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Inhibition of self-grooming induced by sleep restriction in dam rats

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***Background & objectives*: Sleep restriction is a common feature of modern lifestyle and its effects can be extended to pregnancy. Several neurobehavioural consequences of sleep restriction during pregnancy have been reported, among which stand out perinatal depression and maternal fatigue, however, its effects over mother-infant relationship warrant further investigation. Thus, this study was aimed to evaluate the effects of sleep restriction during pregnancy over maternal behaviour and maternal aggression through animal models.**

***Methods*: Eighteen 90-day-old female Wistar rats were distributed in two groups: *(i)* Control - not submitted to any manipulation during pregnancy, and *(ii)* Sleep restriction - submitted to sleep restriction during the entire pregnancy (21 days) through the multiple platforms technique. In the postpartum day 5, resident-intruder paradigm and the latencies test were performed to assess both maternal behaviour and maternal aggression.**

***Results*: The sleep-restricted females displayed grooming in less frequency and duration, and with higher latency when compared to normal animals, while maternal aggression and maternal behaviour parameters remained equivalent between groups.**

***Interpretation & conclusions*: Considering the maintenance of maternal behavioural parameters, the inhibition of grooming seems to exert an adaptive mechanism, enabling sleep-restricted rats to display maternal behaviour properly.**

Key words Grooming - maternal behavior - pregnancy - sleep restriction

Pregnancy is a physiological process and numerous physiological and anatomical changes take place in the body to sustain it. Even in normal conditions, this state predisposes women to lack of sleep, among other consequences[1-3]. During gestation, several sleep complaints and polysomnographic findings become common, ranging from hypersomnia and decreased slow-wave sleep (first trimester) to insomnia, non-restful sleep and diminished rapid eye movement (REM) sleep (third trimester)[2]. These complaints are mainly due to the marked anatomo-physiological changes inherent to this period in addition to alterations of

hormone secretion, greater urinary flow and frequency, difficulty in assuming non habitual sleep positions, among other[1].

The poor sleep quality related to pregnant women becomes more of a cause of concern when contextualized to modern living standards[3,4]. The lifestyle has changed affecting women more severely than man due to added social and professional demands[5]. This situation is aggravated during pregnancy, as pregnant women are subjected to insufficient sleep, promoted by intrinsic gestation circumstances and to impositions of modern lifestyle[3,6].

Sleep curtailment in pregnant women has been associated with long-term impairment and negative outcomes after the parturition[4,7]. Sleep loss during pregnancy leads to neurobehavioural and psychiatric co-morbidities, including perinatal depression[7,8] and maternal fatigue[9]. However, little has been documented about the postpartum consequences induced by sleep deprivation over the offspring as well as over parental behaviour. Recently, Pires *et al*[4] have hypothesized that sleep impairment during pregnancy could lead to deficient mother-infant relationship. However, this concept warrants further investigation as it was theoretically discussed, but not experimentally assayed.

Animal models of maternal behaviour are useful experimental tools to understand the mechanisms underlying mother-infant relationship. However, the studies regarding the interface between maternal behaviour and sleep are incipient[10]. Thus, this study was aimed to examine the effects of sleep restriction during pregnancy over two important issues related to parental behaviour - maternal behaviour *per se* and maternal aggression in rats.

Material & Methods

This study was conducted in the Department of Psychobiology, University of São Paulo, São Paulo, Brazil. For this experiment 18, 90-day old female Wistar rats were obtained from the Center for Development of Experimental Models for Medicine and Biology (CEDEME), Sao Paulo, Brazil. These subjects were kept in monitored rooms with controlled temperature (22±1ºC) and a 12 h light-dark cycle. The experimental protocol was approved by the institutional research ethics committee.

The female rats were mated, and pregnancy was confirmed by the presence of spermatozoa in the vaginal smear associated with compatible estrous cycle condition and, when possible, by the visualization of a vaginal plug. In the first day of pregnancy, the female rats were distributed in two groups: *(i)* Control - not submitted to any manipulation during pregnancy, and *(ii)* Sleep restriction – submitted to sleep restriction during the entire pregnancy (21 days). The control group for this experiment was in accordance to previous studies on maternal sleep deprivation through the platform method. Briefly, this protocol consisted of subjecting the animals to prolonged wakefulness for 18 h per day (from 1600 - 1000h) by the multiple platforms technique, for 21 days. During the remaining 6 h, the animals were returned to their home-cages and allowed to sleep. Sleep restriction was conducted in a tiled water tank (110x41x30cm), which contained 14 platforms, 6.5 cm in diameter and rising 1 cm above water surface[11]. The day of the partum was defined as postpartum day 0 (PPD0) and in the PPD1 the litters were standardized in 8 pups. The dams were subjected to behavioural tests to assess maternal behaviour and maternal aggressive behaviour at PPD3 and PPD5, which corresponds to the ascending phase of these behaviours[12,13]. The protocol was adapted from earlier studies[14,15] and consisted of two different tests, as described below:

Resident-intruder paradigm: This test was performed to assess both aggressive maternal behaviour and maternal behaviour in the presence of an intruder rat. Thus, at the PPD3 and PPD5, a naive 60-day old male rat was introduced in the home-cage with the female rat and its respective litter. This test was able to quantify latency, frequency and duration of behavioral patterns related to maternal behaviour, maternal aggressive behaviour and non-maternal behaviours (Table).

