Archives Italiennes de Biologie, 155: 99-109, 2017. DOI 10.12871/00039829201731

# A descriptive analysis of sleep and wakefulness states during maternal behaviors in postpartum rats

L. BENEDETTO[1], M. RIVAS[1], M. PEREIRA[3], A. FERREIRA[2], P. TORTEROLO[1]

[1] Departamento de Fisiología, Facultad de Medicina, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay;
[2] Sección de Fisiología y Nutrición, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay;
[3] Department of Psychological and Brain Sciences, University of Massachusetts, Amherst, US.

ABSTRACT

*Mother rats spend most of their time nursing their litter during the early stages of the postpartum period, only occasionally leaving the nest. The suckling stimulus from the pups elicits the adoption of nursing postures, during which milk ejection occurs, an event associated with the occurrence of non-REM (NREM) sleep in the rat. Despite this evidence, the characteristics of sleep during different nursing postures along the postpartum period remain unknown. The present study aims to describe the sleep pattern of mother rats while nursing, hovering over their pups and when being away from the pups. For this purpose, lactating females were implanted with electrodes for chronic polysomnographic recording. Simultaneous recordings of sleep-wakefulness cycle and maternal behaviors were performed in both the light and dark phases of the first and second postpartum weeks. Results indicate that while mothers were most of the time awake when hovering over pups and when staying away from pups, they mainly remained in NREM sleep when adopting low kyphosis posture, the most common nursing posture. The sleep-wake pattern during most maternal behaviors was quite stable between the light and dark phases of the first and second postpartum weeks. In addition, the sleep fragmentation was higher during the nursing bouts compared to that observed when mother rats slept without the pups, but sleep depth did not differ between these behaviors. Our results provide an original description of how mother rats synchronize their own sleep-wakefulness cycle with the maternal care of the pups during the postpartum period.*

***Key words***

*Lactation • Polysomnography • Nursing • NREM • REM • Sleep fragmentation*

## Introduction

During the early stages of the postpartum period, mother rats spend most of their time in contact with the litter, occasionally leaving the nest (Grota and Ader, 1969). Throughout this time, rats display maternal behavior, characterized by both active and quiescent nursing behaviors (Stern et al., 2002). In mother rats, upright crouching is the most typical nursing posture, also known as kyphosis (Stern and Johnson, 1990). This posture is elicited by the stimulus of suckling from the pups (Neville, 2006; Wakerley, 1996). Besides, while suckling is also necessary for milk ejection, it is not sufficient to elicit it in the rat. Instead, this stimulus has to be preceded by a slow wave sleep episode (Lincoln et al., 1980; Sutherland et al., 1987; Voloschin and Tramezzani, 1979). In addition, maternal behavior has an inverse circadian rhythm in relation to the motor activity of the lactating rat (Grota and Ader, 1969), suggesting that the pups caring behavior may run in parallel with the sleep circadian rhythm.

*Corresponding author:* Luciana Benedetto, Facultad de Medicina, Universidad de la República, General Flores 2125, 11800 Montevideo, Uruguay - Tel: +598 2924 3414 / +598 2924 3232 - Email: lbenedet@fmed.edu.uy

Although sleep characteristics along the postpartum period (Rocha and Hoshino, 2009; Sivadas et al., 2016) and the link between sleep and milk ejection in the rat has been established, and there are investigations of how sleep restriction modulates maternal behavior (Pires et al., 2010; Pires et al., 2015), there are no descriptions of sleep and wakefulness (W) states during the different nursing behaviors. Also, sleep deprivation and sleep fragmentation during the early stages of the postpartum period are common features of human mothers and also in mother rats (Sivadas et al., 2016; Stremler and Wolfson, 2011). However, there is no description of any of these aspects during maternal behaviors.

Based on this evidence, we hypothesized that mother rats spend most of their nursing time sleeping and that they are mostly awake when they hover over pups or when they are away from pups. Also, the characteristics of sleep may vary during the light-dark cycle or along postpartum period. In addition, we expect that sleep is more fragmented and superficial while nursing than when they are without the pups.

In the present study we aimed to describe how mother rats sleep during different nursing postures, when the mothers hover over pups and when remain away from pups both in the light and dark phases of the first (1stWK) and second (2ndWK) postpartum week. We also aimed to describe the sleep depth and fragmentation when mothers sleep during nursing and when they are away from the pups.

## Methods

### Animals and housing

Six primiparous Wistar female rats (275-315 g) and their pups were included in this study. All animal use and experimental procedures were in strict accordance with the "Guide to the care and use of laboratory animals" (8th edition, National Academy Press, Washington D.C., 2011) and approved by the Institutional Animal Care Committee. All efforts were made to minimize the number of animals used and their suffering.

Two days before giving birth, pregnant females were housed individually in transparent cages (40 x 30 x 20 cm) containing shredded paper towels as nest-building material located in a temperature-controlled room (22 ± 1°C), in a sound-proof recording chamber fitted with slip rings and cable connectors for bioelectrical recordings, under a 12-h light/dark cycle (lights on at 02:30 am), with *ad libitum* access to food and water. On postpartum day 1 (PPD1, birth=day 0), litters were culled to four female and four male pups per mother.

### Stereotaxic surgery

On the morning of PPD1, female rats were implanted under a mixture of ketamine/ xylazine/ acepromazine maleate anesthesia (80/2.8/2.0 mg/kg, i.p.) with cortical electroencephalogram (EEG) electrodes and dorsal neck muscle electromyogram (EMG) electrodes for the assessment of sleep and W states. Recording electrodes for EEG were placed in the frontal cortex (AP=+5.8; ML=1.5), posterior parietal cortex (AP=-4.0, ML=2.2), primary visual cortex (AP=-7.0, ML=3.0), and over the cerebellum as a reference electrode (AP=-11.0, ML=0.0) (Paxinos and Watson, 2005). All electrodes were soldered to a six-pin connector. The connector was cemented to the skull using dental acrylic.

During surgery, that lasted approximately one hour, pups were maintained in their home cage under a heat lamp. Immediately after surgery, each mother was reunited with her pups in the home cage located in the recording chamber until the end of the experiments. Surgical recovery lasted approximately one to two hours. All females remained healthy throughout the experiments, exhibiting typical maternal behaviors, and their pups gained weight and developed normally.

