task (Neese et al., 2010a, 2010b; Wang et al., 2008, 2009; Widholm et al., 2003). The initial training schedule began with cued alternation (CA) training in which a cue light was illuminated to indicate the correct lever on each trial. Each correct cued lever press was reinforced. The lever associated with reinforcement was not altered following an

incorrect response, i.e. the “correct” lever continued to be the lever opposite of the lever associated with reinforcement in the most recent “correct” trial. A 0-sec delay was imposed between trials in this initial cued training phase as levers were retracted and extended between trials. It is important to distinguish a “0-sec” delay from “no delay”. Although the 0-delay is intended to be the “lack” of an intertrial delay, the levers are retracted for a brief (<0.15 sec) delay before insertion back into the chamber. Therefore, the rat is not always in the presence of the stimulus (i.e. the lever), but can respond immediately upon the insertion of the levers back into the chamber following this minimal passage of time (see also Viscardi and Heise, 1986). Rats were trained to a criterion of one session above chance defined as $>60\%$ correct presses. Next, a noncued alternation (NCA) task was presented where the cue light no longer indicated the correct lever, and both cue lights were illuminated when the levers were extended. Correct responses still consisted of alternating right and left lever presses. As for CA, the correct lever was again always the lever opposite of the one on the trial in which the most recent “correct” lever press occurred. Again, a 0-sec delay was imposed between opportunities to press with the levers retracting and extending between presses. Each rat was trained for 10 consecutive sessions on the NCA phase.

Delayed spatial alternation (DSA) testing

The final alternation phase was the variable DSA task. Following each lever press, variable intertrial delays of 0, 3, 6, 9, or 18-sec were randomly imposed between opportunities to press (Neese et al., 2010a, 2010b; Wang et al., 2008, 2009; Widholm et al, 2003). There were 40 trials at each delay and a total of 200 trials per session. Intertrial delays were randomly balanced within each session and any specific delay was not presented on more than three consecutive trials. The correct lever was again always the lever opposite the one on the trial in which the most recent “correct” lever press occurred. Each animal was tested for 40 consecutive sessions.

Statistical Analyses

The behavioral data were analyzed using SPSS for Windows, Version 20.0. Treatment group [OVX treated with estradiol (estradiol) or OVX-untreated (OVX): treatment] and wheel access (wheel or no wheel: wheel) were included in the analyses as between subject factors and significance was set at $p < 0.05$. When appropriate, Tukey post hoc tests were run for pair-wise comparisons. Tukey is a conservative post hoc test that protects for experimentwise error when multiple comparisons are conducted, thus some differences that may appear to be significant on the graphs did not reach statistical significance in the Tukey comparisons. Many of these had p values between 0.05 and 0.10.

For wheel rotations during CA, cumulative distance run was analyzed between the estradiol-wheel and OVX-wheel rats using between-subjects ANOVA for treatment. For NCA, wheel rotations across the ten sessions were analyzed using a 2 (treatment) $\times$ 10 (session) mixed ANOVA where session was the repeated measures factor. For DSA, wheel rotations across the 40 test sessions were first averaged across blocks of 5 test sessions to produce eight 5-session blocks (see also DSA analyses below) and were analyzed with a 2 (treatment) $\times$ 8 (block) mixed ANOVA where block was the repeated measured factor.

For CA, cumulative errors across all sessions served as the main measure of learning, and were analyzed using ANOVA for treatment $\times$ wheel. For NCA, the overall proportion correct across the ten sessions served as the primary measure of learning, and was analyzed using a 2 (treatment) $\times$ 2 (wheel) $\times$ 10 (session) mixed ANOVA where session was a repeated measures factor. Latencies to respond following either a correct or an incorrect lever press during NCA testing were also analyzed using a 2 (treatment) $\times$ 2 (wheel) $\times$ 10 (session) mixed ANOVA where session was a repeated measures factor.

