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Open field behavior and intra-nucleus accumbens dopamine release in vivo in virgin and lactating rats

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Abstract

In adult female mammals, reproductive experience (e.g., mating, pregnancy, parturition, and lactation) has long-term behavioral, endocrine, and neurochemical implications. This experience causes behavioral and neurochemical changes that involve several brain areas important for the expression of maternal behavior. The present study showed that lactating rats exhibited reduced general locomotor activity in the open field test compared with virgin animals. Our hypothesis was that nucleus accumbens dopamine, which regulates maternal behavior in lactating rats, is also involved in the low expression of maternal locomotion in the open field test observed during the early stages of lactation and reflects decreased motivation. Initially we compared open field behavior in virgin and lactating rats to confirm our previous data. Thus, the *in vivo* release of dopamine in the nucleus accumbens in virgin and lactating female rats was measured. Perfusate concentrations of extracellular dopamine and its metabolites showed no differences between virgin and lactating rats. Thus, the reduced general activity observed in lactating rats might not be related to intra-nucleus accumbens dopamine control. **Keywords:** maternal behavior, virgin rats, lactating rats, nucleus accumbens, dopamine.

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Introduction

The maternal brain undergoes remarkable physiological and behavioral changes in the peripartum period to meet the demands of the offspring (Numan & Woodside, 2010; Bosch, 2011). Changes in the physiological state of mammals are usually related to endocrine and neural changes (Numan & Stolzenberg, 2008; Stolzenberg et al., 2007; Nasello, Tieppo, & Felicio, 1995; Felicio, Florio, Sider, Cruz-Casallas, & Bridges, 1996; Bridges, Felicio, Pellerin, Stuer, & Mann, 1993) and reflect behavioral adjustments or alterations (Numan, 1994; Numan & Woodside, 2010; Teodorov, Bernardi, Ferrari, Fior-Chadi, & Felicio, 2010). These changes ultimately reflect the biologically useful appearance of some behaviors and modulation of other behaviors, such as caring for pups and aggression (Maestripieri & D'Amato, 1991; Numan, 1994; Numan & Woodside, 2010).

The open field test was initially proposed to measure emotionality (Hall, 1934), but it is also useful for measuring behavioral responses, such as locomotor activity (Patti et al., 2005), hyperactivity (Fukushiro et al., 2008), and exploratory behavior (Crawley, 1985; Alvarez et al., 2006). The open field is also used to measure anxiety (Prut & Belzung, 2003). Rats and mice tend to avoid brightly illuminated, novel, and open spaces, so the open field environment is an anxiogenic stimulus that allows for the measurement of anxiety-induced locomotor activity and exploratory behavior. The anxiogenic response occurs in the first trial, with little or no impact on the animal's subsequent behavior. When the laboratory conditions are maintained with low illumination and without environmental stimuli, rats or mice express mainly exploratory and motor activity rather than anxiety-like responses.

Much data from the literature suggest that lactating rats exhibit less fear in response to several stimuli (Hard & Hansen, 1985; Pereira, Uriarte, Agrati, Zuluaga, &

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Ferreira, 2005), including in the open field task (Ferreira, Hansen, Nielsen, Archer & Minor, 1989), compared with other stages of the female reproductive cycle. This reduction in general fearfulness observed postpartum appears to be related to the caring of offspring. However, environmental factors may modify open field behavior in lactating dams. Lactating dams of the WAG/Rij (i.e., a genetic model of absence epilepsy) and Wistar rat strains repeatedly placed in the open field arena with their pups showed significantly poorer maternal behavior and were slower in forming the pup location response. Administration of low-dose haloperidol (0.1 mg/kg, i.p.) reduced locomotor activity in dams of both strains and maternal behavior under conditions of bright illumination. Under conditions of red-light illumination, haloperidol increased the number of approaches to the pups and the number of pup transportations in WAG/Rij, but not Wistar, rats and reduced the latencies of these behaviors. Dopaminergic regulation of maternal behavior has been suggested to depend on both genetic and environmental factors (Dobriakova, Dubynin, & van Luijtelaar, 2010). Additionally, we previously found that the physiological state (e.g., lactation) influenced behavior in the open field and elevated plus maze in adult female rats (Silva, Bernardi, Nasello, & Felicio, 1997). Lactating rats exhibited decreased total locomotion in the open field and a decreased percentage of time spent in the open arms of the elevated plus maze, suggesting increased anxiety/emotionality in these behavioral tests.

