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Effects of sleep restriction during pregnancy on the mother and fetuses in rats

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HIGHLIGHTS

- Sleep restriction in pregnancy increases plasma prolactin and oxytocin
- Sleep restriction in pregnancy reduces body weight gain
- Sleep restriction in pregnancy increases BDNF in the hippocampus of the fetuses

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ABSTRACT

The present study aimed to analyze the effects of sleep restriction (SR) during pregnancy in rats. The following three groups were studied: home cage (HC pregnant females remained in their home cage), Sham (females were placed in tanks similar to the SR group but with sawdust) and SR (females were submitted to the multiple platform method for 20 h per day from gestational days (GD) 14 to 20). Plasma corticosterone after 6 days of SR was not different among the groups. However, the relative adrenal weight was higher in the SR group compared with the HC group, which suggests possible stress impact. SR during pregnancy reduces the body weight of the female but no changes in liver glycogen, cholesterol and triglycerides, and muscle glycogen were detected. On GD 20, the fetuses of the females submitted to SR exhibited increased brain derived neurotrophic factor (BDNF) in the hippocampus, which indicates that sleep restriction of mothers during the final week of gestation may affect neuronal growth factors in a fetal brain structure, in which active neurogenesis occurs during the deprivation period. However, no changes in the total reactive oxygen species (ROS) in the cortex, hippocampus, or cerebellum of the fetuses were detected. SR females showed no major change in the maternal behavior, and the pups' preference for the mother's odor on postpartum day (PPD) 7 was not altered. On GD 20, the SR females exhibited increased plasma prolactin (PRL) and oxytocin (OT) compared with the HC and Sham groups. The negative outcomes of sleep restriction during delivery could be related, in part, to this hormonal imbalance. Sleep restriction during pregnancy induces different changes compared with the changes described in males and affects both the mother and offspring.

1. Introduction

Disturbances in sleep, such as a reduction in the duration, poor sleep quality and insomnia, have increased in the general population and have become a health concern [1,2]. In pregnant women, fragmentation and/or sleep disruption increase [3–6]. The impact of pregnancy on sleep is more pronounced during the third trimester, which leads to increased episodes of nighttime awakenings and, as a consequence, a reduction in sleep efficiency. These alterations may remain until the first month following labor [4, and for a review 7]. The physiological changes during pregnancy, which are intensified by environmental and lifestyle changes, may be related to the alterations in sleep patterns [5,8,9].

An important consequence of sleep disturbances during pregnancy is the impact on the fetus. Sleep deprivation is a risk factor for poor fetal growth and preterm delivery [3,10–12]. The mother-infant relationship may also be affected by sleep deprivation during pregnancy. Sleep disturbances have been related to maternal depressive symptoms during the post-partum period, which can affect the infant [13,14]. A healthy emotional state during the postpartum period is important for the establishment of mother-child bonding, which provides a secure

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basis for the child's development [15]. A previous study has demonstrated that SR in pregnant females is causally related to reduced ultrasonic calls in the offspring, which indicates a disturbance in cognitive development [16]. Although several epidemiological studies have reported that sleep disturbances affect the mother and fetus, the mechanisms remain to be analyzed. The causes and consequences of sleep deprivation have been less studied in females compared with males. In animal models, REM sleep deprivation may cause alterations in metabolic [17,18], immune [19], and hormonal [20,21] parameters in male rats. In cycling females, previous studies have demonstrated that SR alters the estrous cycle, plasma levels of corticosterone and progesterone [22] and cognitive performance [23].

In pregnant rats, SR has been related to an increase in the adrenal gland weight on GD 20 [24], which indicates that this procedure may activate the hypothalamic–pituitary–adrenal (HPA) axis in pregnant females, despite its hyporesponsiveness during this period [reviewed in 25]. Pregnancy is characterized by a delicate balance of several hormones, and the hypothalamic–hypophyseal–ovarian axis and the HPA axis are essential for a successful pregnancy, delivery and offspring development [reviewed in 26]. Prolactin (PRL) and oxytocin (OT) are also important for fetal development, labor, lactation and the organization of maternal behavior [27,28]. OT and PRL increase during pregnancy, and at the end of pregnancy, OT plays a crucial role in delivery, whereas PRL plays a role in lactation and the onset of maternal behavior. Moreover, hormones may respond differently in pregnancy vs. non-pregnancy states. It has been well established that the HPA axis exhibits a reduced response to a wide range of physical and psychological stressors during gestation [reviewed in 29]. This hyporesponsiveness appears necessary for the maintenance of pregnancy, maternal behavior, and the development of several fetal organs that are highly sensitive to maternal glucocorticoids [reviewed in 30].