For DSA, the proportion correct across the 40 test sessions was first averaged across blocks of 5 test sessions to produce eight 5-session test blocks. Proportion correct at each intertrial delay across the 40 test sessions was then analyzed using a mixed 2 (treatment) $\times$ 2 (wheel) $\times$ 8 (block) $\times$ 5 (delay) repeated measures ANOVA with block (1 – 8) and delay (0, 3, 6, 9, 18-sec) serving as repeated measures factors. In contrast to NCA training, performance on the DSA task is more variable with rats achieving a slower rate of acquisition than during NCA training. Therefore, averaging the data into 5-session testing blocks provides a clearer index of acquisition of this task. Pearson correlations between DSA performance and wheel rotations were also conducted for any blocks and/or delays in which the performance of the estradiol-wheel or OVX-wheel groups was different from the other experimental groups.

Error pattern analyses were conducted to better understand potential behavioral changes in rats given access to a running wheel. Specifically, these analyses were completed to determine if rats were more likely to respond incorrectly following a correct or an incorrect lever press. A “win-stay” error was defined as an incorrect response on the same lever that had been correct on the previous trial, whereby the rat responded correctly on the $n-1$ trial, but incorrectly on the n^{th} trial. A “lose-stay” error was defined as an incorrect lever press on the same lever that had been incorrect on the previous trial, whereby the rat responded incorrectly on the $n-1$ trial as well as the n^{th} trial. Lose-stay errors thus represent at least the second consecutive press on the lever no longer associated with reinforcement. Win-stay and lose-stay errors were analyzed separately using a mixed 2 (treatment) $\times$ 2 (wheel) $\times$ 8 (block) repeated measures ANOVA with block (1 – 8) serving as a repeated measures factor. The latencies to respond following a correct and an incorrect lever press were also analyzed separately using a mixed 2 (treatment) $\times$ 2 (wheel) $\times$ 8 (block) repeated measures ANOVA with block (1 – 8) serving as a repeated measures factor.

Results

Wheel Rotations

Distance run (in km) for the estradiol-wheel and OVX-wheel groups during DSA training and testing is presented in Table 1. For ease of presentation, distance run during CA is averaged across training sessions. In addition, distance run during NCA is collapsed into two 5-session blocks signifying the first 5 and last 5 sessions of training, respectively. Treatment groups differed in distance run throughout DSA training and testing. A main effect of treatment was uncovered for CA [$F(1,11)=5.867$, $p=0.034$], NCA [$F(1,11)=5.442$, $p=0.040$], and DSA [$F(1,11)=13.137$, $p=0.004$] testing. As Table 1 shows, estradiol-wheel rats ran significantly more than did the OVX-wheel rats. In addition, all rats tended to increase distance run across subsequent sessions of DSA training and testing, with running becoming relatively stable in last blocks of DSA testing. Overall, it does not appear that the shift from CA to NCA training, or NCA training to DSA testing resulted in any significant changes in distance run in either treatment group.

CA and NCA Training

Experimental groups did not differ on errors committed during CA training. This was revealed by non-significant main effects of treatment, $F(1,25)=0.262$, $p>0.05$ and wheel $F(1,25)=1.066$, $p>0.05$, and a non-significant treatment $\times$ wheel interaction, $F(1,25)=2.712$, $p>0.05$.

Experimental groups also did not differ in performance accuracy during NCA training as indicated by a non-significant treatment $\times$ wheel $\times$ session ANOVA, $F(9,225)=1.721$, $p>0.05$. The lack of effect was confirmed by non-significant main effects of treatment and wheel, and a non-significant treatment $\times$ wheel interaction, $p>0.05$. A main effect of session

was uncovered, $F(9,225)=62.64$, $p<0.001$. The performance of all rats improved across subsequent testing sessions.