Latencies test: This test was specific to maternal behaviour. Ten minutes after the end of the resident-intruder paradigm, the female rats were separated from their litter for 5 min. During this time, the litter was sent to the home-cage. When the female rat returned to the cage, the latency to retrieve the first and the last pup was measured. The time between the latencies was calculated.

Both behavioural tests had a cut-off of 10 min and were conducted in the first third of the dark phase. The tests performed at PPD3 were considered as habituation and were not quantified for analysis. The dams were weighed before the parturition and in the PPD5. The results of the tests at PPD5 as well as the dams' weight were compared through the Mann-Whitney test.

Table. Results of comparison between control and sleep deprived females

			Control (n=9)			Experimental (n=9)			
			Mean	SD	Median	Mean	SD	Median	*P*
Resident-intruder paradigm	Maternal aggression parameters	Frontal attack - latency	290.2	211.9	197	294.1	195.2	291	0.93
		Frontal attack - frequency	1.7	1.6	2	1.9	2.4	1	0.71
		Frontal attack - duration	2.6	2.1	2	2.7	3.7	1	0.50
		Lateral attack - latency	168.7	182.0	87	71.3	109.8	44	**0.04**
		Lateral attack - frequency	2.8	1.7	3	6.4	5.4	5	0.26
		lateral attack - duration	2.4	1.6	3	6.7	7.3	2	0.40
		Boxing - latency	558.6	124.3	600	475.4	247.3	600	0.50
		Boxing - frequency	0.1	0.3	0	1.3	3.6	0	0.54
		Boxing - duration	0.1	0.3	0	0.9	2.3	0	0.54
		Submission - latency	478.4	241.6	600	428.7	226.9	600	0.56
		Submission - frequency	0.2	0.4	0	1.2	2.3	0	0.27
		Submission - duration	0.4	1,0	0	6.6	16.4	0	0.27
	Maternal behaviour parameters	Nest building - latency	299.4	246.2	231	169.7	216.3	71	0.23
		Nest building - frequency	6.0	6.3	4	5,0	3.7	4	**0.03**
		Nest building - duration	27.7	32.0	27	9.6	8.2	7	0.48
		Maternal care - latency	179.7	191.5	113	355.4	266.9	489	0.18
		Maternal care - frequency	7,0	6.4	5	4.1	6.2	1	0.18
		Maternal care - duration	64.6	88.5	33	18.4	38.8	3	0.05
	Non-maternal parameters	Grooming - latency	83.7	66.4	64	366.2	225.9	362	**0.01**
		Grooming - frequency	4.7	3.5	4	1.6	1.7	1	**0.02**
		Grooming - duration	31.4	24.7	26	4.4	5.3	4	**0.01**
		Social investigation - latency	31.3	69,0	7	70.6	198.6	3	0.08
		Social investigation - frequency	6.6	6.3	4	4.3	2.2	4	0.79
		Social investigation - duration	15.8	15.2	13	7.1	4.3	8	0.22
		Locomotion - latency	15.8	9.8	16	19,0	17.6	14	0.89
		Locomotion - frequency	10.3	4.2	10	18,0	8.5	18	0.06
		Locomotion - duration	16.2	8.5	18	22.9	11.3	21	0.27
Latencies test		Latency to retrieve first pup	103.4	86.2	104	185.0	198.5	87	0.35
		Latency to retrieve eighth pup	506.6	166.1	600	441.3	190.4	550	0.57
		Diference between latencies	403.1	180.2	493	256.3	173.2	262	0.10

Values for latency and duration are expressed in seconds and frequency represents how many times each behavioural parameter was displayed. The behavioural parameters observed in the resident- intruder paradigm are distributed in categories, according to the type of behaviour they represent. The bold values indicate the statistically significant results. SD: standard deviation

Results

The most affected behaviour by sleep restriction during pregnancy was self-grooming, which was inhibited. Grooming was significantly reduced in the sleep restriction group regarding both frequency (4.7 ± 3.5 vs 1.6 ± 1.7; P=0.02) and duration (31.4 ± 24.7s vs 4.4 ± 5.3 sec; P<0.01) in relation to normal sleep animals. In addition, mean latency of self-grooming was significantly higher in sleep-restricted females (83.7 ± 66.4 sec vs 366.2 ± 225.9 sec; P<0.01), indicating

that these animals delayed the drive to self-groom in the presence of a stressful stimulus (intruder male). Statistically significant results were also obtained in latency for lateral attack (168.7 ± 182.0 sec vs 71.33 ± 109.8 sec; *P*=0.04) and frequency for nest building (6.0 ± 6.3 vs 5.0 ± 3.7; *P*=0.03). There were no significant differences in dam's weight neither before the parturition (336 ± 21.2 vs 322.4±22.8; *P*=0.45) nor in the PPD5 (276.8 ± 28.1 vs 250.3 ± 15.4 g; *P*=0.07).

