Hormones and Behavior 105 (2018) 146–156

Contents lists available at ScienceDirect

Hormones and Behavior

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

The absence of maternal pineal melatonin rhythm during pregnancy and lactation impairs offspring physical growth, neurodevelopment, and behavior

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ARTICLE INFO

Keywords:
Adult neurogenesis
Development
Spatial memory
Working memory
Reference memory
Neuroprotection
Melatonin

ABSTRACT

Maternal melatonin provides photoperiodic information to the fetus and thus influences the regulation and timing of the offspring's internal rhythms and preparation for extra-uterine development. There is clinical evidence that melatonin deprivation of both mother and fetus during pregnancy, and of the neonate during lactation, results in negative long-term health outcomes. As a consequence, we hypothesized that the absence of maternal pineal melatonin might determine abnormal brain programming in the offspring, which would lead to long-lasting implications for behavior and brain function. To test our hypothesis, we investigated in rats the effects of maternal melatonin deprivation during gestation and lactation (MMD) to the offspring and the effects of its therapeutic replacement. The parameters evaluated were: (1) somatic, physical growth and neurobehavioral development of pups of both sexes; (2) hippocampal-dependent spatial learning and memory of the male offspring; (3) adult hippocampal neurogenesis of the male offspring. Our findings show that MMD significantly delayed male offspring's onset of fur development, pinna detachment, eyes opening, eruption of superior incisor teeth, testis descent and the time of maturation of palmar grasp, righting reflex, free-fall righting and walking. Conversely, female offspring neurodevelopment was not affected. Later on, male offspring show that MMD was able to disrupt both spatial reference and working memory in the Morris Water Maze paradigm and these deficits correlate with changes in the number of proliferative cells in the hippocampus. Importantly, all the observed impairments were reversed by maternal melatonin replacement therapy. In summary, we demonstrate that MMD delays the appearance of physical features, neurodevelopment and cognition in the male offspring, and points to putative public health implications for night shift working mothers.

1. Introduction

According to the European Foundation for the improvement of living and working conditions and the US Department of Labor, almost a fifth of the worldwide workforce is engaged in shift work, with 20% of European and American workers engaged in night shifts (Labor, 2005; Parent-Thirion, 2007). Recent data in the human and animal literature suggest that chronodisruption (i.e., disturbance of temporal organization, mainly circadian, of endocrinology, physiology, metabolism, and behavior) during pregnancy has been associated with an increased risk of miscarriage, preterm delivery and low birth weight, in addition to higher incidence of sleep, metabolic and cardiovascular disturbances in the offspring (Knutsson, 2003; Navara and Nelson, 2007; Reiter et al., 2012; Zhu et al., 2004).

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https://doi.org/10.1016/j.yhbeh.2018.08.006
Received 6 December 2017; Received in revised form 2 August 2018; Accepted 12 August 2018

Several social situations (such as work at night, shift work, artificial light exposure at night) cause a reduction in maternal melatonin production and a disturbance of central and peripheral clocks that control circadian rhythms (Dumont and Paquet, 2014). The maternal pineal gland and its secretory product melatonin have a pivotal role in providing photoperiodic information to the fetus, thus influencing the regulation and timing of the offspring's internal rhythms and their preparation for extrauterine life (Torres-Farfan et al., 2011; Velazquez et al., 1992).

In addition to its circadian rhythm modulatory role, maternal melatonin also acts as a neuroendocrine modifier of several physiological systems (e.g., neural, cardiovascular, energy metabolism, immunological and inflammatory responses). It is also a potent direct free radical scavenger and indirect antioxidant and cytoprotective agent at all levels in the maternal–placental–fetal unit. Considering all these data, maternal melatonin seems to be essential for a successful pregnancy (Carrillo-Vico et al., 2013; Cipolla-Neto et al., 2014; Ferreira et al., 2012; Manchester et al., 2015; Vilches et al., 2014) and may represent an important component of the intrauterine environment for perinatal and postnatal programming of brain and behavior (Bale, 2015).

This idea is reinforced by the presence of melatonin receptors in the fetal nervous system, especially in hypothalamic suprachiasmatic and arcuate nuclei and hippocampus (Lacoste et al., 2015). It should be stressed that, in rodents, significant development of hippocampal and hypothalamic neural circuits occurs postnatally during the lactation phase (Bouret et al., 2004; Markakis, 2002; Seress, 2007). One likely mechanism through which melatonin would influence brain development is via modulation of plastic events, such as neurogenesis and neuroprotection. Recent studies show that melatonin administration after birth increases cell proliferation, differentiation and survival of novel neurons in the hippocampus (Kim et al., 2004; Ramirez-Rodriguez et al., 2009; Ramirez-Rodriguez et al., 2011). No investigation of those parameters, however, has been performed to evaluate the intrauterine role of melatonin.