Latencies to respond during NCA training were influenced by experimental group. A significant treatment x wheel interaction was uncovered for both latency to respond after both a correct, $F(1,25)=9.14$, $p=0.006$ (Fig. 1A), and an incorrect lever press, $F(1,25)=6.07$, $p=0.021$ (Fig. 1B). For latency after either a correct and an incorrect press, estradiol-no wheel rats had a longer latency to respond than did the estradiol-wheel rats and the OVX-no wheel rats, $p<0.05$ (Fig. 2). Experimental group did not interact with session, $p>0.05$.

DSA Testing

Statistical analysis of the full model revealed a non-significant treatment x wheel x block x delay interaction, $F(28,700)=2.00$, $p>0.05$. Analysis did reveal a significant treatment x wheel x block interaction, $F(7,175)=3.98$, $p=0.022$. Follow-up analyses across treatment found a significant effect in the estradiol-treated groups only, with the estradiol-wheel rats performing significantly worse than did the estradiol-no wheel group in the 1st block of testing, $p<0.05$ (Fig. 2A). In addition, analyses by wheel found a significant effect in the no wheel groups, with the estradiol-no wheel rats performing significantly worse than did the OVX-no wheel rats in the 6th–8th blocks of testing, $p<0.05$ (Fig. 2C). Follow-up analysis failed to find a significant effect for the OVX-untreated (Fig. 2B) or wheel groups (Fig. 2D), respectively ($p>0.05$). A treatment x block x delay interaction was also uncovered, $F(28,700)=3.06$, $p=0.010$. For ease of discussion, follow-up analyses by treatment group are described by delay below. Finally, wheel access was not found to interact with performance by delay, $p>0.05$.

0-second delay: No significant effects of treatment were uncovered, $p>0.05$, (Fig. 3A).

3-second delay: The estradiol treated rats performed significantly worse than did the OVX only (untreated) rats in the 3rd–7th blocks of testing, $p<0.05$ (Fig. 3B).

6-second delay: The estradiol treated rats performed significantly worse than did the OVX only (untreated) rats in the 3rd and 6th–8th blocks of testing, $p<0.05$ (Fig. 3C).

9-second delay: The estradiol treated rats performed significantly worse than did the OVX only (untreated) rats in the 6th and 8th blocks of testing, $p<0.05$ (Fig. 3D).

18-second delay: The estradiol treated rats performed significantly worse than did the OVX only (untreated) rats in the 6th–8th blocks of testing, $p<0.05$ (Fig. 3E).

Error Patterns

Win-stay errors—A significant treatment x wheel x block interaction was uncovered, $F(7,175)=3.62$, $p=0.032$. Follow-up analyses by wheel found the estradiol-no wheel rats committed significantly more errors than did the OVX-no wheel rats in the 6th–8th blocks of testing, $p<0.05$ (Fig. 4A). Follow-up analyses failed to find differences for the OVX-untreated, estradiol-treated, and wheel groups, $p>0.05$. A main effect of block was also uncovered, $F(7,175)=75.24$, $p<0.001$. All groups committed fewer errors across subsequent sessions of testing.

Lose-stay errors—A significant treatment x wheel x block interaction was uncovered, $F(7,175)=3.69$, $p=0.022$. Follow-up analyses by wheel found that the estradiol-no wheel rats committed significantly more errors than did the OVX-no wheel rats in the 6th–8th blocks of testing, $p<0.05$ (Fig. 4B). In addition, analyses by treatment found the estradiol-wheel rats committed significantly more errors in the 1st block of testing than did the estradiol-no wheel rats, $p<0.05$ (Fig. 4C). Follow-up analyses failed to find differences for the OVX-

untreated and wheel groups, $p>0.05$. A main effect of block was also uncovered, $F(7,175)=131.91$, $p<0.001$. All groups committed fewer errors across subsequent sessions of testing.

