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SOME ASPECTS OF THE SLEEP OF LACTATING RAT DAMS

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ABSTRACT

Background and objective: The sleep of lactating rat dams were evaluated since informations on the subject are scanty.

Methods: Electrographic recordings were made in nine rat dams, in different days after delivery (2nd to 20th day). Normal estrous cycling female rats, with previous motherhood experience, and four adult males were used as controls. Sleep-wakefulness was quantified and expressed as percentages of the total recording time and significance assessed by ANOVA, adopting significance level at the 0.05 level.

Results: Total wakefulness was 43.4 ± 2.6% in control females, 44.3 ± 7.6% in the males and 55.1 ± 4.2% in LRD living with their 8 pups (difference rejected at the limit, p = 0.0547). The mean number of awakening episodes longer than 30 minutes was significantly greater in LRD. Synchronized wave sleep amounted to 46.6 ± 2.1%, 46.1 ± 7.6% and 41.9 ± 4.0%, respectively in control females, males and LRD. Desynchronized wave sleep (REM-sleep) was significantly reduced in LRD (3.0 ± 0.4%) compared to the values found in control females (10.0 ± 1.0%) and males (9.4 ± 0.6%). The average amounts of REM-sleep/hour along the recording period revealed to be constant and significantly lower in the lactating rat dams (3.0 ± 1.3%) comparatively to control females (11.6 ± 3.8%). An average of 1.2 ± 0.5 REM-sleep episodes/hour was also significantly lower in lactating females than in control females (3.2 ± 1.5).

Conclusion: The results suggest an adaptive reorganization of the sleep architecture in the female rat during the nursing period.

Keywords: sleep, nursing rats, lactation.

INTRODUCTION

Sleep, composed by two distinct functional states of the central nervous system in mammals (1), has important relationships with lactation. For instance, milk ejection depends on bouts of synchronized waves sleep (SS) (2,3). Negative relationships, however, seems also to exist since the development of postpartum depression is associated to sleep problems occurring during the lactating period (4,5). Notwithstanding this, there are still only few studies devoted to the subject using polysonographic monitoring (5) although this scantiness has been pointed-out some decades ago (6).

The rat is undoubtedly the animal most used in experimental sleep research (7) and its use has advanced our knowledge on different problems. Despite this, sleep studies using electrographic tools in lactating rat dams (LRD) are also scanty in the literature

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and the absence of data hinders the assessment of an experimental approach to investigate the postpartum depression. As determination of the sleep parameters of female rats in this period of life is necessary for such approach, the present study aims to report some data on the sleep of LRD.

METHODS

The sleep-wakefulness parameters were determined in 9 lactating rat dams, living in their home-cages with 8 pups each. Nine females displaying normal estrous cycles but having previous motherhood experience and 4 males were used as controls. All animals were 5-6 months old and were provided by the Central Breeding House of the Universidade Estadual Paulista (Botucatu). They were albino rats, originally from the Wistar stock, and reared by the Breeding House for more than 20 years.

Chronic electrodes were surgically implanted in all animals for electrocorticographic (ECoG), electromyographic (EMG) and electroculographic (EOG) recordings for determination of sleep-wakefulness parameters. Such determination was made from recordings obtained on days 2, 6, 9, 10, 11, 15, 16 19 and 20 of lactation (using only the first recording made in each animal). Four control female rats were in diestrous, 2 in proestrous and 3 in estrous phase on the morning of the electrographic recording day.

Electrodes for ECoG, EMG and EOG were chronically implanted in the rats under pentobarbital (40mg/kg) and local (Xylocain 2%, with vasoconstrictor) anesthesia. All recommendations for animal use in experimental research (8) were followed. All procedures for surgical implantation of electrodes and recordings followed the descriptions made in previous publications (9,10) and approved by a local Committee. After surgery, the animals were moved to their plastic individual home cages (380x400x160mm), provided with commercial food pellets, potable water and wood shavings. A period of at least seven days for post-surgery recovery was allowed before recordings, maintaining the cages in a controlled 12:12h light-dark cycle (light on at 07:00h) and temperature room (24-25OC).

On the day of electrographic recording, the animal was moved in its home cage to a contiguous room and kept inside a Faraday's cage at 08:00h. Room temperature was kept constant (24-25OC) and a paper cardboard fixed on the wall of the Faraday's cage in order to prevent the direct incidence of the bulb lamp's light on the nest. Electrodes were connected to a Beckman polygraph and recording started at 10:00h and ended at 17:00h. Recordings were made always with paper speed at 5 or 10mm/second.