In the present study, we aimed to examine whether gestational and lactational maternal pineal melatonin absence could induce changes in the offspring somatic and physical growth, neurodevelopment and its effects on cognitive functions and neurogenesis in adulthood. We hypothesized that maternal absence of pineal melatonin during gestation and/or early post-natal period might have more severe consequences, as it may alter brain development and programming, and thereby have long-term consequences for behavior and brain function. To test our hypothesis, we used a model of gestational hypomelatoninemia during pregnancy and lactation resulting from the surgical removal of the pineal gland. Our specific aims were to investigate the effects of maternal pineal melatonin absence and its therapeutic replacement on: (1) somatic, physical growth and neurobehavioral development of offspring; (2) hippocampal-dependent spatial learning and memory of male adult offspring and (3) adult hippocampal neurogenesis.

2. Material and methods

2.1. Animals

Nursing female Wistar rats ($n = 78$) and their male and female offsprings (Animal Facility of the Institute of Biomedical Sciences - São Paulo, Brazil) were kept in a temperature-controlled environment (22 ± 2 °C) under a 12L:12D light–dark cycle (dark to light transition, Zeitgeber time 0 - ZT0 and light to dark transition, Zeitgeber time 12 - ZT12), with food and water ad libitum. All experimental procedures complied with the Brazilian Guidelines for Care and Use of Animals in Education or Scientific Research Activities determined by the National Council of Animal Experimentation Control (DBCA – CONCEA, 2016) and were approved by the Ethics Committee of the Institute of Biomedical Sciences of the University of São Paulo (CEUA Protocol No. 199/116f, book 2).

2.2. Dams and surgical procedures

Eight-week-old female rats were anesthetized with an intraperitoneal injection of ketamine and xylazine (0,4 g/kg and 0,02 g/kg) and subjected to pinealectomy (PINX) as previously described (Ferreira et al., 2012). Briefly, anesthetized animals were placed in a stereotaxic apparatus (David Kopf Instruments, CA, USA) and a sagittal opening was made in the scalp. The skin was pulled apart to expose the lambda confluence, a disc-shaped opening was made around the lambda with a circular drill and the pineal gland was removed with a fine forceps. Meticulous care was taken to avoid injury to the nervous system adjacent to the pineal gland. The disc-shaped piece of bone was replaced and, after brief hemostasis, the scalp was sutured with cotton thread. Animals of the control group (CTL) were subjected to a similar surgical protocol, but their pineal gland was not removed. The integrity of the gland itself after the excision and the post-mortem analysis of the dams' brains allowed us to verify the success of the pinealectomy procedure and the integrity of the adjoining central nervous system.

Immediately after the surgical procedures, PINX rats were randomly assigned to two groups, one received vehicle (PINX) and the other received melatonin (PINX + MEL) diluted in the drinking water. Melatonin (Sigma-Aldrich; St. Louis, MO, USA) replacement therapy was based on a previous study (Ferreira et al., 2012), with the hormone being added to the drinking water exclusively during the dark phase (ZT 12 to ZT 0). CTL and PINX rats received the same concentration of vehicle (ethanol 10^{-8}v:v) in water during the dark phase. During the light phase, all the animals received tap water free of melatonin and vehicle. Melatonin dosage (0.5 mg/kg) was attained by daily correction of melatonin drinking solution concentration based on the previous day measurement of individual body weight and nocturnal water intake.

Thirty days after surgery, female rats were caged with experienced adult males (2:1) across two estrous cycles. The presence of vaginal plugs or sperm was considered indicative of pregnancy and designated as gestational day 0. Pregnant rats were immediately isolated and kept one per cage. Melatonin or vehicle administration was maintained throughout gestation and lactation.

Litters from 30 dams (PINX $n = 10$, PINX + MEL $n = 10$ and CTL $n = 10$), designated PINX-F1, PINX + MEL-F1, and CTL-F1 had their physical and neurobehavioral development (described below) evaluated up to postnatal day (PND) 35. From PND 60 to 77, they were tested for spatial learning memory. The experimental design of the present study is illustrated in Fig. 1.

2.3. Offspring procedures

On PND4, all litters were examined and culled to 8 pups (4 males and 4 females) that were uniquely identified within each litter with a small tattoo on one or more of the paws and the pups were allowed to nurse until PND21.

2.3.1. Neurobehavioral and developmental landmarks

Throughout the lactation period, pups were individually evaluated regarding neurological reflexes development and physical developmental characteristics. Subjects were individually weighed at PND4, 7, 17, 21 and 60.

2.3.2. Physical developmental parameters

The day of each of the following events was recorded for each animal: pinna detachment (PD) (the opening of ear channel), incisor eruption (IE) (observation of superior and inferior teeth), eye opening (EO) (opening of both eyes lids), testis descent (TD) (descent of both testes to scrotum), and vaginal opening (VO) (opening of vaginal channel). The mean day of occurrence of each event for each group was then calculated.