Latencies to lever press

Latencies to lever press were also influenced by experimental group. Similar to NCA training, a significant treatment x wheel interaction was uncovered for latency to respond after a correct press, $F(1,25)=4.61$, $p=0.042$. The estradiol-no wheel rats had a longer latency to press than did the estradiol-wheel and OVX-no wheel rats, $p<0.05$. In addition, for latency to press after an incorrect response, a main effect of treatment was uncovered, $F(1,25)=6.22$, $p=0.020$. The estradiol treated rats had a longer latency to press than did the OVX only (untreated) rats, $p<0.05$. A main effect of block was also uncovered for latency to respond after an incorrect press, $F(7,175)=6.01$, $p=0.003$. Latencies tended to decrease across sessions of testing.

Correlations

Correlations of wheel-running to DSA performance were conducted for the estradiol-wheel rats for the 1st block of testing. Overall, more rotations resulted in worse performance. This was supported by a negative correlation between wheel rotations and DSA performance, $r=-0.728$, $p=0.041$. For comparison, correlations of wheel-running to DSA performance for OVX-wheel rats were conducted for the 1st block of testing. In contrast to the estradiol-wheel rats, no significant relationship was measured between rotations and DSA performance for the OVX-wheel rats in the 1st block of testing, $r=-0.845$, $p>0.05$.

Discussion

The results of this study suggest that voluntary exercise impairs initial DSA acquisition in 17 β -estradiol treated rats. This deficit was driven, in part, by an increase in presses to a lever no longer associated with reinforcement. In contrast, running wheel access did not alter the initial performance of untreated OVX rats, confirming an interaction between wheel running and 17 β -estradiol treatment on this initial DSA deficit. Overall, running wheel access to OVX untreated rats failed to significantly alter DSA performance at any testing time point. Finally, with the extended testing protocol used here, we also observed a significant deficit in performance following the 18-sec intertrial delay in OVX middle-aged 17 β -estradiol treated rats, an effect which we have not previously reported (Neese et al., 2010a, 2013; Wang et al., 2009).

Exercise induced performance deficits in the first block of testing

It is interesting that running wheel access worsened the initial performance of 17 β -estradiol treated rats on the DSA task in comparison to 17 β -estradiol treated rats without running wheel access. As shown in Fig. 2A, there was an impairment in the performance of the estradiol-wheel group in the 1st block of testing. This decrement was less obvious in the 2nd block of testing, with performance of the estradiol-wheel group reaching that of the estradiol-no wheel group by the 3rd block of testing.

Statistical analysis failed to find a significant treatment x wheel x block x delay effect. This suggests an impairment in a domain other than working memory as a deficit specific to working memory would be expected to decline with increasing delay. In this case, the deficit in the first block of testing suggests that the performance of the estradiol-wheel group was disrupted by the change in task contingencies following the shift from non-cued alternation to the variable delay alternation task (Heise and Hudson, 1985).

The deficit in the performance of the estradiol-wheel group in the 1st block of testing was driven by an increase in lose-stay errors as compared to the estradiol-no wheel group, a pattern of lever pressing reflective of response perseveration (see Martinez et al., 1988; Zornetzer et al., 1982). This increase in perseverative responding may be due to an increase in emotional reactivity (i.e. “frustration”: see Skirrow et al., 2009) in response to the addition of unpredictable delays in stimulus presentation and/or the reduction in rewards earned during the early testing sessions of the DSA task (see also Amsel, 1958; Amsel et al., 1964). In particular, a recent study found that omission of a previously rewarded stimulus (food pellet) under the “expectation” of reward produced perseverative responding in mice (Burokas et al., 2012). Specifically, when mice trained to associate a single lever press with both the illumination of a cue light and a food reward were transitioned to an experimental condition in which a lever press only resulted in illumination of the cue light, these mice tended to make a series of unrewarded lever presses during the initial testing sessions. Similarly, an inability to adjust to the new response requirements during the early stages of DSA testing could be responsible for the response perseveration seen during the initial testing block (see also Kelley, 2004), a response pattern also seen in other operant tasks following a change in response contingencies (see Hilson and Strupp, 1997).