### Experimental design

On the morning of PPD5 and 60 minutes before the beginning of the experiment, female rats were connected to the recording system in its own maternal cage until the end of the session day. Each animal was recorded during four days: two in the 1stWK (PPD5-6) and two in the 2ndWK (PPD14-15). During each experimental day, a 120 minute-recording was carried out on the light phase (LPh, 10:00-12:00 am) and another on the dark phase (DPh, 16:00-18:00, under red light) such that data from the two consecutive days were averaged as a single value (per animal) for the light and dark phases separately. Thus, four dependent conditions were analyzed: 1) LPh on the 1stWK (LPh/1stWK), 2) DPh on the 1stWK (DPh/1stWK), 3) LPh on the 2ndWK (LPh/2ndWK) and 4) DPh on the 2ndWK (DPh/2ndWK).

Throughout each experimental session maternal behavior and sleep were recorded simultaneously as shown in Figures 1-2.

### *Maternal behavior*

Using a video tape recording attached to the Spike 2 software (CED, Cambridge, UK), the following behaviors were determined and staged in 5-second epochs: hovering over the pups (dam over the pups while actively engaged in other activity, Figure 1A and 2B), being away from the pups (without contact with pups, Figure 2A) and the following nursing postures: low kyphosis (LKP, the back is flat or slightly arched), high kyphosis (HKP, pronounced dorsal arch and raised ventrum, with limbs extended rigidly) and supine (the dam lies on her side while pups attached to her nipples) (Figures 1B-C and 2C) (Stern and Johnson, 1990). Also, the total time in contact with the pups (hovering over, lying in contact plus nursing) was recorded. Although the time spent lying in contact with pups was annotated, this behavior was displayed by very few animals and for short periods (approximately one minute, only during the 2ndWK) and therefore was excluded from analysis but included in the total time in contact with pups.

### *Sleep*

Bioelectric signals were amplified (x1000), filtered (0.1-200 Hz), sampled (512 Hz, 16 bits) and stored in a PC for further analysis using Spike 2 software. The states of sleep and W were determined manually, by visual inspection of the recordings, in 5-second epochs as: W (low voltage fast waves in frontal cortex, a mixed theta rhythm (4-7 Hz) in parietal cortex and relatively high electromyographic activity); light sleep (LS, high voltage slow cortical waves interrupted by low voltage fast electroencephalographic activity); slow wave sleep (SWS, continuous high amplitude slow frontal waves combined with sleep spindles [11-16 Hz] and a reduced EMG) and REM sleep (low voltage and fast frontal waves, a regular theta rhythm in the parietal cortex, and a silent EMG except for occasional myoclonic twitching) (Benedetto et al., 2013; Lagos et al., 2009).

Total time spent in W, LS, SWS, NREM sleep (LS+SWS) and REM sleep as well as the percentage of time spent in W, LS, SWS, NREM and REM sleep over the total time spent in each maternal behavior during the two-hour-session was analyzed. In addition, the percentage of time in LS and SWS respect to the total NREM sleep time spent while the mother was nursing (kyphosis posture) and away from the pups was analyzed.

### Sleep fragmentation

Sleep fragmentation was analyzed by means of brief awakenings during nursing or when the mother was away from the pups. Brief awakenings were determined as W episodes of $\leq$ 5 seconds during NREM sleep and expressed as number of events in 60 minutes as follow: n° awakenings X 60 minutes / total minutes in NREM sleep during nursing or away from the pups.

### Sleep bout duration

The duration of NREM sleep bouts was analyzed by means of the average length of all NREM sleep episodes during nursing or when the mother was away from the pups.

### Sleep depth

Sleep depth was assessed both by the analysis of the proportion of time spent in LS and SWS respect to the total NREM sleep and by the spectral analysis of the EEG. For the latter, a quantitative analysis of the EEG was performed during the light phase, were rats spend more time sleeping. The power spectrum of the frontal artifact-free EEG traces was analyzed by means of Fast Fourier Transformation (FFT) using the Script COHER_WHOLE of the Spike 2 software, during SWS when the mother rat was nursing in LKP in the 1stWK and 2ndWK, and away from the pups in the 2ndWK in five of the six animals (one mother did not sleep away from the pups). Quantitative analysis of the EEG during 1stWK when the mother was away from the pups was not analyzed due to the lack of sufficient animals sleeping in SWS away from the pups.

The absolute values of power (1 to 40 Hz) were taken from 15 to 45 consecutive epochs obtained from three different episodes. The number of epochs chosen for each animal was limited by the time the mother spent in NREM sleep away from the pups.

