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Reproductive experience alters anxiety-like behavior in the female rat

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Abstract

Reproductive experience (i.e. pregnancy and lactation) results in significant alterations in subsequent hormone levels in female rats. Several studies have demonstrated that circulating hormones can significantly affect anxiety-like behavior. Thus, the purpose of the present study was to determine whether reproductive experience induces alterations in anxiety-like behaviors in cycling female rats and in older, reproductively senescent rats. In Experiment 1, the elevated plus maze (EPM) was used to test young cycling (6–8 weeks post-weaning) and middle-aged (32–36 weeks post-weaning) primiparous rats and their age-matched nulliparous counterparts for anxiety-like responses. In Experiment 2, activity in the open field was used as an additional measure of anxiety-like behavior in young (proestrus) and middle-aged (constant estrus) primiparous and nulliparous rats. For Experiment 3, EPM testing was conducted in separate groups of young and middle-aged animals tested two weeks after ovariectomy. The results revealed that during proestrus, primiparous animals exhibited fewer anxiety-like behaviors on the EPM compared to nulliparous controls. In middle-aged animals, however, parity was associated with increased anxiety-like behavior. In the open field, young, non-lactating primiparous animals again exhibited fewer anxiety-like behaviors compared to nulliparous controls, an effect that was reversed in middle-aged animals. Effects of reproductive experience on the EPM in both age groups were eliminated by ovariectomy. Overall, the findings indicate that reproductive experience significantly alters anxiety-like behavior, effects that are influenced by the endocrine status and/or age of the female.

Keywords: Elevated plus maze; Open field; Proestrus; Constant estrus; Middle-aged; Parity; Aging

Introduction

Reproductive experience (i.e. pregnancy and lactation) is associated with alterations in hormone secretion during subsequent estrous cycles, including reduced estradiol and prolactin on the afternoon of proestrous (Bridges et al., 2005; Byrnes and Bridges, 2005). A number of studies have observed significant variations in anxiety-like behavior across the estrous cycle (Díaz-Véliz et al., 1997; Frye et al., 2000; Marcondes et al., 2001; Mora et al., 1996) using the elevated plus maze task (EPM), with decreased anxiety often associated with proestrus. Moreover, anxiolytic effects of exogenous estradiol and prolactin in female rats have been reported using the EPM (Frye and Walf, 2004; Lund et al., 2005; Marcondes et al., 2001; Torner et al., 2001, 2002). Based on the decreased hormone levels in reproductively experienced female rats, one might predict that anxiety-like behavior would be increased in these females on the afternoon of proestrus when compared to age-matched nulliparous controls. Wartella et al. (2003), however, observed that in an open field task non-lactating, parous females demonstrate fewer anxiety-like behaviors. Since this latter study did not control for the stage of the estrous cycle, the potential role of changes in circulating hormones in this effect remains unknown.

The purpose of the present study was to determine whether anxiety-like behavior following reproductive experience as measured using the EPM task is influenced by alterations in hormone levels during the estrous cycle. In addition, as significant hormonal shifts occur as the female ages, the effects of reproductive experience on anxiety-like behavior were also measured in middle age, reproductively senescent rats. The results indicate that reproductive experience significantly alters anxiety-like behavior in a manner that is dependent on the endocrine status and possibly the age of the female.

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Material and methods

Subjects and mating

One hundred and fifty adult female Sprague–Dawley rats (Crl:CD[SD] BR; 51–52 days of age) were purchased from Charles River Laboratories (Kingston, MA) and housed in our animal facility. One week after arrival in our colony, half of the females were mated with males from our colony. Two weeks after mating, females were individually housed in polypropylene cages (45 × 25 × 20 cm) and monitored for the onset of parturition. On postpartum day 1 (day after parturition), litters were culled to 10 pups (5 males and 5 females). All litters were weaned on postpartum day 21. All other females were not mated and served as age-matched, nulliparous controls. For all experiments, animals were maintained in temperature- (21–25°C) and light- (14:10 light–dark cycle) controlled rooms and food and water were available ad libitum. Animals were maintained in accordance with the National Research Council (NRC) Guide for the Care and Use of Laboratory Animals (© 1996, National Academy of Science), and methods were approved by the Institutional Animals Care and Use Committee of Tufts University, Cummings School of Veterinary Medicine.

Experimental procedures

Experiment 1. Effects of reproductive experience on EPM behavior in intact females

Beginning 4 weeks after weaning (i.e. 18–20 weeks of age), estrous cycles of young primiparous and age-matched nulliparous subjects were monitored by daily vaginal lavage for at least 2 weeks. For middle-aged subjects, estrous cycles were monitored beginning 32–36 weeks post-weaning. Only females demonstrating a consistent 4-day cycle (young) or 7 days of continuous estrous (middle-aged) were used for testing on the EPM. Young females were tested on the EPM during diestrus (DI), proestrus (PRO), or estrus (EST), with each female only tested once.