Two independent scorers, using 2-s epochs, visually analyzed recordings as awakening, SS or REM-sleep. The criteria used for the scoring of each state were similar to those adopted and described in previous quantitative studies (9,11). A third experienced judge solved the cases of discordant scoring segments. All results were expressed as percentages of total recording time (TRT). Analysis of variance (ANOVA) (Statistica Software) was used to evaluate the existence of significant differences among groups and, when necessary, Tukey's honest significant difference (HSD) test was used as the post-hoc test. Significance level was adopted at $p<0.05$.

RESULTS

The prominent behavior of LRD was their permanence in the nest, where sleep, lactation and pup cleansing were displayed in an alternating fashion. The remaining part of the time corresponded to brief episodes of exploratory activity around the cage, nest repair, eating, drinking, sporadic pup retrieval, and sleep episodes outside the nest. These behaviors waned by the time of offspring´s trial to eat food pellets by the 15th day, when adult pattern of locomotion was observed. The mean amount of time spent awake by LRD was 55.1 ± 4.2% of the TRT. The greatest amounts were observed on the 6th and 11th lactation days (75.8 and 72.6%, respectively), whereas the lowest values were observed on the 10th and 18th days (44.5 and 38.6%, respectively). The statistical significance of the general mean presented by the LRD group, comparatively to controls, was rejected at the limit (ANOVA, $F= 3.397$, $p=0.0547$), as shown in Table 1. The increased time spent awake by LRD was determined mainly by frequent pups' demand for suckling. They awoke periodically and synchronously, becoming agitated and presenting uncoordinated movements and strident vocalization while nipple-searching behavior was evident. Although the offspring slept approximately in a synchronized way, many intense phasic muscular jerks of sleep displayed by the newborns disturbed the lactating dam. In such cases, dams changed posture in order to facilitate nipple access or to attend other demands. Such facts increased the number of awakenings longer than 30 minutes in LRD (mean of 1.37 ± 0.31 episodes/hour) that was significantly greater ($F(2,19) = 10.16$, $p<0.05$) than those observed in control females (0.84 ± 0.27 episodes/hour). After eyes opening by the 11th-12th day of age, lactation demand became progressively silent; however, nipple-searching movements became more vigorous, which also awakened the dams. LRD progressively refused to lactate when pups started to try solid food ingestion. However, dams continued to sleep on an overcrowded environment.

Synchronized sleep amounted to 41.9 + 4.0% of the TRT in the LRD. This value was not significantly different from that found in control groups (Table 1).

The individual amount of REM-sleep in LRD never surpassed 5% of the TRT and the group reached an average of 3.0 ± 0.4%. The amount presented by the LRD group was significantly lower ($F = 24.948$, $p<0.05$) than the means of control females (Tukey HSD test, $p<0.05$) and males ($p<0.05$), as presented in Table 1. The mean amounts of REM-sleep/hour, throughout the recording period, revealed to be steadily and significantly lower in lactating rat dams (3.0 ± 1.3%) comparatively to control females (11.6 ± 3.8%) ($F(1,12) = 32.770$, $p<0.05$). The number of REM-sleep episodes presented by LRD ranged from 3 to 17, showing relatively higher values on the last days of lactation (mean 12.6 episodes) compared to the initial days (mean 6.6 episodes). Control female rats displayed about 20 episodes each during the similar 7 hours of recordings. The mean number of 1.2 ± 0.5 episodes of REM-sleep/hour was significantly lower in LRD than that observed in control females (3.1 ± 1.5) ($F= 9.434$, $p<0.05$). All longer episodes of REM-sleep observed in lactating dams were manifested outside the nest. In such case, they laid down alone and stretched to full length on the wooden shavings, distant from the pups.

Table 1. Parameters of diurnal sleep-wakefulness cycles in lactating rat dams and in control animals. Values are expressed as mean percentages (+ s.d.) of the total recording time (from 10:00h to 17:00h). W = wakefulness; SS = synchronized sleep; REM-sleep = Rapid Eye Movements sleep; nc = non computed

Parameter	Lactating mothers (n=9)	Cycling females (n=9)	Males (n=4)
I – Amount of states (%)			
a) Waking	55.1 ± 4.2[A]	43.4 ± 2.6	44.3 ± 7.6
b) SS	41.9 ± 4.0	46.6 ± 2.1	46.1 ± 7.6
c) REM-sleep	3.0 ± 0.4*	10.0 ± 1.0	9.4 ± 0.6
II - Mean number of episodes per hour:			
a) Waking longer than 30 min	1.4 ± 0.3*	0.8 ± 0.3	nc
b) REM-sleep	1.2 ± 0.5*	3.1 ± 1.5	nc

*** Significant difference (ANOVA plus Tukey's test, p<0.05);;**

[A] Significant at the limit (p=0.0547)

DISCUSSION

The results obtained in the present study seem to disclose two important and interrelated changes in the sleep-wakefulness cycles of LRD. First, they displayed more awakening episodes longer than 30 min than their controls; second, they manifested lower amounts of REM-sleep.