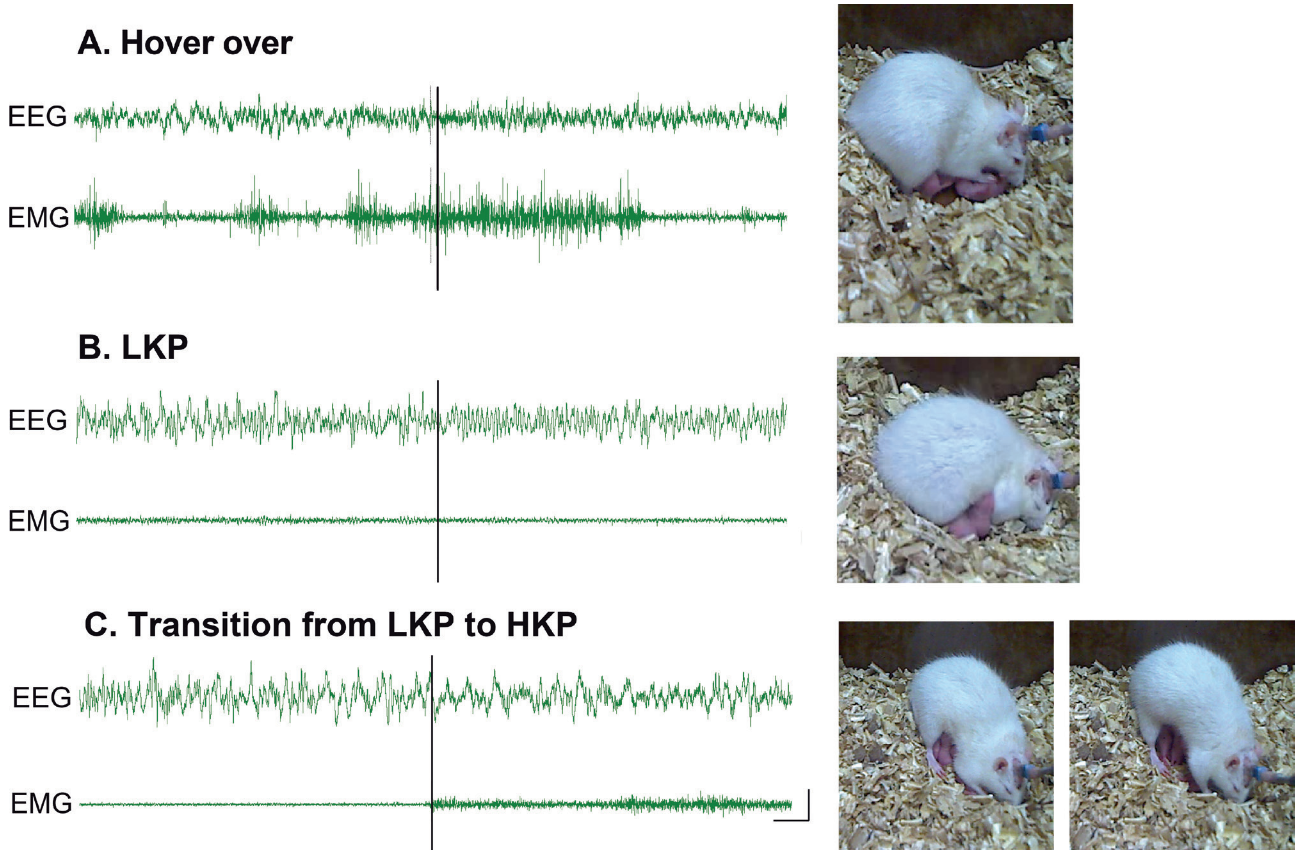


Fig. 1. - Mother rat during the first postpartum week. The electroencephalogram (EEG) and the electromyogram (EMG) of a mother rat in hover over while licking a pup (A), nursing in low kyphosis posture (LKP, B) and in the transition from LKP to high kyphosis (HKP, C) are shown. The vertical lines indicate the moment when the photos were taken. A. Note that while licking a pup, the mother is awake, with muscular activation and low amplitude in the EEG. B. At the beginning of LKP it can be seen the mother rat in slow wave sleep and gradually enters to REM sleep, with muscle atonia and theta activity. C. The second photo was taken in the exact moment of the transition from LKP to HKP. Note that in the transition from LKP to HKP there is muscle activation with a slight reduction in amplitude in the EEG. Horizontal scale bar: 1 sec.; vertical scale bar: 0.2 mV.

### *Statistics*

All values are presented as mean ± standard error (S.E.M). As data follow a normal distribution (Kolmogorov-Smirnov test, $p > 0.05$), parametric tests were utilized.

Maternal behaviors performed for less than five minutes in 120 minutes are shown but excluded from the sleep and W analysis.

We employed a one way repeated measures ANOVA followed by Tukey test to compare the total time among maternal behaviors in each condition.

In addition, the analysis of the total time and the percentages of time in W, NREM and REM sleep within each maternal behavior between conditions (LPh/1stWK vs. DPh/1stWK, LPh/2ndWK vs. DPH/2ndWK; LPh/1stWK vs. LPh/2ndWK and DPh/1stWK vs. DPh/2ndWK) were performed using the Student t test for dependent samples.

The comparisons of briefs awakenings and sleep bout durations while nursing (kyphosis posture [LKP+HKP]) and when the mother was away from the pups were performed by means of the Student t test for dependent samples, both within each condition and between conditions.

The total power frequency in bands during SWS while the mothers was nursing and away from the pups between first and second postpartum week (light phase only) was compared by the Student t test for dependent samples.

## Results

### *Maternal behavior*

The total time spent in the different maternal behaviors at each condition is shown in Table 1A.