Dopamine affects motivation via the mesolimbic system, acting mainly in the nucleus accumbens (NAc; Carlezon & Thomas, 2009; Anstrom, Miczek, & Budygin, 2009). Maternal motivation is also regulated by the NAc (Champagne et al., 2004; Afonso, Grella, Chatterjee, & Fleming, 2008; Numan & Wodside, 2010). In rodents, ongoing maternal behavior requires dopamine receptor activation. We previously showed that dopamine receptors in the NAc play a role in ongoing maternal behavior (Silva, Bernardi, Cruz-Casallas, & Felicio, 2003).

We hypothesized that NAc dopamine, which regulates maternal behavior in lactating rats, is also involved in the decreased expression of maternal locomotion in the open field observed in the early stages of lactation, reflected by decreased motivation. We initially compared open field behavior in virgin and lactating rats to confirm our previous data. Thus, *in vivo* dopamine release in the NAc in virgin and lactating female rats was measured. A microdialysis experiment was performed in another group of rats because this procedure requires a specialized system to collect the dialysate.

Methods

Subjects

Fifteen virgin rats and 14 lactating rats (Department of Pathology, School of Veterinary Medicine, University of São Paulo, Brazil) were divided into four groups: eight virgin and seven lactating rats were used in the behavioral studies, and seven virgin and five lactating rats were used for the microdialysis studies. For lactating rats, 13 pregnant Wistar rats from our own colony, weighing 200-250g each, were used (gestational day [GD] 0 = spermatozoa in the vaginal smear). The dams were individually housed in polypropylene cages (38 x 32 x 16 cm) with controlled room temperature (22°C), humidity (65-70%), and artificial lighting (12 h/12 h light/dark cycle; lights on at 6:00 AM) with free access to Nuvilab rodent chow (Nuvital, São Paulo, Brazil) and filtered water. Sterile and residue-free wood shavings were used as the bedding in the cages. After birth (day 2), the pups were culled to eight pups per litter, and no cross-fostering procedure was used. The animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo, Brazil (protocol 109, sheet 6, Book 2). Virgin rats (*n* = 15, two females per cage) were maintained in the same laboratory conditions as lactating rats until the beginning of the experiments.

Drugs

Anesthetics (2% xylazine hydrochloride and 5% ketamine hydrochloride; Konig) were administered intraperitoneally according to the body weights of the rats (1:1). Benzathine benzylpenicillin (Bayer) was administered subcutaneously at a dose of 75 U per animal in a single dose in the dorsal region immediately after stereotaxic surgery.

Stereotaxic surgery

Stereotaxic surgery was performed on GD14 to implant guide cannulae aimed directly at the left NAc (Figure 1). We used the following coordinates relative to bregma (Paxinos & Watson, 1986): dorsal/ventral, 6.4 mm; rostral/caudal, + 1.2 mm; lateral, 1.4 mm.

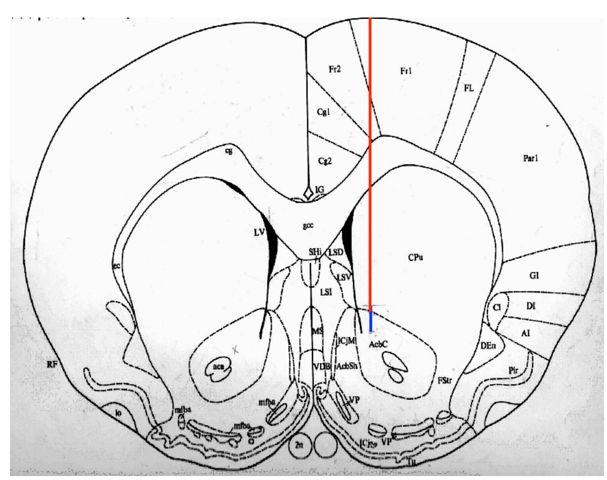

Figure 1. Location of the NAc in the cutting of brain, showing the location of the cannula implanted in NAc, according to the coordinates above.