The present study aimed to analyze the effects of sleep restriction (SR) from gestational day (GD) 14 to 20, using the multiple platform method, in the pregnant females and their fetuses, as well as the mother and offspring following birth in rats. The effects of SR were analyzed after 6 sessions of partial sleep deprivation for 20 h per day per session. This experimental sequence was adopted to mimic a more realistic situation considering that sleep disturbances are typically repeated. In the females, we analyzed the effects of SR on the plasma corticosterone, PRL, progesterone and OT on GD 20 and subsequently during lactation on postpartum day (PPD) 7. Previous studies have identified a decrease in body weight in male [31] and pregnant female rats submitted to SR [24]; thus, the levels of triglycerides, glycogen and cholesterol were analyzed in the liver and the soleus muscle on GD 20 to investigate the potential causes of weight loss. Considering the period of synaptogenesis during the final week of gestation in the rat [reviewed in 32], and that pre-natal stress may affect brain development in the offspring [reviewed in 33, 34], we measured brain-derived neurotrophic factor (BDNF) in the fetuses on GD20. Reactive oxygen species (ROS) were simultaneously analyzed to assess the potential cellular damage by oxidants. Following birth, maternal behavior was assessed to investigate the effects of SR on the mother-offspring relationship. Moreover, in the 7-day-old male and female pups, the behaviors of the pups were evaluated using the nest odor preference test.

2. Materials and methods

2.1. Subjects

Female (113) and male (24) rats approximately 70 days old from the Centre for Reproduction and Animal Experimentation Laboratory of the Universidade Federal do Rio Grande do Sul, UFRGS were used. The animals were group-housed (4 per cage of 40 × 33 × 17 cm) in a temperature (21 ± 1 °C) and humidity-controlled (60%) animal facility. The rats were maintained on a 12-h light/dark schedule (lights on at 06:00 h) with access to water and rodent chow (Nuvilab Cr2, Colombo, Brazil) ad libitum. The experimental protocol was approved by the Ethics Committee in Use of Animals (CEUA) of the UFRGS (No. 23948/2013). The experimental procedures were performed following the Guidelines for Animal Care and Use of Laboratory Animals of the National Institutes of Health (2011).

2.2. Sleep restriction (SR)

SR was induced using the multiple platform method, according to the protocol described in [23,24]. The paradigm consisted of placing 4 rats of the same group in a 90 × 50 × 50 cm tank filled with water. Ten cylinder platforms (7.5 cm in diameter and 10 cm in height) were placed in the water tank and were arranged at distances of 11 cm from one another. These platforms protruded 2 cm above the water surface, which enabled the animals to move from one platform to another. To better isolate the sleep restriction variable, one group of animals was utilized to control for the environmental parameters of the multiple platform method. In this Sham group, tanks with the same characteristics and dimensions as the tanks used to induce SR were used; however, instead of water, the floor was covered with sawdust. These tanks also contained the 10 platforms of equal size, and the animals had to climb to a platform to reach food and water. Pregnant females were submitted to sleep deprivation or exposed to the new environment for 20 h per day (14:00 to 10:00 h next day) from GD 14 to 20. In the present sleep restriction protocol, the animals could recover sleep; thus, the design comprised partial sleep deprivation.

2.3. Experimental procedure

After 4 regular estrous cycles, 90-day-old virgin females (n = 74) were mated with males on the evening of proestrus. The presence of spermatozoids in the vaginal smear the following morning was considered a positive indicator of pregnancy (n = 64), and this day was designated GD 0. The pregnancy was monitored daily, and the body weight of each pregnant female was measured using an electronic balance (SHIMADZU BL3200H, Tokyo, Japan) between 10:00–10:30 h every day. On GD 1, the animals were randomly assigned to the following groups: home cage (HC, n = 26), Sham (n = 17), and sleep restriction (SR, n = 21). On GDs 12 and 13, at 14:00 h, the pregnant rats in the 3 groups were transported to a room with the tanks for sleep restriction, which was maintained at similar conditions to the animal facility. The SR rats were placed together on platforms inside the tanks (4 animals from the same cage per tank) for 30 min. The same procedure was adopted for the Sham group. The HC rats were left in their home cages (40 × 33 × 17 cm) in the same room for the same period as the Sham and SR rats. The purpose of this procedure was to habituate the animals to the new environment of the tanks with the platforms. On GD 14 at 14:00 h, the HC, Sham and SR rats were again transported to the experimental room. The SR rats were placed on the platforms in the water tank, where they remained until 10:00 h of the next day. The Sham rats were placed in tanks that contained shavings and were arranged with multiple platforms. The HC rats were maintained in their home cages without manipulation in the same room as the other groups (Sham and SR). On the following day at 10:00 h, the SR and Sham rats were removed from the tanks and placed back in their respective home cages. The 3 groups were returned to the animal facility where they remained for 4 h (10:00 to 14:00 h), when the animals could recover sleep. This procedure was repeated daily until GD 20. During the sleep restriction period, food and water were offered ad libitum. Food pellets were left on a compartment in the grid (similar to the home cage) above the platforms.

On GD 20 at 10:00 h, following the conclusion of the SR period, the pregnant rats were returned to the animal facility and weighed. Immediately afterwards, females from each group (HC = 10, Sham = 6 and SR = 7) were randomly selected; they were decapitated, and blood was collected. The fetuses were subsequently removed via