Running wheel activity varies across the estrous cycle in female rodents, reaching a peak on the day of estrous (Albers et al., 1981; Kent et al., 1991; Stern and Murphy, 1972; Wollnik and Turek, 1988). A similar increase in running is seen in OVX rats following 17 β -estradiol treatment (Gorzek et al., 2007; Kadi et al., 2002; Morgan and Pfaff, 2001 and see below), an effect confirmed in the current study. Thus, the desire to run could have diminished the motivation of the estradiol-treated wheel rats to perform the DSA task, an effect which may have been most visible during the initial DSA testing sessions that were the first to require “wait” periods between opportunities to respond.

Chronic 17 β -estradiol treatment has been found to reduce prefrontal tissue dopamine content, an effect thought to be driven by enhanced dopamine turnover in this brain region (Luine et al., 1998; Pandaranandaka et al., 2006). Interestingly, exercise has also been shown to alter dopamine signaling in the brain, with some studies reporting an increase in dopamine metabolism in distinct regions of the brain, including the frontal cortex (see Chauloff, 1989). Stress models also provide evidence of altered dopamine signaling in the rodent brain (see Claustre et al., 1986). Therefore, as discussed above, the frustration (i.e. stress) caused by the change in response contingencies in the shift from NCA training to DSA testing may have further increased dopamine metabolism in the estradiol-wheel rats, providing one mechanism by which running wheel access may have impaired the performance of the estradiol-wheel rats in comparison to the estradiol-no wheel rats during the initial DSA testing sessions.

Correlational analyses also confirmed a negative relationship between number of wheel rotations and proportion correct in 17 β -estradiol treated rats in the 1st block of DSA testing. It does not appear that the change in task demands influenced distance run as an appreciable change in wheel rotations was not seen in the transition from the NCA to the DSA conditions in either treatment group (Table 1). Research suggests a differential impact of “strenuous” (or intense) exercise on cognition as one study found forced intense/exhaustive running impaired memory processes in young adult male mice (Rosa et al., 2007), while overtraining in another study improved memory in 28 month old rats (Ogonovsky et al., 2005). The running in the current study was voluntary in nature and it is therefore difficult to determine if the distance run per day by 17 β -estradiol treated rats would be considered “intense” in nature. At this time, there is little information on what an “optimum level” of exercise would be to enhance cognitive processes in aging rodents, above which potential harm could occur.

Overall, the results from these initial blocks of DSA testing suggest the potential for an interactive effect of voluntary exercise and 17 β -estradiol exposure in the deficit measured in the acquisition of this task. This hypothesis is supported by the fact that a similar deficit in performance was not measured in the early testing blocks in OVX-untreated wheel rats.

Performance in later blocks of testing

An unexpected result of this study was the significant performance deficit measured in the 17 β -estradiol treated groups in comparison to the untreated OVX groups following the 18-sec intertrial delay in the 6th – 8th blocks of testing. In our previous studies assessing 17 β -estradiol treatment to OVX middle-aged rats on this same DSA task testing occurred for only 25 sessions, resulting in five 5-session testing blocks (Neese et al., 2010a, 2013; Wang et al., 2009). In the present study, we extended the testing for 15 additional sessions, and found that with this extended testing the untreated OVX groups showed a significant improvement in their performance following an 18-sec intertrial delay beginning in these three additional 5-session testing blocks. In contrast, the estradiol treated groups continued to perform near 50% accuracy in these later testing blocks, showing little improvement with extended testing. This unique finding suggests that 17 β -estradiol can have a negative impact on performance following longer intertrial delays that engage the hippocampal memory system in a time dependent manner (Chudasama and Muir, 1997; Dunnett, 1985; Izaki et al., 2008; Kirkby and Higgins, 1998; Maruki et al., 2001). The performance deficit we measured between the estradiol treated and untreated OVX groups at the 18-sec intertrial delay was paralleled by performance deficits at the 3-, 6-, and 9-second intertrial delays in these later blocks of testing, and was due to increases in both win-stay and lose-stay errors. In our previous work, 17 β -estradiol induced deficits following short intertrial delays (3, 6, and 9-sec) were also driven by a combination of both types of errors (Neese et al., 2010a, 2013; Wang et al., 2009). Therefore, the underlying error pattern measured with chronic 17 β -estradiol treatment following the 18-sec intertrial delay is similar to what we have reported previously following shorter intertrial delays. Finally, as the performance deficits measured between the 17 β -estradiol and untreated OVX rats were most evident between those rats not given wheel access (see Fig. 2C and error patterns), wheel running may have a subtle effect on DSA performance in later testing blocks not statistically detected in the current study.

