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Dynamic changes in sleep pattern during post-partum in normal pregnancy in rat model

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HIGHLIGHTS

- First report on sleep-wakefulness during pregnancy-postpartum continuum.
- Sleep fragmentation during last trimester of pregnancy and post-partum.
- Non-REM sleep delta power was increased during late pregnancy and after parturition.
- Post-partum sleep and anxiety were reduced in contrast to ante-partum.
- An animal model for drug trials and study of sleep disorders during pregnancy.

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ABSTRACT

To develop an animal model for studies on peri-partum sleep disorders, sleep patterns in female Wistar rats during pregnancy, post-partum and after weaning, were assessed and associated adaptive changes in their anxiety were examined. Adult nulliparous female rats, maintained in standard laboratory conditions with *ad libitum* food and water, were surgically implanted with electroencephalogram and electromyogram electrodes under anaesthesia for objective assessment of sleep-wakefulness (S-W). After post-surgical recovery, three control recordings of S-W were taken for 24 h before the animals were kept for mating. After confirmation of pregnancy, S-W recordings were acquired during different days of pregnancy, post-partum lactation/nursing days, and also after weaning. Their anxiety levels were tested in the elevated plus maze. During pregnancy, sleep increased primarily due to increase in light non-REM sleep during dark period. There was an increase in non-REM sleep delta power after parturition, though the sleep was fragmented, especially during daytime. Simultaneous behavioural recording showed increased anxiety during third trimester of pregnancy and gradual reversal of it after parturition. This is the first report where diurnal and nocturnal variations in S-W and delta power, along with adaptive changes in anxiety, were studied before, during and after pregnancy. This study also provides an animal model for drug trials and studies on sleep disorders during *peri*-partum window.

1. Introduction

Sleep during pregnancy and post-partum is crucial in shaping the neural development of babies. To study the sleep loss related pathophysiological changes in human situations, it is necessary to develop a model in rats after identifying the lacunae in the existing knowledge in the *peri*-partum changes in sleep in altricial species. Pregnancy is accompanied by an increase in nocturnal sleep in rats [1]. There is enhancement in both non-REM (NREM) and REM sleep during the dark period which is the normal waking period for the rats [2]. Though there are no changes in the daytime sleep till late pregnancy, a reduction in REM sleep, is reported towards the end of the gestation [3–5]. Although there are several studies on sleep changes during gestational period, post-partum changes in sleep-wakefulness (S-W) in rats are not well investigated. According to single brief report, based on 7 h of S-W light period recording, there

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was reduction in NREM and REM sleep in the lactating rats [6]. There is no report where post-partum changes in S-W were recorded for 24 h, during day and night during different nursing/lactation days. Recording of NREM delta power is considered a gold standard for assessment of homeostatically regulated sleep [7]. However, information on changes in NREM delta power during pregnancy and post-partum are also lacking.

Sleep changes during gestation and post-partum are also accompanied by other behavioural changes in the rodents. There are several reports on anxiety related behaviour in pregnant and parous females [8–13]. Though some studies showed enhanced anxiety-like behaviour during later stages of pregnancy [11,12], there are also contrary findings [8,14]. Rodent females display novel, pup-caring maternal behaviour immediately following parturition [15]. In addition to modifications in foraging behaviours, the lactating females exhibit enhanced exploratory behaviours and increased aggression, which are important for meeting the post-partum challenges [8,16]. This behavioural phenotype observed during pregnancy and lactation is very distinct and important for mother-child bonding.

Importance of sleep during pregnancy is highlighted in a recent report that showed depression like symptoms in babies of REM sleep deprived rats [17]. Acute sleep deprivation during third term of pregnancy resulted in high risk-taking behaviour and impulsivity in infants [18,19]. These studies not only indicate that sleep during pregnancy and post-partum is crucial in shaping the behaviour of babies, it also highlights the need for developing a small animal model to study the pathophysiological changes in human situations.

Adaptive changes in maternal S-W profiles would be required during the period of nursing and foetal growth. However, no reports are available on changes in S-W during gestation-lactation continuum. The pathophysiology associated with sleep deprivation in rats is similar to that observed in humans [20,21]. Therefore, in the present study the changes in S-W, NREM delta power and anxiety levels during pregnancy, post-partum and after weaning were studied in an animal model. This study would provide a baseline for the sleep profile of the rat in the peri-natal period. As this would be similar to changes during the periods of pregnancy, gestation and lactation in a woman, we can better understand sleep disorders related changes during the peri-natal period in humans, with this rat model.