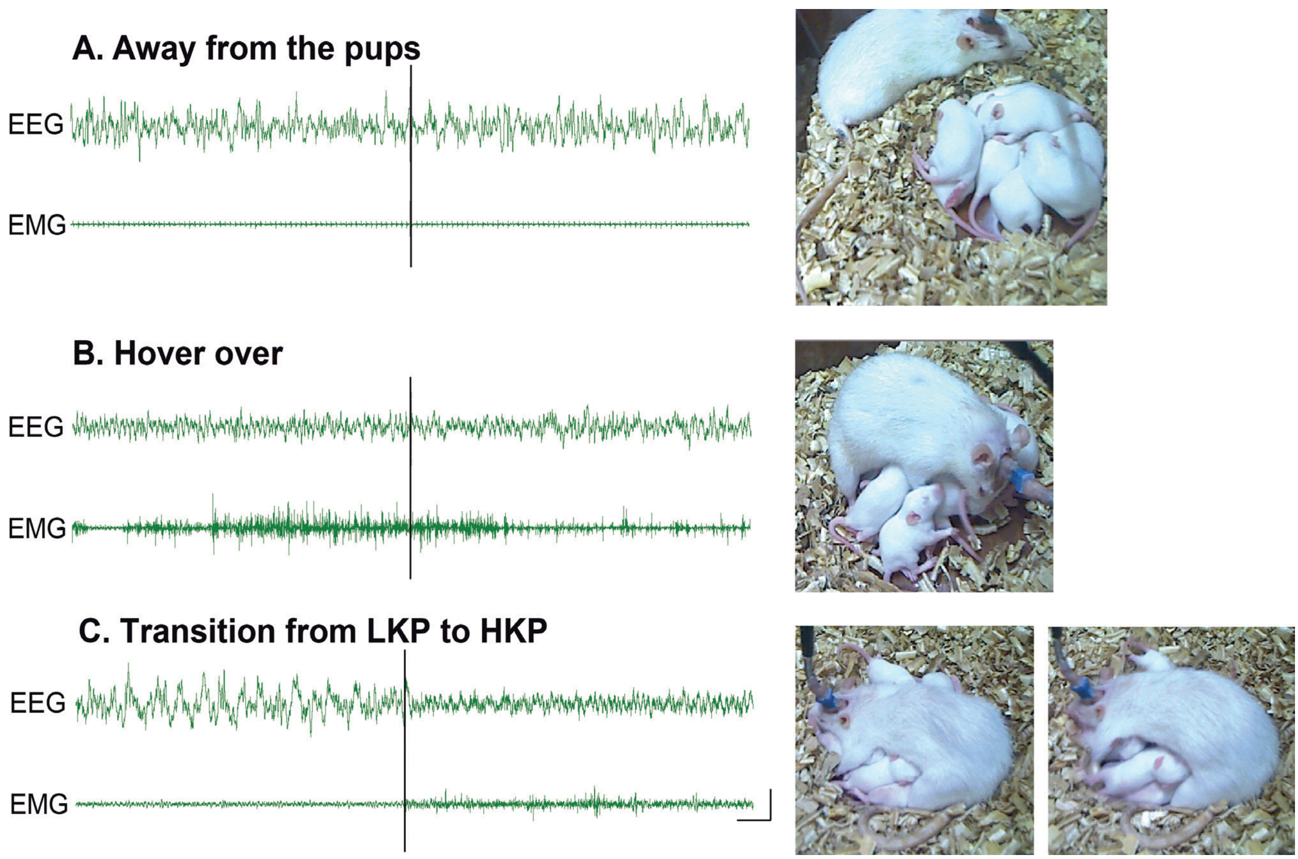


Fig. 2. - Mother rat during the second postpartum week. The electroencephalogram (EEG) and the electromyogram (EMG) of a mother rat sleeping away from the pups (A), in hover over while licking (anogenital) a pup (B) and during the transition from low kyphosis (LKP) to (HKP) are shown. The vertical lines indicate the moment when the photos were taken. A. The mother rat is sleeping in light sleep away from the pups, with moderate amplitude in the EEG and reduced activity in the EMG. B. Note that while licking a pup, the mother is awake with muscular activation and low amplitude in the EEG. C. The second photo was taken in the exact moment of the transition from LKP to HKP. Note that in the transition from LKP to HKP there also a transition from sleep to wakefulness. Horizontal scale bar: 1 sec.; vertical scale bar: 0.2 mV.

Comparisons among conditions within each maternal behavior show that the time that lactating females spent in contact with their litter during the LPh/1stWK tended to be higher compared to DPh/1stWK (t=2.12, p=0.087) and was significantly higher compared to LPh/2ndWK (t=3.39, p=0.019). Mothers remain similar amount of time away from the pups during all conditions but during LPh/1stWK that tended to be lower compared to DPh/1stWK (t=2.12, p=0.087) and significantly lower compared to LPh/2ndWK (t=3.39, p=0.019). Respect to the time the mothers remained in LKP, this time was higher during LPh/1stWK compared to DPh/1stWK (t=4.21; p=0.014) and tended to be higher compared to LPh/2ndWK (t=2.70; p=0.054).

When comparing the time spent in each maternal behavior among them, significant differences were found in all conditions: LPh/1stWK ($F_{(4,\ 20)}$=53, p=0.00000), DPh/1stWK ($F_{(4,\ 20)}$=4.29, p=0.012), LPh/2ndWK ($F_{(4,\ 20)}$=14.81; p=0.00001) and during DPh/2ndWK ($F_{(4,\ 20)}$=3.46; p=0.026). The most significant observation during LPh/1stWK is that LKP was the main maternal behavior (all p=0.0001). Specific significant differences between maternal behaviors are indicated in Table 1A.

### *Total sleep and wake time*

The time spent in W and sleep is shown in Table 1B. As expected, we found significant differences across dark-light phases, mainly during 1stWK. The time spent in NREM sleep was higher during the LPh/1stWK in comparison to that spent during the DPh/1stWK (t=3.84, p=0.012; Table 1B). Specifically, both LS (t=2.44, p=0.058) and SWS (t=2.18, p=0.081)

Tab. 1. Total time spent in the different maternal behaviors (A) and in sleep and wakefulness (B) during the 120 minutes recording time.

| Total time (mins.) | LPh/1stWK | DPh/1stWK | LPh/2ndWK | DPh/2ndWK |
|---|---|---|---|---|
| *A. Maternal behavior* | | | | |
| Away from the pups | 15.9 ± 5.5* | 48.7 ± 13.7[d,e] | 52.8 ± 7.8[b,d,e] | 47.0 ± 12.0[d] |
| Hover over | 20.8 ± 3.1 | 28.8 ± 8.9 | 14.0 ± 2.9 | 27.1 ± 11.7 |
| Low kyphosis | 74.8 ± 4.4[#a,b,d,e] | 35.7 ± 10.1 | 44.2 ± 8.4 [b,d,e] | 30.5 ± 6.4 |
| High kyphosis | 8.5 ± 2.5 | 4.3 ± 2.0 | 4.7 ± 1.1 | 3.8 ± 1.2 |
| Supine posture | 0.0 ± 0.0 | 2.5 ± 1.6 | 3.2 ± 1.6 | 10.3 ± 4.1 |
| Lie in contact | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.1 ± 0.7 | 1.3 ± 0.9 |
| Total time with pups | 104.1 ± 5.5* | 71.3 ± 13.7 | 67.2 ± 7.8 | 73.0 ± 12.0 |
| *B. Sleep and wakefulness* | | | | |
| W | 48.6 ± 2.4 | 74.5 ± 10.6 | 56.1 ± 8.7 | 74.7 ± 10.1 |
| LS | 24.9 ± 5.6 | 16.3 ± 3.2 | 22.1 ± 4.7 | 14.1 ± 2.6 |
| SWS | 40.4 ± 5.9 | 19.5 ± 5.2 | 34.8 ± 7.8 | 26.2 ± 6.9 |
| NREM | 65.3 ± 1.5[#] | 35.8 ± 7.1 | 56.9 ± 6.9 | 40.3 ± 8.7 |
| REM | 6.1 ± 1.7 | 9.7 ± 4.3 | 7.0 ± 2.3 | 5.0 ± 2.1 |