Latencies to lever press

The estradiol-no wheel group took longer to make successive lever presses during NCA and DSA training. Latencies to lever press can be indicative of many things, including locomotor activity level, attentional processes, and even frustration (Carli and Samanin, 1992, 2000; Fujiwara et al., 2009; Kondrad and Burk, 2004). An exercise or 17 β -estradiol induced alteration in locomotor activity could potentially alter latency to lever press, but unrestricted access to a running wheel produces inconsistent effects on locomotor activity assessed in open-field tasks (Burghardt et al., 2004; Dishman et al., 1996; Salam et al., 2009; Zheng et al., 2006). Furthermore, while 17 β -estradiol can increase locomotor activity in the open field, these effects are short-lived following Silastic implant (typically <2 weeks: Gulinello et al., 2006; Luine et al., 1998). As latency to lever press did not interact with testing block, this longer latency to press does not appear to be related to the performance differences measured on the DSA task.

Comparison to previous DSA and 17 β -estradiol research

We have published a series of papers describing a 17 β -estradiol induced deficit in the performance of OVX rats on 25 sessions of testing on this DSA task. In our previous studies deficits were present following 3, 6, and 9-sec intertrial delays that emerged after the 2nd block of testing (Neese et al., 2010a, 2013; Wang et al., 2009). In the current study, the effects of 17 β -estradiol were less consistent following these delays during early testing, but

emerged in later blocks of testing. The sample size in this study ($n=5-8$ per group) was limited in comparison to our previous research ($n=16$), reducing statistical power to detect differences. Visual inspection of the learning curves in Figs. 3B, 3C, and 3D indicates a trend in the same direction as our previously published findings (Neese et al., 2010a, 2013; Wang et al., 2009).

In addition, housing conditions may have influenced our data. We typically pair-house rats, but due to the nature of this study rats were single-housed. Single-housed rats perform differently than do group housed rats on a variety of maze-based tasks (Einon, 1980; Pacteau et al., 1989; Schrijver et al., 2002; Voikar et al., 2005), evidence that the changed housing conditions may have impacted the outcome of this study. Importantly, the large effect we measured following the 18-sec intertrial delay with this limited sample size speaks to the robustness of this deficit.

Summary and Conclusions

In summary, free access to a running wheel impaired the initial acquisition of this variable delay operant alternation task in middle-aged OVX rats treated with 17β -estradiol, an effect not seen in untreated OVX rats with running wheel access. A significant performance deficit was present in the 1st block of testing in 17β -estradiol treated rats given access to a running wheel in comparison to 17β -estradiol treated rats without wheel access. This is contrary to previous research that suggested a positive relationship between fitness levels and cognitive outcome in menopausal women taking longer-term HRT (Erickson et al., 2007). However, it is difficult to draw direct comparisons between the human study and this rodent study. Both studies used tasks that engage the prefrontal cortex, but the DSA task measures working memory, while the Wisconsin Card Sorting Task used in the human study measures set-shifting/cognitive flexibility (Choi and Kurtz, 2009). These distinct aspects of executive function have been shown to engage specific subregions of the prefrontal cortex in both rodents and humans (see Berman et al., 1988; Floresco et al., 2009). In addition, we report here for the first time a significant deficit following the hippocampally-sensitive 18-sec intertrial delay in OVX rats treated with 17β -estradiol on this DSA task.

