Behavioural Brain Research 292 (2015) 414–427

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Behavioral and pharmacological investigation of anxiety and maternal responsiveness of postpartum female rats in a pup elevated plus maze

Yu Yang[a,b], Jingxue Qin[a,b], Weihai Chen[a,b], Nan Sui[c], Hong Chen[a,b,**], Ming Li[d,*]

[a] Key Laboratory of Cognition and Personality (Southwest University), Ministry of Education, China
[b] Faculty of Psychology, Southwest University, Chongqing, China
[c] Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, Beijing, China
[d] Department of Psychology, University of Nebraska-Lincoln, Lincoln, NE 68588-0308, USA

HIGHLIGHTS

- Under the no pup condition, dams entered the open arms more than nulliparous rats.
- With pups, dams retrieved pups, entered the open arms more and had a higher speed.
- Haloperidol and fluoxetine decreased the time spent in the open arms and velocity.
- Diazepam did not affect pup retrieval, open arm time or entry in lactating rats.

ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form 28 June 2015
Accepted 2 July 2015
Available online 6 July 2015

Keywords:
Postpartum anxiety
Maternal responsiveness
Elevated plus maze
Haloperidol
Fluoxetine
Diazepam

ABSTRACT

The present study investigated the validity of a novel pup-based repeated elevated plus maze task to detect reduced anxiety and increased maternal responsiveness in postpartum female rats and explored the roles of dopamine D_2, serotonin transporter and GABA/benzodiazepine receptors in the mediation of these processes. Sprague–Dawley postpartum or nulliparous female rats were tested 4 times every other day on postpartum days 4, 6 and 8 in an elevated plus maze with 4 pups or 4 pup-size erasers placed on each end of the two open arms. When tested with erasers, untreated postpartum mother rats entered the open arms proportionally more than nulliparous rats. They also tended to spend more time in the open arms, indicating reduced anxiety. When tested with pups, postpartum rats retrieved pups into the closed arms, entered the open arms and closed arms more and had a higher moving speed than nulliparous rats, indicating increased maternal responsiveness. Both haloperidol (0.1 or 0.2 mg/kg, sc) and fluoxetine (5 or 10 mg/kg, ip) dose- and time-dependently decreased the percentage of time spent in the open arms and speed, but did not affect the percentage of open arm entries. Diazepam (1.0 or 2.0 mg/kg, ip) did not affect pup retrieval, open arm time/entry in lactating rats. Thus, the percentage of open arm entries appears to be the most sensitive measure of anxiety in postpartum female rats, while speed could be used to index maternal responsiveness to pups, which are likely mediated by the dopamine D_2 and serotonin transporter systems.

1. Introduction

Pregnancy, parturition and lactation bring about numerous changes in the female's brain, body and behavior which are essential for the survival and health of the offspring and necessary for the female to successfully respond to the new demands of her changed environment [1]. Animal work has identified several adaptive changes from molecules to behaviors, including decreased responsiveness of the hypothalamic pituitary adrenal (HPA) axis to stressors, decreased corticotropin-releasing hormone (CRH, a stress hormone) mRNA and binding in the hypothalamic paraventricular nucleus (PVN), increased oxytocin and its receptor mRNA expressions in the PVN, reduced sensorimotor gating (an attentional filtering function) as measured in prepulse inhibition (PPI), reduced acoustic startle response, increased pup-directed maternal responses (pup retrieval and nursing), indicative of increased

* Corresponding author at: Associate Professor, Department of Psychology, University of Nebraska-Lincoln, 238 Burnett Hall, Lincoln, NE 68588-0308, USA.
** Corresponding author at: Professor, Faculty of Psychology, Southwest University, Tianshen Road 2, Beibei District, Chongqing 400715, China.
E-mail addresses: chenhg@swu.edu.cn (H. Chen), mli2@unl.edu (M. Li).

http://dx.doi.org/10.1016/j.bbr.2015.07.010
0166-4328/

maternal motivation, and decreased anxiety and enhanced memory functions [1–6]. Research also suggests that disruption of these normal adaptations could lead to postpartum mood, anxiety and memory disorders [7]. It is estimated that approximately 5–12% of mothers worldwide display postpartum anxiety [8], 5–25% postpartum depression [9], and 0.1% postpartum psychosis [10]. Some individuals also show impairments in prospective memory [11]. Therefore, pharmacological interventions are often required to manage these mental disorders.

In laboratory rats, reduced anxiety and increased maternal responsiveness to pup cues in postpartum females are often examined using an elevated plus maze (EPM) [12] and pup retrieval test, respectively [13]. The EPM is a canonical rodent test of anxiety-like behavior [14]. The percentage of testing time subjects spend in the open arms of the maze and number of entries to the open arms are thought to be inversely correlated with level of anxiety. Acute anxiolytic treatments targeting the GABA/benziodiazepine receptor complex are known to increase the number of entries and time spent in the open arms as compared to the closed arms [15]. The pup retrieval test, typically conducted in the home cage, provides two important indices of maternal responsiveness: the number of pup retrieved and pup retrieval latency. Drugs targeting dopamine D_2 and serotonin 5-HT_{2A} and 5-HT_{2C} receptors are shown to decrease the number of pup retrievals and increase the pup retrieval latency, implicating their involvements in maternal motivation [16–21].