Data is presented as mean ± standard error. Numeral (#) symbol represents significant differences compared to dark phase of the first postpartum week (DPh/1stWK); asterisks (*) represents significant differences compared to light phase of the second postpartum week (LPh/2ndWK; dependent t-test, p < 0.05). Significant differences among the different maternal behaviors are indicated with superscript letters (significant differences compared to away from pups [a], hover over [b], low kyphosis [c], high kyphosis [d] or supine posture [e]; Tuckey test, p < 0.05). W, wakefulness; LS, light sleep; SWS, slow wave sleep; NREM, no-REM sleep (LS+SWS); REM, REM sleep.

tended to be higher during LPh/1stWK compared to DPh/1stWK. The time spent in W tended to be higher during the DPh/1stWK compared to LPh/1stWK (t=2.28, 0.071). During LPh/2ndWK, NREM sleep tended to be higher compared to DPh/2ndWK (t=2.32, p=0.068).

We did not find significant differences in the total time spent in W, NREM or REM sleep according to the postpartum week (all p=n/s, see Table 1B).

### *Sleep and wakefulness within each maternal behavior*

Figure 3 shows the percentages of W and sleep states in each maternal posture across conditions. In this regards, it is important to consider the time spent in each maternal posture detailed in Table 1A.

#### Away from the pups

An example of a mother rat during LPh/2ndWK is shown in Figure 2A. Sleep and W percentages while mother rats stayed away from the pups were similar during all conditions but during the LPh/2ndWK.

Lactating females were mostly awake (80-90% of time) while they were away from the pups in all conditions but during the LPh/2ndWK, when the W time was approximately 50%. In this latter condition, the proportion of time in W was lower in comparison with that of DPh/2ndWK (t=3.27, p=0.022) and LPh/1stWK (t=3.27, p=0.022; see Figure 3A). However, is worth noting that as the total time away from the pups during LPh/1stWK is three times less compared to the other conditions (see Table 1A), the total time in W in this behavior during the LPh/1stWK is approximately 15 minutes compared to the approximately 30 minutes of W during LPh/2ndWK.

The percentage of time the mother rats spent in NREM sleep while they were away from the pups at LPh/2ndWK was higher than during the DPh/2ndWK (t=4.37, p=0.007) and the LPh/1stWK (t=3.58, p=0.016; see Figure 3A).

#### Hover over

Figure 1A and 2B show two examples of awaked mothers in hover over during the 1stWK and 2ndWK respectively. Accordingly, this female exhibits high muscular activation, as well as low amplitude and high frequency in the EEG while licking a pup.

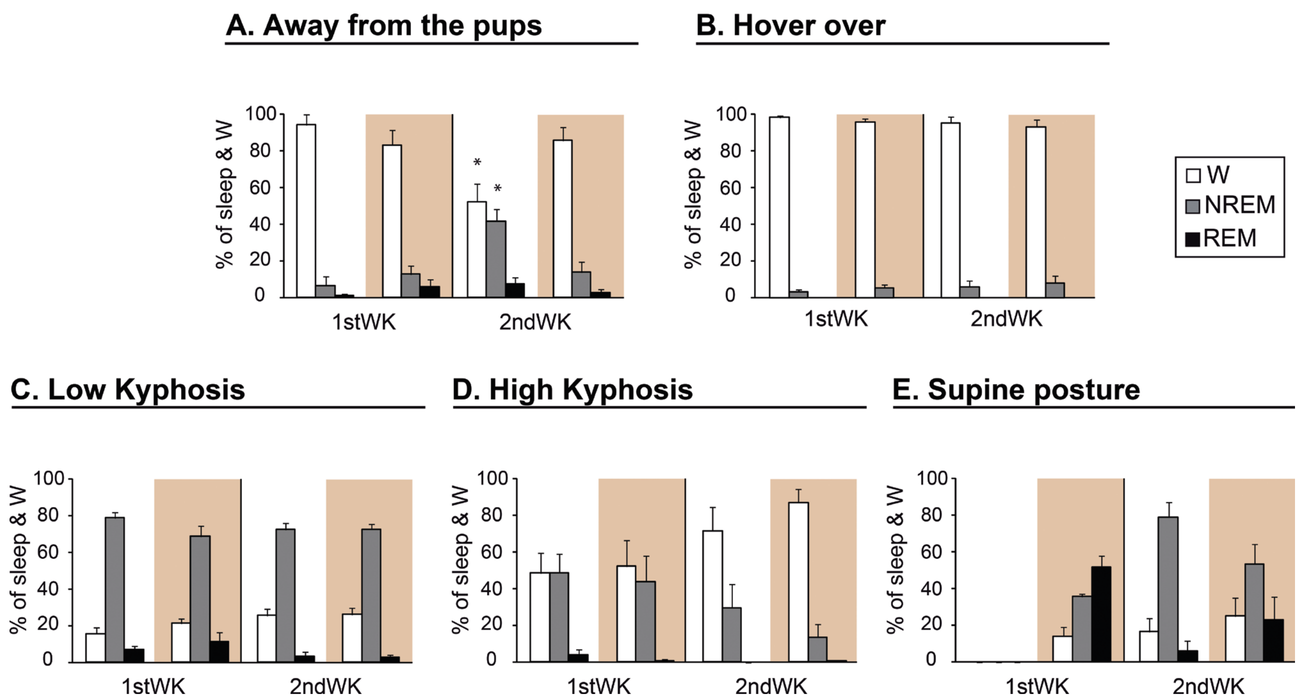


Fig. 3. - Graphic charts showing the percentage of time spent in wakefulness (W), NREM and REM sleep at the different maternal behaviors. Comparisons of the percentage of time of sleep and W within each maternal behavior according to the postpartum week in the same light/dark phase or according to the light/dark period within the same postpartum week were made using a dependent t-test. White backgrounds correspond to light phases and pink backgrounds to dark phases. Asterisks (*) represent significant differences compared to dark phase of the second postpartum week (DPh/2ndWK) and to the light phase of the first postpartum week (LPh/1stWK; all $p < 0.05$).