The increase in the number of longer awakening episodes observed in LRD is similar to the ones observed in human mothers during the first month of lactation, as revealed by actigraphic and sleep-log monitoring techniques (12) or in the first two postpartum weeks as determined by EEG monitoring (6). The increase in wakefulness episodes showed by LRD does not seem to be a secondary consequence of the overcrowded condition inside the nest. Overcrowding was also reported to induce an increase in the number of wakefulness episodes (13); however, in such case, animals resume sleep. Awakening episodes in LRD are frequently devoted to several kinds of maternal care, and they may last more than 30 minutes, as observed in the present study. As constant maternal care is essential for the newborns, it may be thought the increase in awakening episodes as being an important adaptation for the lactation period.

Preservation of SS amounts in LRD seems to be also a significant adaptation for the nursing period. Milk-ejection reflex depends on previous bout of SS, being sleep probably induced by a rise in prolactin release (2,3,14). If one considers that increased amounts of wakefulness and preservation of SS are important adaptive manifestations during lactation, the reduced amount of REM-sleep in LRD, observed in the present study, may be taken as a mere remainder time of the recording period. This, however, does not seem to be the case. Neither the case to attribute such fact to the impact of delivery and concomitant psychological changes as stated by Karacan and colleagues (6) for the marked suppression of REM-sleep that occurs in the first postpartum day in humans. This REM-sleep change in humans is an acute suppression, therefore, not similar to that observed in LRD in which the state of sleep is manifested chronically and in reduced amounts during the lactation period.

Sleep in normal male adult rats is manifested mainly during the diurnal phase of the circadian cycle. In females the circadian distribution also exists, with a small variation in the REM-sleep amount associated to a change in the estrous cycle phase (15-17). A regular daily amount of REM-sleep is necessary, and a need to compensate for its loss develops after a total or partial deprivation period (18). Maternal care is required by rat pups during the entire circadian cycle and this seems to impose a rearrangement on the mother's sleep-wakefulness architecture during the lactation period. Such reorganization explains and allows considering all results obtained in the present study as parts of the same adaptive mechanism. The need for a daily amount of REM-sleep seems to shift part of its diurnal manifestation to the nocturnal period, allowing the fulfillment of the need to spent more time awake and to preserve diurnal amounts of SS. In addition, SS needed for the milk-ejection reflex seems granted at night, since the lack of REM-sleep induces somnolence and promotes also an increase in SS amount during compensation. This parallel increase in SS amounts for REM-sleep compensation is a well-known fact (19). Although attractive, our few trials to detect signs of REM-sleep compensatory rebound in LRD were considered negative since no significant changes were observed when they were allowed to sleep alone after pups' removal.

REM-sleep episodes manifested by LRD outside the nest seems an important topic among the behavioral data obtained in the present study. REM-sleep manifestation in stretched posture has a temperature-dissipating role (11) and such behavior in LRD suggests an important involvement of thermoregulatory mechanisms in their sleep organization. Body temperature in LRD is relatively higher (20,21) and this is important for pups' development (22). Prolonged maintenance of elevated back-posture on the nest overcrowded by pups seems the cause of overheating. Such overheating is possible since thermoregulatory capacity in LRD is lower (23). The elevated basal temperature of LRD seems to be determined by the higher immune activity occurring in the LRD organism. Many agents of the immune system induce hyperthermia and promote SS, and may reduce REM-sleep (24-26). Sleep reduction may contribute also for the hyperthermia since its deprivation promotes an increase in body temperature (27-29). These properties of immune agents suggest their possible involvement in the sleep architecture reorganization observed in LRD.

It is important to note that our more recent studies indicate that the strain of Wistar rats used in the present study has some particular characteristics (30), like increased level of anxiety (31) and is more susceptible to anxiety disorder-like manifestations when submitted to REM-sleep deprivation (32,33). Increased anxiety during the lactation period may also explain, at least partially, the results reported in the present paper. In this case, the significant increase in the number of longer episodes of awakenings observed in LRD may be interpreted as being the result of sleeplessness induced by an increased anxiety level. Postpartum insomnia seems to correlate to the emergence of psychiatric disorders (6) and such considerations makes the LRD, forgotten in sleep research, a fascinating subject for investigation.

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