



Gene expression in the CNS of lactating rats with different patterns of maternal behavior



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ABSTRACT

For most mammalian species, maternal behavior has an essential role in the development of the offspring. The frequency of licking/grooming (LG) the pups has been used as a parameter to evaluate maternal care, having mothers with high (HL) or low (LL) frequencies of LG. This study aimed to analyze the gene expression of the receptors for dopamine (Drd1a), prolactin (Prlr), serotonin (Htr1a, Htr1b), estrogen (Esr1, Esr2), and of Bdnf in the olfactory bulb (OB), hippocampus (HP), prefrontal cortex (PFC), and striatum (ST) of Wistar rats from three groups: LL ($n=8$); HL ($n=8$); virgin females in diestrus (D; $n=6$). Maternal behavior was studied between the 1st and 7th postpartum days. Brain parts were analyzed by qRT-PCR. LL showed a decrease in the frequency of nursing, and an increase of remaining off the pups. There was an increase in gene expression of Drd1a, Prlr, Htr1a, Htr1b and Esr1 in the OB of HL, compared to LL. In the HP, Drd1a, Prlr and Htr1a were differently expressed when comparing HL, or LL, with D. The main finding is that HL had higher gene expression levels in the OB, which is a crucial structure to promote behavioral differences.

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1. Introduction

For mammals, maternal care has an essential role in the development of the offspring. Rodents, such as rats, constitute ideal model systems to manipulate and investigate the maternal behavior, when mothers practice actions such as: crouching over the young for nursing and to keep them warm, nest building and retrieving the pups to the nest, body and genital licking and grooming the pups (Rosenblatt, 1975; Stern and Johnson, 1990; Numan and Insel, 2003). As well, lactating females, during this period, show an enhanced aggressive behavior toward intruders that approach the nest site, aiming to protect the offspring (Numan and Numan, 1994; Giovenardi et al., 1998; Souza et al., 2010).

Maternal behavior is expressed in different modalities, physiological conditions, and contexts. It is the result of a highly motivated

brain that allows the female to flexibly adapt her caring activities to different situations and social demands (Olazábal et al., 2013). In rats, sensory stimuli from pups, such as sight, sound and odor contribute to contact-seeking, whereas physical contact from pups to dam elicit essential maternal behavioral reflexes involved in retrieval, licking, and the quiescent, upright nursing posture (Stern, 1997).

Lévy et al. (2004) suggest that olfaction has an essential role in the modulation of mother/pups relationship, referring to the influence of the neuroendocrine mechanism in the accessory and main olfactory system as a mediator of this behavior. A profound change in the regulation of olfaction, modulated by physiological changes that occur at the end of pregnancy and in the parturition, allows the female to respond positively to the stimulus coming from the newborn (Lévy et al., 2004).

Although olfaction is not necessary for the initiation of maternal behavior in parturient rats, once maternal responsiveness is established, olfaction is critical for the organization of some dam's specific behaviors. In fact, it was demonstrated that a specific olfactory cue regulates licking, a behavioral pattern crucial for pup survival (Lévy et al., 2004). Studies of bilateral bulbectomy

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performed before parturition interferes with the normal onset of maternal behavior, with profound disturbances of licking behavior and total time spent with pups (Lévy and Keller, 2009).

During the first postpartum week, lactating female rats engage in a high frequency of pup licking/grooming (LG). This behavior serves to stimulate pups, modify body and brain temperature, and allows the dam to reclaim salt and water to meet the physiological demands of lactation (Gubernick and Alberts, 1983). The LG frequency has been used as a parameter to evaluate maternal care (Liu et al., 1997; Caldji et al., 1998; Champagne et al., 2003; Parent and Meaney, 2008; Veenema and Neumann, 2009; Lenz and Sengelaub, 2009), and it has a normal distribution when assessed in a large sample (Champagne et al., 2003), where mothers with high frequencies of LG (HL) and low frequencies of LG (LL) represent the two extremes of the same population (Champagne, 2008).

HL and LL dams differ in mesolimbic dopaminergic activity associated with mother–pup interactions. Such differences may serve as neural substrates for individual differences in the motivational component of maternal behavior (Champagne et al., 2004). HL mothers spend more time taking care of the offspring, display a high frequency of biting intruders of the nest area (Ruthschilling et al., 2012), and their pups are less fearful; while LL have more sexually receptiveness, and pups are more able to react to stressful situations. Thus, HL uses strategies to obtain an offspring with more quality, whereas LL uses strategies to maximize the quantity of the offspring. Both strategies are important, depending on the environmental needs and conditions, and represent different forms of maintaining their own species (Cameron et al., 2008). These variations in maternal care are transmitted across generations, such that the lactating adult offspring of HL mothers are themselves high in pup LG, whereas the offspring of LL mothers exhibit decreased levels of pup LG (Francis et al., 1999; Champagne et al., 2001).