As expected from its definition, mother rats were mostly awake (more than 90% in all conditions) as they hover over their pups while engaged in other activities (see Figure 3B). During this behavior, mothers had briefs NREM sleeping episodes (≈ 10%) and no REM sleep.

### Low kyphosis

Figure 1B shows a transition from NREM to REM sleep in LKP during the 1stWK. During this nursing posture, lactating female rats spent most of the time sleeping, predominantly in NREM sleep (approximately 70% in all conditions, see Figure 3C). The percentages of time spent in W, NREM and REM sleep during LKP was stable along the two postpartum weeks and the light and dark phases (Figure 3C).

### High kyphosis

Figure 1C and 2C depicted the transition from LKP to HKP during the 1stWK and 2ndWK correspondingly. While HKP was performed by all lactating females in all conditions, the time the mother rat spent in this posture was low (under five minutes, see Table 1A) except for the LPh/1stWK. Thus, we will focus only in this latter condition.

Mother rats during LPh/1stWK spent almost one half of the time of HKP in W and the other half in NREM sleep, with little time in REM sleep (Figure 3D). Although not analyzed, it was observed that at the transition from LKP to HKP, lactating females invariably woke up if they were asleep (see Figures 1C-2C). It is interesting to note that this waking period was either a somnolent W (with some slow waves in the EEG) accompanied by active EMG as depicted in Figure 1C, or active W (Figure 2C). After this arousal, mother rats gradually fell asleep if they return to LKP. As a result, most W episodes correspond to the beginning of HKP and NREM sleep to the culmination of this behavior.

### Supine posture

The supine posture was not performed during LPh/1stWK, was observed in three rats during DPh/1stWK and four during LPh/2ndWK and DPh/2ndWK. The total time in this posture was scarce except during the DPh/2ndWK (see Table 1A). Thus, the latter was the most suitable period to describe this posture. During this posture mother rats principally slept. In particular, mother rats remained half of the total time in NREM sleep. Curiously they exhibited a relatively high percentage of REM sleep (Figure 3E).

### *Sleep fragmentation*

When briefs awakenings during NREM sleep were studied, it was found that sleep fragmentation was higher during kyphosis postures (LKP+HKP) than when the mothers were away from the pups during the LPh/1stWK (t=3.77, p=0.013), DPh/1stWK (t=3.62, p=0.015), LPh/2ndWK (t=2.87, p=0.035) and DPh/2ndWK (t=4.56, p=0.006; see Table 2). In addition, sleep fragmentation while nursing was higher during LPh/2ndWK compared to LPh/1stWK (t=3.82, p=0.012). Moreover, sleep fragmentation while the mother rats were away from the pups did not vary significantly along the postpartum period and across light-dark cycle (Table 2).

### *Sleep bout duration*

NREM sleep bout duration while nursing during LPh/1stWK was significantly longer compared to while the mothers slept away from the pups in the same condition (t=3.04, p=0.028; see Table 2) and had a tendency to be longer compared to nursing during LPh/2ndWK (t=2.31, p=0.068). Also, it was found a tendency to had longer sleep bouts while sleeping away from the pups during LPh/2ndWK compared to the same behavior during LPh/1stWK (t=2.19, p=0.079) and while nursing during LPh/2ndWK (t=2.03, p=0.097).

### *Sleep depth*

The percentage of time spent in LS or SWS respect to the total NREM sleep did not differ between nursing (kyphosis postures) and when the mothers were away from the pups. There was also no significant variation according to the condition within each maternal behavior (see Table 3). The EEG power spectrum in SWS during nursing and when the mother rats were away from the pups are shown in Figure 4. No significant differences were found in any frequency during nursing compared to when the rats were away from the pups or according to the postpartum week in each behavior.

## Discussion

In the present study we provided a detail description of sleep characteristics in relation to huddling and nursing behaviors. We show that sleep and wake percentages for most maternal behaviors were quite constant between light and dark phases and between the first and second week of the postpartum period. Particularly, while mothers were most of the time awake when hovering over pups and when they were away from pups, they mainly remained in NREM sleep in low kyphosis, the most common nursing posture. Results also demonstrate that mother rats had more sleep fragmentation during nursing compared to when they slept without the pups, but sleep depth did not differ between both behaviors.

As previously described, during the 1stWK lactating females spent most of the time with their litter and this time decreased with the progression of lactation (Grota and Ader, 1969). The present results further show that this high amount of time with their litter during 1stWK relied on an elevated amount of time during LPh, while during the DPh/1stWK was similar to both phases of the 2ndWK.

Total sleep and W times were quite stable across the postpartum period in the two-hour-recording. In this sense, Voloschin and Tramezzani (1979) reported that sleep and W percentages over a 24-hour-period of lactating female rats at PPD15-17 did not differ from that of non-maternal females during LPh or DPh. We also show that total times of W, NREM and REM sleep during the light-dark cycle on the 1stWK did not differ from those in the 2ndWK. In addition, these parameters during DPh were similar to that recorded in males in a previous study of our group (Benedetto et al., 2013). Together, we could consider that total time in sleep and W of lactating females in the first two postpartum weeks may not differ from that of non-maternal rats. However, we recorded only two hours during each phase, and Rocha and Hoshino (2009) and recently Sivadas et al. (2016) have shown some differences in total sleep time in the postpartum compared to pre-mating values (Sivadas et al., 2016).

While the sleep-wake pattern was quite constant between light and dark phases and between the first and second week of the postpartum period for most maternal behaviors, some differences emerged when the mothers were away from the pups during the LPh/2ndWK, when the percentage of NREM sleep

Tab. 2. - Sleep fragmentation during NREM sleep and NREM sleep bout duration.

| | LPh/1stWK | DPh/1stWK | LPh/2ndWK | DPh/2ndWK |
|---|---|---|---|---|
| Brief awakenings (episodes/hour of NREM) | | | | |
| Nursing | 15.4 ± 2.2* | 15.6 ± 3.5* | 20.4 ± 1.9*# | 18.2 ± 3.5* |
| Away from the pups | 2.1 ± 1.3 | 2.1 ± 0.7 | 7.5 ± 2.0 | 3.0 ± 1.2 |
| NREM bout duration (mins) | | | | |
| Nursing | 1.00 ± 0.12* | 0.66 ± 0.06 | 0.68 ± 0.09 | 0.76 ± 0.13 |
| Away from the pups | 0.32 ± 0.12 | 0.53 ± 0.19 | 0.98 ± 0.17 | 0.81 ± 0.16 |

Mean ± standard error. Asterisks (*) represent significant differences compared to away from pups in the same condition. Numeral (#) indicates significant difference compared to nursing during LPh/1stWK. The data was analyzed by means of a dependent t-test; the level of significance is $p < 0.05$.