Experiment 2. Effects of reproductive experience on open field behavior in intact females

Experiment 2 was conducted to measure the effects of reproductive experience on another measure of anxiety-like behavior, the open field test. The design for this experiment was identical to that of Experiment 1, using groups of young (proestrous) and middle-aged (constant estrous) primiparous and age-matched, nulliparous rats.

Experiment 3. Effects of reproductive experience on EPM behavior in ovariectomized females

The experimental groups in Experiment 3 were similar to those used in Experiments 1 and 2. However, to determine the role of ovarian hormones in the effect of reproductive experience on anxiety-like behavior, all subjects were ovariectomized under isoflorane anesthesia 2 weeks prior to EPM testing.

Elevated plus maze behavior

EPM testing was conducted between 1300 and 1500 h in a quiet behavioral testing room. At the time of testing, animals were placed individually into the center of a fully automated EPM (Hamilton–Kinder; Poway, CA) consisting of two open arms (38 L × 5 W cm) and two closed arms (38 L × 5 W × 15 H cm) with a central intersection (5 cm × 5 cm). The apparatus was elevated 75 cm above the floor. Movement through the maze was detected by 48 equally spaced photocells embedded in each arm of the apparatus. Each test lasted 5 min during which animals were able to explore the maze. Ethanol (70%) was used to clean the apparatus between individual test sessions. All data were automatically collected and quantified using MotorMonitor® software (Hamilton–Kinder). Measures included overall activity (beam breaks) and percent of time spent on the open and closed arms. Increased time in the open arms was interpreted as reflecting reduced anxiety-like behavior (Lister, 1990; Pellow et al., 1985).

Open field activity

Open field activity was monitored with the SmartFrame® Open Field Activity System (Hamilton/Kinder) in a quiet behavioral test room. The apparatus included a Plexiglas cage (40 L × 40 W × 38 H cm) surrounded by a frame consisting of 32 photocells (16 L × 16 W, spaced 2.5 cm apart) which continuously tracks the animal's movement. The peripheral region was defined as 7.5 cm from any wall, while the center was defined as the 25 × 25 cm region in the middle of the field. At the time of testing (1300–1500 h), animals were placed individually into the center of the open field arena and their movement recorded continuously for 5 min. The apparatus was cleaned with ethanol (70%) between tests. Photobeam breaks were recorded as a measure of horizontal activity within the open field using MotorMonitor® software (Hamilton/Kinder). Measures included overall activity (beam breaks), total distance traveled (cm), and percent time spent in the center, with less time and distance in the center indicating anxiety-like responses.

Hormone assays

Approximately 1–2 weeks following behavioral testing, a subset of intact young (proestrous) and middle-aged, primiparous and nulliparous females from each experiment were sacrificed by rapid decapitation (1400–1600 h). Blood collections were not conducted immediately following behavioral testing due to the potential confound of the possible stress of testing on basal PRL secretion. Trunk blood was collected into heparinized tubes, centrifuged, and plasma stored at −20°C until assayed for PRL, estradiol, and progesterone. PRL concentrations were measured in duplicate using the NIDDK rat PRL kit that included reference preparation NIDDK-rPRL-RP-3 and anti-rat prolactin S-9 supplied by Dr. A.F. Parlow through the National Hormone Pituitary Program. Assay sensitivity averaged 0.2 ng/ml. Plasma estradiol and progesterone levels were also measured by radioimmunoassay using commercially available kits (Diagnostics Products Corporation, Los Angeles, CA) according to the respective protocol.

Statistical analyses

EPM data for Experiment 1 were analyzed using a two-way factorial ANOVA with endocrine status (diestrus (DI), proestrus (PRO), estrus (EST), or constant estrus (CE)) and reproductive experience (nulliparous or primiparous) as independent factors. A separate two-way factorial ANOVA was conducted with age (young collapse across the cycle stage and middle-aged) and reproductive experience (nulliparous or primiparous) as independent factors. In Experiment 2, a two-way factorial ANOVA with age (young or middle-aged) and reproductive experience (nulliparous or primiparous) as independent factors was used to analyze open field data. For Experiment 3, EPM data from ovariectomized females were subjected to two-way factorial ANOVA with age (young or middle-aged) and reproductive experience (nulliparous or primiparous) as independent factors. Finally, hormone data FSwere also analyzed using a two-way factorial ANOVA with age (young or middle-aged) and reproductive experience (nulliparous or primiparous) as independent factors. All post hoc tests were performed using the Tukey's test.

Results

Experiment 1. Effects of reproductive experience on EPM behavior in intact females

In young subjects, there were no main effects of endocrine status or reproductive experience on percent time and distance traveled on the open arms (all Fs < 1). However, there was a significant interaction between endocrine status and reproductive experience ($F_{[3,84]} = 3.423$, $P = 0.02$). These data are illustrated in Figs. 1A and B. Post hoc analyses indicated that during proestrus, primiparous females spent significantly more