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Estradiol impairs response inhibition in young and middle-aged, but not old rats

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ABSTRACT

Estrogens have been shown to have a strong influence on such cognitive domains as spatial memory, response learning, and several tasks of executive function, including both working memory and attention. However, the effects of estrogens on inhibitory control and timing behavior, both important aspects of executive function, have received relatively little attention. We examined the effects of estradiol on inhibitory control and timing behavior using a differential reinforcement of low rates of responding (DRL) task. Ovariectomized young (3 month), middle-aged (12 month), and old (18 month) Long–Evans rats were implanted with Silastic implants containing 0, 5 or 10% 17β-estradiol in cholesterol vehicle and were tested on a DRL task requiring them to wait 15 s between lever presses to receive a food reinforcer. The ratio of reinforced to non-reinforced lever presses did not differ across age in the cholesterol vehicle group. Conversely, 17β-estradiol impaired learning of the DRL task in young and middle-aged rats, but the learning of old rats was not impaired relative to vehicle controls following either 5% or 10% 17β-estradiol treatment. Overall, old rats also made fewer lever presses than both the young and middle-aged rats. These results provide new evidence that estrogens impair inhibitory control, an important aspect of self regulation, and add to existing evidence that estrogens differentially affect cognition at different ages.

1. Introduction

Extensive research in rodents suggests a strong influence of estrogens on such cognitive domains as working memory and place learning (see Dohanich et al., 2009; Frick, 2009; Korol, 2004). In contrast, relatively little is known about the effects of estrogens on behavioral inhibition. Behavioral inhibition consists of several related phenomena including the ability to withhold a response and the ability to estimate time (Evenden, 1999a), and is one of a subset of cognitive processes commonly referred to as executive functions (Miller and Cohen, 2001).

In animal models behavioral inhibition is often assessed using operant tasks in which the animal must "withhold" a behavioral response for a specific period of time to earn a reinforcer (Evenden and Ryan, 1998; Evenden, 1999b; Monterosso and Ainslie, 1999; Sanabria and Killeen, 2008; Wang et al., 2008). Of these, the differential reinforcement of low rates (DRL) of responding operant schedule has proven to be a sensitive task to measure the ability to delay previously reinforced behavior (Arce and Santisteban, 2006; Evenden, 1999b; Monterosso and Ainslie, 1999). In the operant DRL, a response (i.e. a lever press) that results in reinforcement must be withheld for a fixed period of time before that response will again be reinforced (Sable et al., 2009; Wang et al., 2008). Premature responses that occur during this fixed period are not reinforced and result in a "resetting" of the wait period.

Research addressing the effects of estrogens on the ability to learn DRL tasks is sparse and conflicting (Beatty, 1973; Lentz et al., 1978; Wang et al., 2008). In one study (Beatty, 1973), ovariectomy (OVX) was found to impair the ability of young rats (5 months) to learn a DRL task as compared to intact controls. Specifically, OVX rats had a lower efficiency ratio (reinforced presses/total presses), earned fewer reinforcers, and made more lever presses than intact controls (Beatty, 1973). However, Lentz et al. (1978) citing a personal communication from Beatty (1973) described the findings of a follow-up study in which Beatty failed to replicate the deleterious effect of OVX on DRL learning (see discussion in Lentz et al., 1978). Lentz et al. (1978) also failed to uncover an OVX-induced difference between young (6 months) adult OVX rats and intact control rats on a similar DRL task (Lentz et al., 1978). Lentz et al. (1978) further found that chronic treatment of OVX rats with a supraphysiological level of estradiol (10 ng/ml in the serum; typical physiological levels in rodents range

Abbreviations: DRL, Differential reinforcement of low rates of responding; OVX, Ovariectomy.

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from 3 to 90 pg/ml (Overpeck et al., 1978)) failed to produce any effect on DRL efficiency.

We recently completed a study in which OVX young adult rats (6 months) treated chronically with a physiological level of estradiol (86 pg/ml in the serum) performed worse than did cholesterol vehicle controls during acquisition of a DRL task (Wang et al., 2008). Estradiol-treated rats made more lever presses and had a lower efficiency than both OVX cholesterol controls and intact rats. In agreement with Lentz et al. (1978), performance did not differ between OVX cholesterol controls and intact rats. Our results are also consistent with a recent study showing hormone effects on timing estimates in a peak-interval task (Sandstrom, 2007). In that study, OVX adult female rats (3 months) were initially trained on a fixed-interval procedure in which rats were free to lever press at any time, but a lever press was only associated with reinforcement either 7 or 21 s after the illumination of a cue light. Rats were then exposed to the peak-interval procedure, in which they were again free to respond at any time following the onset of the cue light, but only half of the presses occurring after the target interval were associated with reinforcement. On the non-reinforced trials the lever remained extended for a duration of 2.5–3 times the target interval, allowing assessment of the peak interval for responding. Although both DRL and peak-interval tasks assess timing behavior, there is no "resetting" of the time interval associated with peak-interval tasks. The animal is free to respond at any time without negative consequence and peak time of responding is dissociable from overall response rate (Roberts, 1981). As such, peak time tasks allow measurement of both over- or undershooting of the target time interval. Following acquisition of the peak-interval task, rats were exposed to estradiol treatment (proestrus levels: peak serum levels of 88 pg/ml) and again exposed to the peak-interval procedure. Estradiol produced a leftward shift in the peak response time for both 7- and 21-second target intervals, resulting in an undershooting of both target intervals (Sandstrom, 2007), suggestive of an increase in the speed of the internal clock (Meck, 1996; Roberts, 1981; Sandstrom, 2007).

