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Long lasting alteration in REM sleep of female rats submitted to long maternal separation

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Abstract

Early adverse experiences represent risk factors for the development of anxiety and mood disorders. Maternal separation can induce biobehavioral alterations in male rodents similar to those seen in depressed humans, such as hyperresponsiveness to stress and sleep disturbances. Nonetheless, no study has yet explored the effects of early life events on the relationship between stress and sleep in female rats. Whole litters of Wistar rats were submitted to brief- or long maternal separations (15 [BMS] or 180 min/day [LMS], from postnatal days 2–14) or kept undisturbed with their mothers (CTL). When adults, female rats were sleep-recorded for 22 h before (baseline) and after a 1 h exposure to cold stress (post-stress). Additional subsets of animals were sacrificed before, 1 or 3 h after the stressor for plasma corticosterone determination. No differences in baseline sleep were observed among the groups. Female rats submitted to LMS exhibited a significant increase of REM sleep on the night following a 1 h exposure to cold stress, whereas the sleep of BMS rats was barely altered by stress. All groups exhibited similar basal and stress-induced corticosterone levels. The present results are compared to a previous study performed in male rats, and corroborate that manipulations applied during infancy modify the expression of stress-induced sleep rebound.

Keywords: Maternal separation; Sleep; Sleep rebound; Stress; REM sleep; HPA axis; Depression; Female

1. Introduction

Early adverse life events constitute one of the major risk factors for the development of mental disorders, such as anxiety, post-traumatic stress disorder, and major depression. Moreover, it may also increase an individual's vulnerability to substance abuse latter in adult life [1]. Several animal models have been used to elucidate the functional and biochemical consequences of early life adversity, and have revealed a number of persistent effects including changes in the activity of the hypothalamic-pituitary–adrenal axis (HPA) [2,3]. Among the paradigms used to study early life events, prenatal stress is the best studied in regards to sleep outcomes. It has been shown that prenatal stress predisposes rats to disturbances that persist throughout adulthood and parallels, to a large extent, those found in depressed patients, such as cognitive impairment [4], increased anxiety behavior [5], hypercortisolemia and sleep alterations, that may be related to stress-inducing events [6]. Prenatally stressed rats show an increase in rapid eye movement (REM) sleep duration at all phases of the light–dark cycle, and this is positively correlated to basal and stress-induced plasma corticosterone (CORT). In addition to the major effects on REM sleep, prenatal stress results in sleep fragmentation, diminished slow wave sleep (SWS), lower delta EEG power and reduced REM sleep latency [6,7]; changes that are similar to those observed in depressed patients.

The existence of a close relationship between stress hormones and sleep disorders has been established, although the temporal sequence of events, and what is the cause and what is the consequence remains unknown. On the one hand, CRH and ACTH, hormones of the hypothalamic-pituitary–adrenal (HPA) axis, impair sleep, for they are pro-waking hormones [8,9], although the effects of glucocorticoids on sleep are rather more complicated, exhibiting an inverted U-shaped relationship [9]. On the other hand, chronic insomniac patients exhibit high

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levels of ACTH and cortisol during the nadir of the circadian rhythm, i.e., between 20:00 and 02:00 h, but not immediately after waking [10], suggesting that persistently elevated cortisol levels at a time when they should be the lowest may predispose the subjects to poor sleep quality. Although hyperactivity of the HPA axis is associated to several pathologies, including mood and anxiety disorders, and drug abuse, and these are also more prevalent in women [11,12], there is no clear cut consensus that the reactivity of the HPA axis is greater in women than in men [13]. A recent study showed that men secrete more ACTH and cortisol than women in response to a psychological stressor, but the opposite is observed with the use of naloxone, which blocks the opioid negative feedback at the hypothalamic level [14]. There is a large amount of evidence for sexual differences in basic neural processes and behaviors. Thus, the use of females in experimental studies has become an important issue, since it has been demonstrated the existence of differences between females and males in many aspects of health, such as manifestation of some psychiatric illnesses. Indeed, gender-dependent consequences of early life events have also been previously shown. In prenatally-stressed rats, depressive behavior is greater in females than in males [15,16]. In adolescent female rats, neonatal handling produces elevated stress-induced hormone concentrations, the opposite effect being observed in males [17]. Besides, handling provides males with a greater capacity to actively face chronic stressors, whereas in females it increases their susceptibility to express depressive-like behavior [18]. Finally, sex-differences are also found in anxiety-like and other emotional behaviors in adult rats [19,20]. Moreover, community-based studies have consistently shown a higher prevalence of insomnia among women than among men [21–24].