One limitation of this study is that rats were given unlimited access to a running wheel. As distance run per day was negatively correlated with DSA performance in the 1st block of testing, future research should determine if limited access to a running wheel may have a different effect than the unlimited access in the current study. In addition, the effects of 17β -estradiol and exercise on other aspects of executive function in middle-aged OVX rats, including cognitive flexibility, should be explored.

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Highlights

- Exercise effects on an operant alternation task were measured.
- Middle-aged rats were ovariectomized and treated with an estradiol or blank implant.
- Running wheel access impaired acquisition in ovariectomized estradiol-treated rats.
- Running wheel access did not alter performance in untreated ovariectomized rats.
- Exercise impairs initial acquisition of an alternation task in estradiol-treated rats.

Figure 1.

(A) Latency to lever press following a correct lever press. Estradiol-no wheel rats took longer to respond, $*p < 0.05$. (B) Latency to lever press following an incorrect lever press. Estradiol-no wheel rats took longer to respond, $*p < 0.05$.

Figure 2.

(A) Proportion correct across eight 5-session blocks of testing for the 17β -estradiol treated groups. Estradiol-wheel rats performed worse than did the estradiol-no wheel rats in the 1st block of testing, $*p < 0.05$. (B) Proportion correct across eight 5-session blocks of testing for the OVX-untreated groups. No significant differences measured. (C) Proportion correct across eight 5-session blocks of testing for the no wheel groups. Estradiol-no wheel rats performed worse than did the OVX-no wheel rats in the 6th–8th blocks of testing, $*p < 0.05$. (D) Proportion correct across eight 5-session blocks of testing for the wheel groups. No significant differences measured.

Figure 3.

Proportion correct across eight 5-session blocks of testing, sorted by intertrial delay, for treatment group. (A) 0-second delay. No significant differences measured. (B) 3-second delay. Estradiol-treated rats performed significantly worse than did the OVX-untreated rats in the 3rd–7th blocks of testing, $*p < 0.05$. (C) 6-second delay. Estradiol-treated rats performed significantly worse than did the OVX-untreated rats in the 3rd and 6th–8th blocks of testing, $*p < 0.05$. (D) 9-second delay. Estradiol-treated rats performed significantly worse than did the OVX-untreated rats in the 6th and 8th blocks of testing, $*p < 0.05$. (E) 18-second delay. Estradiol-treated rats performed significantly worse than did the OVX-untreated rats in the 6th–8th blocks of testing, $*p < 0.05$.

Figure 4.

(A) Win-stay errors committed across eight 5-sessions blocks of testing for the no wheel groups. Estradiol-no wheel rats committed more errors than did the OVX-no wheel rats in the 6th–8th blocks of testing, $*p < 0.05$. (B) Lose-stay errors committed across eight 5-session blocks of testing for the no wheel groups. Estradiol-no wheel rats committed more errors than did the OVX-no wheel rats in the 6th–8th blocks of testing, $*p < 0.05$. (C) Lose-stay errors committed across eight 5-session blocks of testing for the 17 β -estradiol treated groups. Estradiol-wheel rats committed more errors than did the estradiol-no wheel rats in the 1st block of testing, $*p < 0.05$.

Table 1

Distance run in km (Mean±SEM) by DSA training and testing phase (* $p<0.05$).

	CA All Sessions	NCA First 5 Sessions	NCA Second 5 Sessions	DSA Block 1	DSA Block 2	DSA Block 3	DSA Block 4	DSA Block 5	DSA Block 6	DSA Block 7	DSA Block 8
Estradiol-Wheel	4.1±0.6*	4.6±1.3*	5.0±1.0*	5.4±1.1*	5.5±1.0*	5.0±0.9*	6.2±0.9*	6.9±0.9*	7.1±0.9*	7.2±1.1*	6.5±0.8*
OVX-Wheel	0.9±0.2	1.3±0.2	1.2±0.2	1.4±0.6	2.3±0.9	1.6±0.6	1.8±0.5	2.2±0.5	2.6±0.9	2.6±1.0	1.6±0.4