Although there are some conflicting findings, the majority of studies in the literature report that postpartum rats are less anxious than virgins in behavioral tests of anxiety [7,22]. However, some important issues have not been completely resolved. How pup presence affects anxiety-like behavior [22,23], and whether physical contact with pups is essential [24] need to be further examined. Also, which measure of open-arm behaviors is more sensitive to reveal the reduced anxiety in postpartum rats has not been settled [25]. Because the anxiety-like behavior of lactating rats is modulated by the presence of pups, which inevitably activates the motivational system, it seems reasonable to assume that anxiety-like behavior, as tested in a variety of behavioral paradigms, is often influenced by the motivational level of mother rats [22]. A reciprocal interaction between emotion regulation and maternal responsiveness likely exists, that is, reduced anxiety in mother rats could facilitate their motivation to care for their offspring, and increased maternal responsiveness could also mitigate mother rats' natural fearfulness. Unfortunately, studies designed to study emotion regulation and maternal responsiveness in a single paradigm and examine their interaction are rare. The underlying neurochemical bases of their interaction are also not well understood.

The present study introduced a novel pup-based elevated plus maze (EPM) paradigm. In this task, 4 pups or pup-size erasers were placed at each end of two open arms. Each rat (postpartum or nulliparous female) was repeatedly tested 4 times every other day for 3 days in order to track changes in anxiety and motivation over time. We first identified two distinct sets of behavioral indices for maternal anxiety (Experiment 1) and maternal responsiveness (Experiment 2) by comparing primiparous females with nulliparous ones on the basis of their performances on the EPM and their responses to pups. We then explored the roles of dopamine D_2, serotonin transporter and GABA/benzodiazepine receptors in the regulation of maternal anxiety and responsiveness by examining the effects of haloperidol (a dopamine D_2 receptor antagonist), fluoxetine (a selective serotonin reuptake inhibitor), and diazepam (a GABA/benziodiazepine agonist) treatment on various measures of maternal anxiety and responsiveness (Experiment 3). Because these drugs are also psychotherapeutic drugs used to treatment psychosis, depression and anxiety, respectively, this study also possesses clinical implications for the understanding of behavioral mechanisms of action of these drugs.

2. Materials and methods

2.1. Animals

Female Sprague–Dawley rats, approximately 200–250 g in weight (8–10 weeks old), purchased as virgins from the Experiment Animal Center (Chongqing Medical University, China) were used in this study. After arrival, they were housed in transparent polycarbonate cages (two per cage) under 12-h light/dark conditions (light on between 0800 am and 2000 pm). Room temperature was maintained at $22 \pm 2\,^{\circ}C$ with a relative humidity of 45–75%. Standard laboratory rat chow and water were available ad libitum. After a 7-day acclimation period, some virgin female rats were placed into the cage of a proven stud male for ten days to ensure pregnancy. Following the mating procedure, pregnant females were singly housed until parturition after which they were housed together with their litters for the remainder of the experiment. Non-mated virgin females ($n = 6$, 4 used in Experiment 1 and 2 in Experiment 2) and non-pregnant females (mated but not pregnant, $n = 10$, 4 used in Experiment 1 and 6 in Experiment 2), combined as the control nulliparous group, were also singly housed during the same period. We combined them as a single control group because they both did not go through the gestation, parturition and lactating processes, and did not have any maternal experience with pups. In addition, they did not differ significantly in any of the EPM measures. Their estrous cycles were not monitored as they were tested on 3 days over a 5-day period, presumably covering all the phases of the cycle. Experiments were conducted during the light cycle (between 0830 am and 1800 pm). A total of 94 postpartum female rats were tested. Data from 14 dams were not included in the final analysis because they failed to retrieve 8 pups at the baseline test on postpartum day (PP) 4 or their tests were not recorded due to malfunction of the camcorder. All animal procedures were approved by the animal care and use committee at Southwest University, China. Every effort was made to minimize the number of animals used and the suffering of the animals.

2.2. Drugs

Haloperidol (HAL, 5.0 mg/ml Ampoules, Hunan Dongting Pharmaceutical Co., Ltd., Hunan, China), fluoxetine (FLU, Sigma–Aldrich, St. Louis, MO, USA) and diazepam (DZ, 5.0 mg/ml Ampoules, Tianjin Jinyao Anjisuan Pharmaceutical Co., Ltd., Tianjin, China) were obtained by mixing the drugs with sterile water. HAL was administered subcutaneously (sc), whereas FLU and DZ were administered intraperitoneally (ip). Based on our previous work [18–20] and published work on FLU and DZ [26–28], we chose to test HAL at doses of 0.1 and 0.2 mg/kg, FLU at doses of 5 and 10 mg/kg, and DZ at doses of 1 and 2 mg/kg.

2.3. Elevated plus-maze apparatus

The EPM consisted of two open arms (50 cm × 10 cm), two enclosed arms (50 cm × 10 cm) and a central platform (10 cm × 10 cm) made of black Plexiglas. Each arm was supported by a sturdy plastic leg and was elevated 70 cm above the floor. The two enclosed arms had high walls (40 cm in height), while the two open arms had raised edges (1.0 cm in height) along each side and end to decrease the possibility of falling during drug testing.