Open field test

The apparatus was similar to Broadhurst (1960) and consisted of a round 96 cm diameter arena surrounded by a 25 cm high wall painted white, with the floor divided into 19 painted black parts. Hand-operated counters were used to score locomotor frequency (i.e., number of squares crossed with all four paws) and rearing frequency (i.e., number of times a rat stood erect on its hindlegs with its forelegs in the air). A chronometer was used to measure the duration of immobility (i.e., total time in seconds without spontaneous movements). Two groups of female rats (i.e., virgin group and lactating group) were individually placed in the center of the arena, and the behavioral parameters were observed for 5 min on day 5 of lactation. One hour before the experiment, the pups of the lactating rats were maintained separately in another room. The virgin and lactating rats were intermixed and observed. These observations were made between 2:00 PM and 5:00 PM.

Microdialysis

A continuous infusion pump (Harvard Apparatus, model 2274) was equipped with a syringe (Hamilton Model 1002 LT, 2.5 ml) connected to the microdialysis membrane by polyethylene tubes. The female rats were previously habituated to the microdialysis system manipulation. On the day of the experiment, 1 h before the experiments, the dams were separated from their pups and placed in the microdialysis system in another room. After 1 h of acclimation to the system, dialysate was collected at 0, 20, and 40 min. The perfusion fluid entered an inlet tube, exited an outlet tube, and then was collected in Eppendorf tubes that contained 5 µl of 1 M perchloric acid ($ClHO_4$) placed at the end of the outlet tube. The samples were frozen at -80ºC for at most 1 month until they were injected into a high-performance liquid chromatography (HPLC) system with electrochemical detection to quantify monoamine levels.

Confirmation of cannula placements

The rats were deeply anesthetized. Using a peristaltic pump (Cole Parme), the rats were perfused intracardially through the left ventricle with 150 ml of 0.9% saline, followed by 500 ml of 10% paraformaldehyde fixative solution diluted in milli-Q water. The brains were then removed and transferred to a saturated solution of sucrose in 10% paraformaldehyde, where they remained for approximately 24 h. Serial frontal sections, 30 µm thick, were then obtained using a freezing microtome (Leica). The sections were collected sequentially in six compartments so that the distance between the cuts in the same compartment was 180 mm. The sections were stained using the Nissl method and viewed under an optical microscope to determine the placement of the guide cannula in the region of interest. Only data from animals with correct cannula placements were considered for the statistical analysis.

Determination of neurotransmitter and metabolite levels

The samples were analyzed by HPLC (Shimadsu) with an injector, column (Chromolith RP-18e, Merck), and electrochemical detector (1049 A, Hewlett Packard). The levels of dopamine, 3,4-dihidroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were measured to calculate absolute values (pmol) and DOPAC/dopamine, HVA/dopamine and (DOPAC + HVA)/dopamine ratios.

Statistical analysis

The general locomotor activity results were analyzed using an unpaired *t*-test, and the neurochemical data were analyzed using two-way analysis of variance (ANOVA) followed by the Bonferroni *post hoc* test. In all cases, the results were considered significant at $p < .05$.

Results

Experiment 1: general activity in virgin and lactating rats in the open field

Table 1 shows the general activity of virgin and lactating rats in the open field. Compared with virgin rats, the unpaired *t*-test revealed decreased locomotor frequency ($F = 5.495$; $p = .006$) and rearing frequency ($F = 1.129$; $p = .0002$). No significant differences were detected between groups in the duration of immobility ($F = 33.648$; $p = .388$).

Table 1. General activity of virgin rats and in lactating rats observed in the open field. Data are presented as means ± *SEM*.