Discussion

The significant results in this study were found in the self-grooming. A decrease was found in lateral attack latency and in nest building frequency in sleep-restricted females, pointing to an increase in the defensive component of maternal aggression and decrease in a maternal care-related behaviour, respectively. The data regarding the lateral attack latency partially corroborated the results of Sandrin and Hoshino[16], who have described an increase of defensive aggression in sleep-deprived rats. However, the findings related to nest building and lateral attack were isolated and not accompanied by complementary behavioural parameters (latency and duration), in contrast to the results for grooming. The hypothesis that the findings related to lateral attack and nest building were occasional and had no practical relevance can be raised, despite the statistical significance. Thus, both maternal aggression and maternal behaviour in sleep-restricted females were equivalent to the control group, as the majority of parameters related to these behaviours were not significantly different.

The sleep pattern in pregnant rats is similar to what is seen in women, showing dynamic oscillations in total sleep time and in other features[17]. Moreover, the sleep restriction methodology through the platform method is specific to REM sleep[18], promoting sleep fragmentation rather than total sleep deprivation[11]. Accordingly, REM sleep deprivation provides a better framework to mimic disrupted sleep patterns during pregnancy than does total sleep deprivation because pregnant women show a marked REM sleep curtailment, mostly in the third trimester of gestation[2]. Hence, sleep restriction by the platform method in animals can be taken, with caution, as analogue to the sleep deficit scenario experienced by pregnant women. These results corroborate, in some degree, the hypothesis proposed by Pires *et al*[4], since behavioral alterations related to mother-infant relationship were observed as consequence to sleep restriction. However, the behavioral alterations did not occur in maternal behavior *per se*, but in behavioral adaptive mechanisms, thus enabling effective maternal care.

The data were also analyzed in the perspective of the approach-avoidance model of maternal behavior[19,20]. This model proposes that the maternal behaviour occurs when the tendency of approach and interaction with the litter is greater than the tendency to avoid pup stimuli. According to this model, maternal behaviour is a result of both maternal motivational and non-specific systems, the interaction between these systems, and the influence of external stimuli over them.

Self-grooming is a highly complex behaviour[21] with a well known association with high stress situations[22]. In rodents, the grooming analysis is an indirect approach to infer about anxiety[23]. In general, the increase in self-grooming, mainly in frequency, composes the behavioural profile of rodents under an anxiogenic condition[23]. Considering the approach-avoidance model, anxiety-like behaviour and stressful conditions could be considere d as possible factors inducing the avoidance of the litter by the dam, or, more generally, anxiety and stress could compose the antagonistic system, avoiding the maternal behaviour as a whole. Since sleep deprivation and sleep restriction are closely related to stress[24,25], a clear deficit in maternal behaviour would be expected under these situations, however, it was not observed, suggesting an adaptive mechanism. The anxiolytic condition observed in the sleep restricted rats, evidenced by the increase in grooming latency and decrease in frequency and duration, could be responsible for allowing these females to display both maternal aggression and maternal behaviour properly.

The relationship between sleep and anxiety has been reported as a feature of adaptive behaviour mechanisms[26]. In the context of maternal coping, a marked stress hyporesponsiveness during lactation is described[27] as well as a dramatic reduction in levels of anxiety-like behaviour[28]. These effects seem to be more prominent in the face of environmental challenges[29]. It might be possible that in an acute environmental challenge (the exposure to a male intruder in the resident-intruder paradigm), the anxiolytic conditions expected for lactating rats are even more prominent in those animals subjected to sleep restriction during pregnancy. Thus, the inhibition of self-grooming due to sleep restriction could be interpreted as one of the several adaptive mechanisms by which maternal care toward the litter is modulated in accordance with environmental demand[30].

There are some methodological limitations in this study. First, one may inquire whether the results could be due to the stress promoted by sleep loss or to the sleep rebound that occurs after the sleep restriction protocol, rather than to sleep loss *per se*. However, it should be assumed that both sleep rebound and stress are conditionally associated with sleep deprivation. Even if one considers stress and sleep rebound as the causes of the described data, it is acceptable to state that these results are consequences of the context of sleep deprivation. Second, no sleep recording was performed in this study because the surgery and the recording equipment could represent additional sources of stress for the dams, leading to biased results. Also, the present results are strictly related to pregnancy and puerperium contexts and should not be extended to other conditions.

The present data should not be taken as conclusive findings, but rather as background to further investigation. Several aspects in this behaviour still warrant further studies, addressing in particular the extent to which grooming plays an adaptive role. Moreover, these studies could be even more useful if conducted with animal models that allow translational applicability. It must also be pointed out that the maternal bond and parental behaviour are far more complex in humans than in animals. Parental behaviour in humans is inserted into a social context that is not conceivable in animal experimentation. The adaptive responses observed in animals could give rise to neurobehavioural co-morbidities in humans, such as postpartum depression and maternal fatigue. Thus, the acquired data should be extrapolated to human behaviour with caution. Despite these considerations and of the preliminary nature of these findings, the results are of clinical importance regarding the relationship between disrupted sleep during pregnancy and mother-infant relationship.

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