We have recently shown that male rats submitted to brief maternal separation (BMS, i.e., 15 min of separation daily) exhibit a faster and longer rebound of REM sleep after 1 h of restraint stress, although the baseline sleep pattern is similar to that of non-manipulated rats [25]. In addition, in a previous study [26] we assessed the effects of LMS and of brief maternal separation (BMS) on baseline and cold stress-induced sleep rebound in male rats. Baseline sleep pattern revealed that LMS male rats exhibit more REM sleep, and in response to 1 h of cold stress, control, BMS and LMS rats exhibit a similar sleep rebound during the nighttime period, indicating that long periods of maternal separation in male Wistar rats increases spontaneous REM sleep, but does not interfere in the sleep response to acute cold stress.

Based on the evidence presented above, we hypothesized that if LMS induced a hyper-reactive HPA axis in females, then it should result in stress-induced impairment on sleep architecture. To test this hypothesis we assessed baseline and stress-induced sleep pattern of BMS, LMS and control female rats. Our results showed that baseline sleep was similar among the groups, but LMS rats exhibited more REM sleep in response to cold stress.

2. Materials and methods

Animal studies were approved by the Animal Care and Use Committee of UNIFESP (CEP# 0823/03) and were in accordance with National Institutes of Health guidelines on animal care.

2.1. Subjects

Female Wistar rats were mated in the animal facility of the Department of Psychobiology and inspected twice a day for the presence of pups. The day of birth marked postnatal day (PND) 0, when the litters were randomly distributed into 3 groups: control non-manipulated (CTL), BMS, and LMS. On PND 1, the litters were culled to 4 males and 4 females per dam. The animal facility, including breeding, experimental, and recording rooms, was maintained in a controlled 12-hour light–dark cycle (light on at 7:00 and off at 19:00) and temperature (23 °C± 2 °C). Animals had free access to chow and tap water at all moments of the study.

2.2. BMS and LMS procedures

From PND 2 to 14, whole litters were removed from the nest for a period of 15 min (BMS) or 180 min (LMS) and placed as a group in separate cages on a heating pad set at 33 °C. The mothers remained in the home cage, and at the end of the separation period, litter and mother were reunited. CTL litters remained with the mothers, and were manipulated only during cage cleaning (once a week), when half of the sawdust shavings were changed. Rats were weaned on PND 22 and housed with their sex-mate siblings until adulthood. To avoid possible litter effect, only 2 females from each litter were submitted to implantation of electrodes for the sleep study. The remainder of the females and those from additional litters were used for determination of corticosterone plasma concentrations. Males were used in a previously published study [26].

2.3. Implantation of electrodes

At approximately PND 75, CTL, BMS, and LMS animals were anesthetized with diazepam (5.5 mg/kg, intraperitoneal, Roche Chemicals and Pharmaceuticals, Brazil) and ketamine chloride (140 mg/kg, intraperitoneal, Agener União, Brazil), and their head and neck were shaved. Each animal was attached to a David Kopf stereotaxic apparatus, and the perforation of the skull was performed according to the following coordinates: Electrode 1: 1 mm posterior to bregma, 3 mm to the left of the sagital suture; Electrode 2: 3 mm anterior to bregma, 1 mm to the right of the sagital suture; Electrode 3: 1 mm anterior to lambda, 4 mm to the left of the sagital suture; Electrode 4: 4 mm anterior to lambda, 1 mm to the right of the sagital suture. For electroencephalogram, two ipsilateral stainless steel screws were implanted and for electromyography recording, one additional pair of nickel–chromium fine wire electrodes were inserted the neck muscle. After the surgery, the animals received 0.25 mL of Pentabiotic (intramuscular, Fort Dodge Animal Health, Ltd., Brazil) to avoid infection and sodium diclofenac (25 mg/mL, intraperitoneal, Ariston, Brazil) to induce analgesia.

After recovering from anesthesia, the animals were placed individually in transparent cages (34 cm in height × 31.5 cm