The different patterns of LG come from variations in the maternal care that involves sensorial, neuroendocrine and neural systems, in addition to genetic mechanisms. Several structures in the central nervous system (CNS), such as medial preoptic area (MPOA), medial (MA) and cortical nucleus of amygdala (CA), nucleus accumbens (NAC), paraventricular nucleus of hypothalamus (PVN), olfactory bulb (OB), hippocampus (HP), among others, are responsible for the development and maintenance of the maternal behavior (Consiglio and Lucion, 1996; Champagne et al., 2003; Teodorov et al., 2010; Ruthschilling et al., 2012). In addition to the neuroendocrine changes observed in lactating rats, important interactions among neurotransmitter systems occurs in the CNS, such as dopamine (DA), prolactin (PRL), serotonin (5-HT), estrogen, oxytocin (OT), norepinephrine, and gamma-aminobutyric-acid (GABA) (Spielewoy et al., 2000; Numan and Insel, 2003; Teodorov et al., 2010; Ruthschilling et al., 2012). Certain deficiencies in these substances or in their receptors show a modification in the maternal behavior, such as deficits in retrieval and decreased time in nest (Numan and Insel, 2003).

Molecular basis involved in postpartum behavioral changes and in the regulation of signaling pathways in the CNS are not fully elucidated. Understanding these mechanisms has been the basis of many behavioral studies in neuroscience. Such studies aim to address how a pattern of behavior is controlled by the expression of a candidate gene or group of genes. The present study aimed to analyze the gene expression, at transcriptional levels, of the receptors for dopamine (Drd1a), prolactin (Prlr), serotonin (Htr1a, Htr1b), estrogen (Esr1, Esr2), and of brain-derived neurotrophic factor (Bdnf) in different structures of the CNS, such as olfactory bulb (OB), hippocampus (HP), prefrontal cortex (PFC), and striatum (ST) of lactating females with different patterns of maternal behavior.

2. Materials and methods

2.1. Animals

Primiparous lactating Wistar rats ($n=92$), approximately 90 days old, from the animal house of the Universidade Federal de Ciências da Saúde de Porto Alegre were used. During the last week of gestation, female rats were single-housed in 46 cm × 17 cm × 31 cm Plexiglas cages that allowed a clear view of all activities within the cage. Food and water were provided *ad libitum*. Animals were housed under controlled temperature ($21 \pm 1^\circ\text{C}$) and light (12:12 light-dark cycle with lights off at 5 pm) conditions. Litters were standardized with eight pups one day after the parturition. Virgin females ($n=6$) showing three regular estrous cycles, in the diestrus phase, were used in the experiment (group D).

All procedures were performed in conformity with Conselho Nacional de Controle de Experimentação Animal (CONCEA) for the care and use of laboratory animals, and this study was approved by the Ethics Committee of the UFCSPA (protocol number 788/09).

2.2. Maternal behavior

The behavior of each mother was daily observed during observation periods of 75 min for the first 7 postpartum days (PPD). Observers were trained to ensure a high level of inter-rater reliability. Observations occurred at regular hours each day, with three periods during the light (10:00 am, 1:30 pm and 3:00 pm) and one period during the dark (5:30 pm) phases of the light/dark cycle. The distribution of these observation periods was based on findings that nursing in rats occurs more frequently during the light phase of the cycle. Within each 75 min observation period, the behavior of each mother was scored every 3 min (25 observations/period × 5 periods/day = 100 observations/mother per day) as previously described (Myers et al., 1989; Ruthschilling et al., 2012). The following behaviors were recorded: mother licking/grooming the pup; mother nursing the pups in an arched-back posture; a “blanket” posture in which the mother lays over the pups, or a passive posture in which the mother is lying either on her back or side while the pups nurse; and the mother remaining off the pups. The behavioral categories were not mutually exclusive – a mother could be licking/grooming while nursing the pup.

For the cohorts ($n=92$) used in the present study, the mean ± SEM percentage of licking across the first 7 PPD was 7.99 ± 2.49 . Thus, HL ($n=8$) were defined as females whose frequency scores for LG were greater than 1 SD above the mean (10.48), and LL ($n=8$) were defined as females whose frequency scores for LG were greater than 1 SD below the mean (5.50), as previously described (Champagne et al., 2003; Cameron et al., 2008; Ruthschilling et al., 2012).

2.3. Brain tissue samples

At the 11th PPD the LL and HL rats, also the virgin females (D), were decapitated and brains were quickly removed. The OB, HP, ST and PFC (*only the front portion*) from the left hemisphere were manually dissected as described below, *with the use of a stereomicroscope, on ice, using sterile materials*. Samples were always collected in the morning, during the light cycle and in a noiseless room.

The structures dissection was performed as previously described and illustrated by Chiu et al. (2007), following the coordinates stated in the maps and guides to dissection published by Palkovits (1988) and Swanson (2004) to separate each specific brain area of interest for the present study. After removing the brain, RNA-later (Ambion) was dropped on the tissue. The brain was cut

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