General activity parameter	**Virgin (8)**	**Lactating(7)**
Locomotion frequency	135 ± 6.66	79 ± 16.69*
Rearing frequency	22 ± 1.64	10 ± 1.65**
Immobility	25 ± 3.99	46 ± 21.65

Unpaired *t* test. * p< 0.01; ** p< 0.001 in relation to the virgin group; ( ) = number of rats.

Experiment 2: in vivo dopamine release in the NAc in virgin and lactating rats

Figure 2 shows in vivo dopamine and dopamine metabolite concentrations obtained by microdialysis in virgin and lactating rats. The two-way ANOVA of the concentrations of dopamine (lactating vs. virgin: $F_{1,30}$ = 0.67; p = .41; sessions: $F_{2,30}$ = 1.18; p = .32; interaction: $F_{2,30}$ = 1.17; p = .32), HVA (lactating vs. virgin: $F_{1,30}$ = .96; p = 0.33; sessions: $F_{2,30}$ = 0; p = 1.0; interaction: $F_{2,30}$ = .49, p = .62), and DOPAC (lactating vs. virgin: $F_{1,30}$ = .92, p = .34; sessions: $F_{2,30}$ = .65; p = 0.95; interaction: $F_{2,30}$ = 1.24; p = .30) did not reveal differences between virgin and lactating rats. The DOPAC/dopamine (lactating vs. virgin: $F_{1,30}$ = 0.41; p = .52; sessions: $F_{2,30}$ = 1.80; p = .18; interaction: $F_{2,30}$ = 1.26; p = .77), HVA/dopamine (lactating vs. virgin: $F_{1,30}$ = 0.25; p = .52; sessions: $F_{2,30}$ = 0.94; p = .40; interaction: $F_{2,30}$ = 0.66; p = .52), and HVA+DOPAC/dopamine (lactating vs. virgin: $F_{1,30}$ = 0.10, p = .75; sessions: $F_{2,30}$ = 1.14; p = .75; interaction: $F_{2,30}$ = 0.50; p = .61) ratios were also not significantly different between groups.

Discussion

The present study confirmed previous results by showing that lactating rats exhibit decreased locomotor frequency in the open field compared with virgin animals (Silva et al., 1997). Rearing frequency in lactating rats also decreased compared with virgin females. No significant difference was detected in the duration of immobility, suggesting a lack of effect on motor function.

When confronted with a novel environment, the behavior of rats in the open field is determined by two conflicting behaviors: the drive to explore and motivation to avoid potential danger (Bertoglio & Carobrez, 2000). Rats with high levels of emotionality exhibit decreased locomotion and rearing. Thus, the decreased locomotor and rearing behavior observed in the present study may be attributable to increased emotionality in lactating rats compared with virgin females. However, Silva et al. (1997) studied the influence of this physiological state (i.e. lactation) in the elevated plus maze and open field in adult female rats and showed conflicting data. In the open