DRL tasks are sensitive not only to hormone status but also to age at testing. However, it is important to point out that the effects of aging have only been assessed in male rats. Aged male rats (24–25 months) showed lower response rates and a higher efficiency ratio than did young adult rats (3- or 7-months old) when trained and tested on DRL tasks with intervals ranging from 5 to 20 s (Lejeune, 1989; Soffie and Lejeune, 1991). These age-related differences in DRL learning were most clearly seen during initial training sessions of both DRL-5 (Soffie and Lejeune, 1991) and DRL-20 (Lejeune, 1989) schedules. With extended training these differences diminished (Lejeune, 1989; Soffie and Lejeune, 1991). Performance of a peak-interval task was also found to be affected by age at testing, with aged male rats (24–26 months) showing a rightward shift in peak response time based upon a 20-second target interval, resulting in aged animals overshooting the target interval (e.g., underestimating the passage of time, (Meck, 1996; Roberts, 1981)) in comparison with younger rats (4–6 months) (Lejeune et al., 1998; Meck, 2006).

Although these studies were conducted with male rats, these findings do suggest that an effect of estradiol on DRL acquisition might be moderated by age at testing in female rats. Therefore, a goal of this study was to expand our previous finding that chronic estradiol treatment impaired learning of a DRL task in young female rats to determine whether different results would be obtained in old rats. In addition, because little is known about the effects of estradiol on DRL acquisition at middle-age, a time during which significant changes in reproductive status, associated hormonal levels, and cognition begin to occur (Markowska and Savonenko, 2002; Wu et al., 2005), we also included middle-aged (approximately 14-months old at time of testing) rats in the study.

2. Methods

2.1. Animals and exposure

A total of 144 female Long–Evans rats were obtained from Harlan (Indianapolis, IN), and were housed in a temperature and humidity controlled room (22 °C, 40–55% humidity) under a 12-hour reverse light–dark cycle (lights off 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 Institute for Laboratory Animals Research, 2003).

The rats were received in two cohorts of 72 animals each, separated by 6 months. Due to the extensive number of rats needed to complete this study and the limits of available operant testing chambers (see below), it was necessary to conduct the study in two cohorts balanced for age and estradiol treatment. Each cohort consisted of 24 young (3 month old) virgin females, 24 middle-aged (12 month old) retired breeders and 24 old (18 month old) retired breeders. Each age group was divided into three estradiol treatment groups (cholesterol vehicle, 5% estradiol, and 10% estradiol) (Luine et al., 1998) with 8 rats per age per dose in each of the two cohorts, or a total of 16 rats in each dose group at each age. Rats from the same treatment groups were pair-housed in standard polycarbonate cages ($45 \times 24 \times 20$ cm) with corncob bedding.

After a one-week period of acclimation to the vivarium, rats were OVX and a Silastic capsule containing 5% or 10% 17β-estradiol in cholesterol (Sigma, St. Louis, MO) or cholesterol vehicle alone (as described in Luine et al., 1998) was implanted subcutaneously at the nape of the neck under isoflurane gas anesthesia (VetEquip, Pleasanton, CA). One end of the Silastic capsule (1.5 mm i.d., 1.96 mm o.d.) was plugged with 0.25 cm silicone and dried overnight before packing with 1 cm of the estradiol/cholesterol mixture or cholesterol vehicle after which the other end was plugged with 0.25 cm silicone. Capsules were soaked in sterile saline at 37 °C overnight before insertion during surgery.

The diet was switched from standard rat chow to AIN-93G on the day of OVX surgery. This was done to ensure that the rats were not exposed to additional estrogens via the diet, which becomes increasingly important for long-term behavioral studies. Research has shown that the amount of soy phytoestrogens in standard lab chow can vary greatly, even across different lot numbers from the same supplier (Brown and Setchell, 2001; Thigpen et al., 2004, 2007). Beginning one week after surgery, rats were food restricted to and maintained at 85% of their free-feeding body weights. The average weight in grams (Mean ± SEM) following restriction to 85% for young rats was 223.42 ± 2.82, for middle-aged rats was 279.06 ± 3.80, and for old rats was 287.22 ± 5.71. After the rats had been food restricted for one week they were tested for a single day on a dual solution T-maze task that tests for biases in learning strategy. Rats were trained to collect a food reinforcer located in one arm of a T-maze (goal arm). The start and goal arms were held constant with respect to extra-maze cues. After training to criterion, the rats were given a single probe trial in which the start arm was rotated 180° from the position used during training. Rats were assumed to be using a "place" strategy if they entered the arm in the spatial location that was correct during training, while a "response" strategy was indicated if the rat made a turn in the same direction that was correct during training. Although previous studies using shorter term higher dose estradiol treatment produced a shift towards a place strategy to solve this task (Korol and Kolo, 2002), we failed to uncover any differences in the response strategies used by 17β-estradiol- and the cholesterol vehicle-treated rats (data not reported). Operant training began the