Tab. 3. - Mean percentage ± standard error of the percentage of time spent in light sleep (LS) and slow wave sleep (SWS) respect total time of NREM sleep during nursing and when the mothers were away from the pups.

| Behavior | Light phase | | Dark phase | |
|---|---|---|---|---|
| First postpartum week | | | | |
| | LS (%) | SWS (%) | LS (%) | SWS (%) |
| Away from the pups | 25.1± 20.3 | 74.9 ± 20.3 | 24.1 ± 13.8 | 75.9 ± 13.8 |
| Nursing | 38.0 ± 8.7 | 62.0 ± 8.7 | 42.5 ± 6.1 | 57.5 ± 6.1 |
| Second postpartum week | | | | |
| Away from the pups | 43.7± 10.4 | 56.3 ± 10.4 | 48.3 ± 10.9 | 51.7 ± 10.9 |
| Nursing | 43.5 ± 10.8 | 56.5 ± 10.8 | 40.6 ± 7.7 | 59.4 ± 7.7 |

Differences between groups were evaluated using dependent t-test; no significant differences were found (all $p > 0.05$).

was about 40% (compared to less than 20% in all other conditions). In addition, we found a tendency to decreased the time spent in LKP from LPh/stWK to LPh/2ndWK (approximately 40% less), a nursing behavior with elevate percentage of NREM sleep. These two findings may compensate each other, resulting in that the total sleep time did not vary between first and second week.

Interestingly, the sleep and wake percentages observed when mothers were away from pups, hovering over or nursing pups exhibited particular characteristics. Mother rats spent above 80% of the time in W when hovering over pups and when they are away from pups for most conditions, with no REM sleep during hover over.

Regarding sleep and W during nursing postures, mother rats remained over 65% in NREM sleep during LKP, the most common nursing posture. During the condition where mother rats express more time in HKP (LPh/1stWK), they spent similar amount of time in sleep and W, while in supine posture (during DPh/2ndWK, the condition where it was exhibit for 10 minutes,) they spent half of the time in NREM sleep and a particular high amount of time in REM sleep (approximately 20%). In this regard, Lincoln et al. (1980) mentioned that lactating females appear to be somnolent when nursing. However, no measures or distinction among nursing postures were done before. Interestingly, the fact that all nursing postures were characterized by high proportion of NREM sleep would be a benefit, presumably allowing milk ejection occurrence. In this line, it has been proposed that NREM sleep is necessary for milk ejection to occur (Lincoln et al., 1980; Sutherland et al., 1987; Voloschin and Tramezzani, 1979). Additionally, in spite we chose two time periods where total sleep time differ (Ray et al., 2004), sleep pattern during most maternal behaviors were quite stable along the light and dark phases in both postpartum weeks. Thus, it is probable that sleep pattern during each maternal behavior should be maintained along the day. However, we cannot discard some differences if the analysis were performed in other periods of the light-dark cycle.

Moreover, the supine posture was characterized by a quite high proportion of REM sleep. This posture

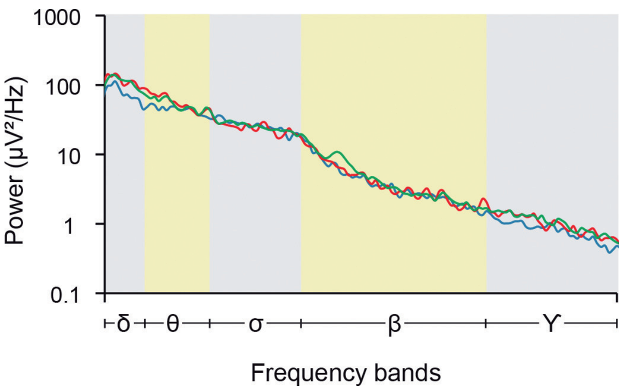


Fig. 4. - EEG power profiles during SWS in different maternal behaviors. Mean power values are represented in logarithmic scale (absolute values) for the different frequency bands: δ (delta: 1-4 Hz), θ (tetha: 4-9 Hz); σ (9-16 Hz); β (beta: 16-30 Hz); γ (gamma: 30-40 Hz). Blue line represent nursing during light phase in the first postpartum week; red line corresponds to nursing during light phase in the second postpartum week; green line corresponds to sleeping away from pups during light phase in the second postpartum week. Comparisons were made between nursing according to the postpartum period and between the different behaviors in the same postpartum week, using a dependent t-student. No significant differences were found in any frequency band.

was displayed a considerable amount of time only during DPh/2ndWK. Although the time spent in supine posture was three times less than in LKP during DPh/2ndWK, the time spent in REM sleep in supine posture almost triples that of in LKP. One possible explanation for this elevated time spent in REM sleep is that mother rats are slightly REM sleep deprived during some postures (e.g. hover over and HKP) and partially recover this debt during the supine posture where muscles can lose their tone.

While no differences were found in sleep fragmentation when comparing LKP with when the mother was away from the pups (data not shown), sleep fragmentation became evident when considering kyphosis posture as a whole (LKP and HKP together). In fact, we believe that sleep fragmentation relies on the transition from LKP to HKP, because at that precise moment mother rats invariably woke up, and progressively felt asleep while returning to LKP. This is in accordance with the observation of Rocha and Hoshino (2009), who found that movements of the litter disturbed the sleep of the mother rat, resulting in longer awakenings bouts compared to control females and males (Rocha and Hoshino, 2009). It can be argued that this sleep fragmentation we found during nursing was compensated when the mother rat slept away from the pups. However, this is not probable, as the amount of time the mother slept away from the pups was scarce in most conditions studied. Interestingly, this particular sleep characteristic resembles human mother sleeping pattern (Hunter et al., 2009).