2. Materials and methods

2.1. Animals

The study was conducted on 14 adult nulliparous female Wistar rats (body weight 230–270 g) acclimatized to 12:12 h light:dark schedule at ambient temperature of 26 ± 1 °C with *ad libitum* supply of food and water. Seven of them were chronically implanted with electrodes for recording S-W during pregnancy and post-partum. S-W in these rats was monitored for 24 h on three consecutive days prior to conception. S-W was also recorded during pregnancy (gestation days 2, 8, 15, 19), post-partum (lactation days 2, 4, 6, 10, 18) and post-weaning periods (days 7, 14) as shown in the protocol (Fig. 1). The gestational days 2, 8, 15 were selected to evaluate changes in sleep during each of the 3 trimesters, and day 19 was taken to assess sleep toward late pregnancy. Their anxiety levels were tested every week in elevated plus maze during the entire study (Fig. 1). Seven other non-pregnant female rats were tested once a week, for nine weeks, in the elevated plus maze, to assess whether there was any change in anxiety levels by repeated testing. The study was approved and performed in accordance with the guidelines laid down by the Institutional Animal Ethics Committee of the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, Kerala.

2.2. Procedure for recording sleep wakefulness

To assess S-W in rats, the electroencephalogram (EEG) and electromyogram (EMG) electrodes were implanted under anaesthesia (Ketamine 50 mg/kg and Xylazine 5 mg/kg body weight, im) as described previously [22,23]. In brief, miniature screws electrodes (1 mm shaft diameter) were implanted bilaterally on the skull above the parietal cortex for recording EEG. Loop electrodes were sutured in the nuchal muscle for recording EMG, and a miniature screw electrode was implanted in the nasal plate to serve as reference electrode. The electrodes were connected to an IC socket and the whole assembly was fixed on the skull using dental cement. After post-operative recovery of ten days, the rats were individually given the opportunity to get habituated to the sleep recording chamber.

2.3. Experimental protocol

Three 24 h (6 am to 6 am next day) baseline control recordings (C1, C2, C3) of S-W were taken on the consecutive days, prior to mating. After confirming that the sleeping patterns during these three days are comparable, the female rats were kept for mating with a male Wistar rat of the same age. The day of vaginal plug formation was noted as day 1 of gestation. After confirmation of pregnancy, the rats were housed individually in polystyrene cages. Their body weights were monitored regularly and their S-W recordings for 24 h were taken on various days of pregnancy, post-partum and post-weaning as per the recording protocol (Fig. 1). The day of delivery was marked as post-delivery day 0. Litter size was maintained at 6–7 for all dams to ensure uniformity in maternal care. Throughout the study, the litters were kept with the mother rats, even during S-W recording, to provide the dams with natural conditions.

2.4. Recording and analysis of S-W

The S-W signals were acquired using data acquisition system MP-150 (Biopac System). The signals were amplified (× 5000), filtered (EEG: 0.1–35 Hz; EMG: 1–500 Hz), and digitized at 1 kHz. The S-W recordings of 24 h were scored manually (offline) taking epochs of 30 s duration. The S-W stages were classified into wakefulness consisting of active wake (W1) and quiet wake (W2), non-rapid eye movement (NREM) sleep (having light NREM sleep as S1 and deep NREM sleep as S2 components), and REM sleep, as described previously [24]. For each stage of S-W, the percentage time, frequency and average bout durations were calculated. Day and night values (%) of NREM (S1 and S2) and REM sleep, in three hour bins, were compared to find out their circadian alterations. The mean delta power was calculated from artefact-free NREM sleep on all the recorded days. The mean delta power was calculated for every 30 s epoch of NREM in 24 h EEG and was averaged in 3 h bin for light phase (06.00 h till 18.00 h) and dark phase (18.00 h till 06.00 h the next day). The delta power obtained from NREM sleep was normalized by dividing it with the delta power obtained in the REM sleep of the same bin of 3 h [25]. The area under curve (AUC) for NREM delta power was calculated for all recording days and compared with averaged control values. The delta power data for all gestational and post parturition days was also pooled to obtain the key trends.

2.5. Emotional state testing

To find the state-dependent alterations in emotional behaviour in rats, anxiety levels were tested in elevated plus maze (EPM) using