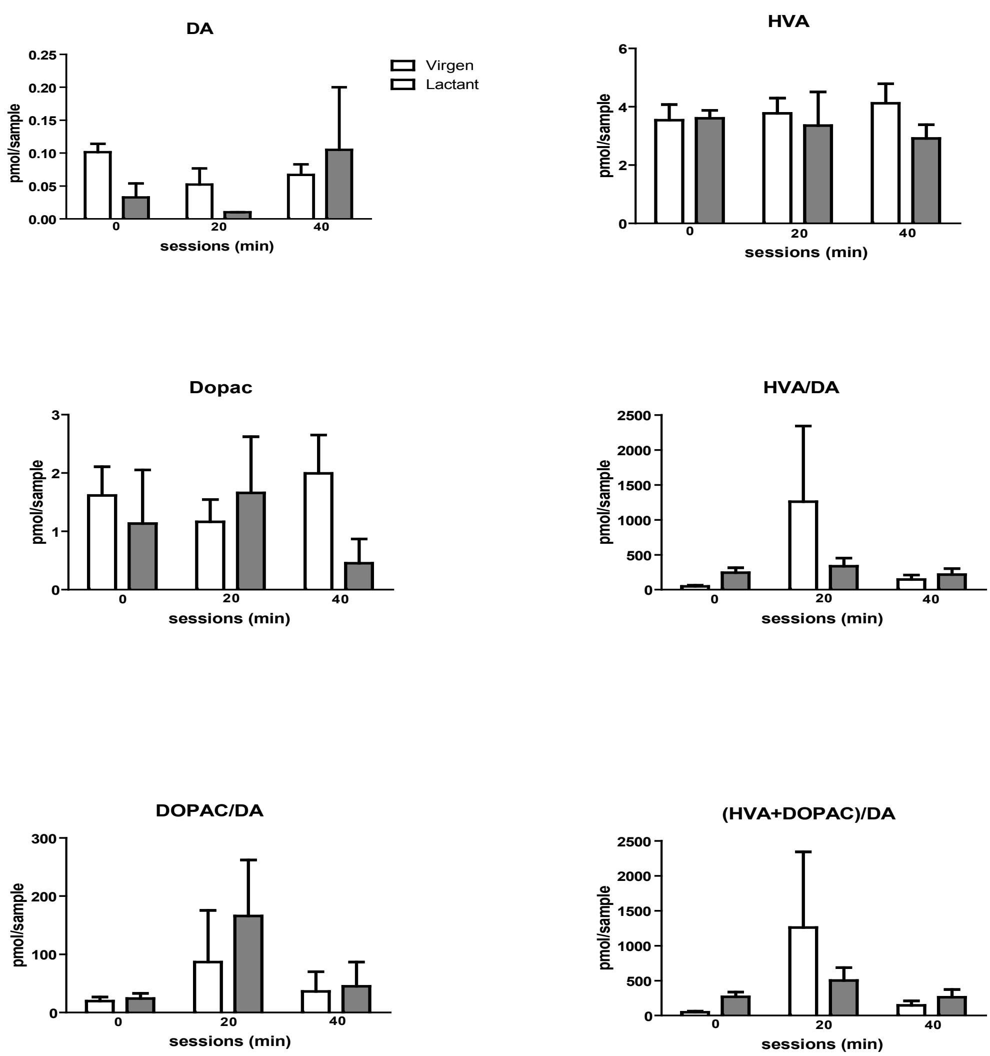

Figure 2. *In vivo* release of DA in NAc of virgin rats and in lactating rats. Data are presented as means ± SEM of absolute values of DA, DOPAC, HVA, DOPAC/DA, HVA/DA e (DOPAC+HVA)/DA in virgins rats (7 rats) and lactating rats (5 rats).Two way ANOVA.

field, total locomotion significantly decreased, and central locomotion (i.e., a parameter related to anxiety) did not differ from virgin rats. In the elevated plus maze, lactating rats displayed a significant reduction in the percentage of time spent on the open arms and a trend toward a reduction in the percentage of entries into the open arms, suggesting an increased anxiogenic-like state. The discrepancies between the two studies may be attributable to the different methods used to assess anxiety levels.

In the present study, both locomotor frequency and rearing frequency decreased in lactating rats compared with virgin rats. Rearing is one of several components of a rat's behavior that, together with other components, comprise what is commonly referred to as general activity (Walsh & Cummins, 1976). Rearing is also a part of stereotyped behavior (Setler, Sarau, Zirkle, & Saunders, 1978) induced in rats by activation of postsynaptic dopaminergic receptors (Randrup & Munkvad, 1974). Decreases in open field locomotion and rearing frequency without changes in the duration of immobility could be interpreted as reduced exploratory behavior or increased anxiety. Exposure to the open field in conditions of intensive light and a noisy environment can have aversive connotations. In contrast, when an animal is exposed to this apparatus under less extreme conditions, such as low illumination and an absence of noise (i.e., conditions used in the present study), the duration of locomotion may reflect and be used to quantify other types of behavior, such as exploratory and motor behavior. Clearly, the emotional component does not completely disappear in such conditions but becomes less relevant. In the present study, general activity in lactating and virgin rats was observed in conditions of low illumination and the absence of noise. Thus, the lactating female rats may have had a decreased motivational response to novelty compared with virgin rats.