In relation to sleep depth, the comparison of NREM sleep characteristics while nursing or being away from pups shows that neither delta power nor the total time in SWS differed between both behaviors. As it is well established that delta power is an electrophysiological marker of sleep intensity or depth and one of the main determinants of SWS (Borbély, 2011), we conclude that sleep depth did not differ while nursing and being without the pups. From the endocrinological point of view, we could expect that sleep was deeper while nursing, as prolactin levels are positively correlated with NREM sleep, and NREM sleep is enhanced in humans with prolactinoma (Frieboes et al., 1998; Spiegel et al., 1995). However, prolactin secretion in rats begins to rise at 1-3 minutes and reach the maximum 10 minutes after the initiation of suckling, falling when nursing ends (Freeman et al., 2000). Thus, nursing and away from the nest may not be separated enough in time to be differentially affected by prolactin secretion. In addition, taking into account the presence of the pups, it is reasonable to observe high sleep fragmentation and no deeper NREM sleep during nursing. However, it has been recently report that overall delta power of lactating rats is higher compared to pre-mating female rats (Sivadas et al., 2016).

The overall profile of our findings provide a first descriptive overview of how mother rats cope with maternal care of the pups and its own physiology of sleep. Future studies will be focused on determined the neural circuits that allow the coordination of these behaviors.

## Acknowledgements

This study was partially supported by the "Programa de Desarrollo de Ciencias Básicas" (PEDECIBA), Uruguay.

## References


Benedetto L., Rodriguez-Servetti Z., Lagos P., D'Almeida V., Monti J.M., Torterolo P. Microinjection of melanin concentrating hormone into the lateral preoptic area promotes non-REM sleep in the rat. *Peptides*, **39**: 11-15, 2013.

Borbély A. Sleep Homeostasis and Models of Sleep Regulation. pp. 431-444. In: Kryger M.H., Roth T., Dement W.C., (Eds). *Principles and Practice of Sleep Medicine*. St. Louis, Missouri, Elsevier, 2011.

Freeman M.E., Kanyicska B., Lerant A., Nagy G. Prolactin: structure, function, and regulation of secretion. *Physiol. Rev.*, **80**: 1523-1631, 2000.

Frieboes R.M., Murck H., Stalla G.K., Antonijevic I.A., Steiger A. Enhanced slow wave sleep in patients with prolactinoma. *J. Clin. Endocrinol. Metab.*, **83**: 2706-2710, 1998.

Grota L.J., Ader R. Continuouus Recording of Maternal Behavior in Rattus Norvergicus. *Animal Behav.*, **17**: 722-729, 1969.

Hunter L.P., Rychnovsky J.D., Yount S.M. A selective review of maternal sleep characteristics in the postpartum period. *J. Obstet. Gynecol. Neonatal Nurs.*, **38**: 60-68, 2009.

Lagos P., Torterolo P., Jantos H., Chase M.H., Monti J.M. Effects on sleep of melanin-concentrating hormone microinjections into the dorsal raphe nucleus. *Brain Res.*, **1265**: 103-110, 2009.

Lincoln D.W., Hentzen K., Hin T., van der Schoot P., Clarke G., Summerlee A.J. Sleep: a prerequisite for reflex milk ejection in the rat. *Exp. Brain Res.*, **38**: 151-162, 1980.

Neville M.C. Lactation and Its Hormonal Control. pp. 2993-3054. In: Knobil E. Neill J.D., eds. *The Physiology of Reproduction*. New York, Elsevier, 2006.

Paxinos G., Watson C. *The Rat Brain in Stereotaxic Coordinates*. San Diego, California: Elsevier Academic Press, 2005.

Pires G.N., Andersen M.L., Giovenardi M., Tufik S. Sleep impairment during pregnancy: possible implications on mother-infant relationship. *Med. Hypotheses.*, **75**: 578-582, 2010.

Pires G.N., Tufik S., Andersen M.L. Effects of REM sleep restriction during pregnancy on rodent maternal behavior. *Rev. Bras. Psiquiatr.*, **37**: 303-309, 2015.

Ray B., Mallick H.N., Kumar V.M. Changes in thermal preference, sleep-wakefulness, body temperature and locomotor activity of rats during continuous recording for 24 hours. *Behav. Brain Res.*, **154**: 519-526, 2004.

Rocha L., Hoshino K. Some aspects of the sleep of lactating rat dams. *Sleep Sci.*, **2**: 88-91, 2009.

Sivadas N., Radhakrishnan A., Aswathy B.S., Kumar V.M., Gulia K.K. Dynamic changes in sleep pattern during post-partum in normal pregnancy in rat model. *Behav. Brain Res.*, **320**: 264-274, 2016.

Spiegel K., Luthringer R., Follenius M., Schaltenbrand N., Macher J.P., Muzet A., Brandenberger G. Temporal relationship between prolactin secretion and slow-wave electroencephalic activity during sleep. *Sleep*, **18**: 543-548, 1995.

Stern J.M., Johnson S.K. Ventral somatosensory determinants of nursing behavior in Norway rats. I. Effects of variations in the quality and quantity of pup stimuli. *Physiol. Behav.*, **47**: 993-1011, 1990.

Stern J.M., Yu Y.L., Crockett D.P. Dorsolateral columns of the spinal cord are necessary for both suckling-induced neuroendocrine reflexes and the kyphotic nursing posture in lactating rats. *Brain Res.*, **947**: 110-121, 2002.

Stremler R., Wolfson A. The postpartum period. pp. 1587-1591. In: Kryger M.H., Roth T., Dement W.C., (Eds). *Principles and Practice of Sleep Medicine*. Canada, Elsevier, 2011.

Sutherland R.C., Juss T.S., Wakerley J.B. Prolonged electrical stimulation of the nipples evokes intermittent milk ejection in the anaesthetised lactating rat. *Exp. Brain Res.*, **66**: 29-34, 1987.

Voloschin L.M., Tramezzani J.H. Milk ejection reflex linked to slow wave sleep in nursing rats. *Endocrinology*, **105**: 1202-1207, 1979.

Wakerley J.B. Milk Ejection and Its Control. pp. 3129-3191. In: Knobil E., J.D.N., (Eds). *The Physiology of Reproduction*. New York, Academic Press, 1996.