During the lactation period, the typical behaviors directed toward pups increase, whereas less relevant behaviors, such as environmental exploration, decrease. Such adaptations in the maternal brain are necessary to optimize the care toward the offspring. Motherhood in rodents induces reduced reactivity to normally aversive stimuli (Fleming & Luebke, 1981; Ferreira, Hansens, Nielsen, Archer, & Minor, 1989). Lactating rats are less inclined to run away from an intruder (Fleming & Luebke, 1981) and exhibit lower reactivity in classic conflict tests (Ferreira et al., 1989). In a light/dark choice situation, lactating mice displayed less avoidance of the aversive illuminated environment than virgin animals (Maestripieri & D'Amato, 1991). Changes in anxiety-like behavior during lactation might reflect other adaptations of the maternal brain. Therefore, a link between maternal behavior and reduced maternal anxiety has been repeatedly postulated, suggesting the existence of changes in the motivational state for some behaviors (Silva et al., 1997; Lonstein, 2005, 2007; Numann & Wodside, 2010).

Neumann (2003) reported that during pregnancy and lactation several neuroendocrine alterations occur that are accompanied by marked behavioral changes, including emotional responsiveness to external challenging situations. A downregulation of hypothalamic-pituitary-adrenal axis responses to physical or emotional stimuli, particularly anxiety-related behavior, was reported in pregnant and lactating rats (Toufexis, Tesolin, Huang, & Walker, 1999; Johnstone et al., 2000). Reduced anxiety levels could be observed only after separating the lactating rats from their pups immediately before testing in the elevated plus maze after transportation to a neighboring test room, but this was not observed when the rats were kept in the testing room for at least 24 h and separated from their pups 2 h before testing (Picazo & Fernandez-Guasti, 1993; Neumann, 2003).

The NAc is a region rich in dopaminergic inputs. Several authors stated that this limbic site modulates emotional behaviors, including aggression and defensive tasks (Garattini, Giacalone, & Valzelli, 1967; Louilot, Le Moal, & Simon, 1986; Oliver et al., 1987; Almeida & Lucion, 1997; Boer, Lesourd, Mocaer, & Koolhaas, 1999; Volavka, 1999). Dopamine affects motivation via the mesolimbic system, mainly in the NAc (Willner & Scheel-Kruger, 1991; Robbins & Everitt, 1996), and reproductive experience decreases prolactin levels in lactating rats. We investigated the possible relationship between the reduction in open field behaviors and NAc dopamine concentration using *in vivo* methods. Earlier reports showed that PRL stimulates the *in vitro* release of dopamine from the rat superfused corpus striatum (Chen & Ramirez, 1982) and increases the *in vivo* output of DOPAC from the rat caudate nucleus when directly infused into the brain (Ramirez, 1985). However, in the present study, the release of dopamine and its metabolites in the NAc was not different between virgin and lactating female rats.

Previous work showed that lesions of the NAc affected the behavior of retrieving pups to the nest (Hansen, Harthon, Wallin, Lofberg, & Swensson, 1991). Administration of the dopamine receptor antagonist haloperidol and dopamine D_2 receptor antagonist pimozide affected search behavior, including grouping offspring and rebuilding the nest. The effects of pimozide on maternal behavior were less pronounced compared with the effects of haloperidol (Silva, Bernardi, & Felicio, 2001; Silva et al., 2003). Our hypothesis is that NAc dopamine, which regulates maternal behavior in lactating rats, is involved in the low maternal locomotion observed in the open field during the early stages of lactation, possibly reflecting decreased motivation. The present study found reduced locomotor and rearing frequency in the open field and no changes in NAc dopamine levels in lactating rats compared with virgin rats, suggesting that the motivational changes observed in lactating rats might occur independently of dopamine release in NAc.

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