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Sleep science

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editorial

This issue of Sleep Science contains some articles published in the journal Hypnos, which represented the Brazilian Sleep Association and Federation of Latinamerican Sleep Societies. Hypnos did not have it registry at the ISSN and therefore did not count on editorial officialization. And because of the high quality of some of those papers we thought it best to make them official in Sleep Science.

We thank the authors for their permission to do so and we feel honored to be able to offer this extension of knowledge to the readers of the papers.

Dr Lia Rita Azeredo Bittencourt
Editor in chief

ERRATUM:
On the last edition of Sleep Science (Vol 2, Issue 1), at the first page, of Contents Index, the author of the article *Effects of naps at work on the sleepiness of 12-hour night shift nursing personnel* **is Flavio N. S. Borges.**

contents

Guide for authors: how to submit your manuscript

SCOPE AND POLICY

The SLEEP SCIENCE journal, published every three months, is the official organization of Associação Brasileira de Sono (ABS) and of Federação Latino Americana de Sociedades de Sono (FLASS) for publication of scientific papers concerning sleep, chronobiology, and related topics.

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The accuracy of all concepts presented in the manuscript is the exclusive responsibility of the authors.

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The title page should include the title in English; the full names and institutional affiliations of all authors; complete address, including telephone number, fax number and e-mail address, of the principal author; and a declaration of any and all sources of funding.

Abstract: The abstract should present the information in such a way that the reader can easily understand without referring to the main text. Abstracts should not exceed 250 words. Abstracts should be structured as follows: Objective, Methods, Results and Conclusion. Abstracts for review articles and case reports may be unstructured. Abstracts for short communications should not exceed 100 words.

Keywords: Three to six keywords in English defining the subject of the study should be included.

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Journal articles

1. Tufik S, Lindsey CJ, Carlini EA. Does REM sleep deprivation induce a supersensitivity of dopaminergic receptors in the rat brain?Pharmacology. 1978;16(2):98-105.

2. Andersen ML, Poyares D, Alves RS, Skomro R, Tufik S. Sexsomnia: Abnormal sexual behavior during sleep. Brain Res Rev. 2007; 56: 271-282.

Abstracts

3. Moreno CRC, Carvalho FA, Matuzaki LA, Louzada FM. Effects of irregular working hours on sleep and alertness in Brazilian truck drivers [abstract]. Sleep. 2002; 25:399.

Chapter in a book

4. Andersen, ML; Bittencourt, LR. Fisiologia do sono. In: Tufik S, editor. Medicina e Biologia do Sono. 1a ed. São Paulo: Manole; 2007. p. 48-58.

Official publications

5. World Health Organization. Guidelines for surveillance of drug resistance in tuberculosis. 2nd ed. Geneva: WHO; 2003. p. 1-24.

Thesis

6. Bittencourt L. Avaliação da Variabilidade do Índice de apnéia e hipopnéia em pacientes portadores da Síndrome da Apnéia e Hipopnéia do Sono Obstrutiva [tese]. São Paulo: Universidade Federal de São Paulo;1999.

Electronic publications

7. Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 [cited 2002 Aug 12];102(6):[about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm.

Homepages/URLs

8. Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc., c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/

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ORIGINAL ARTICLE
Category: Sleep Deprivation - Basic

SLEEP FRAGMENTATION DIFFERENTIALLY MODIFIES EEG DELTA POWER DURING SLOW WAVE SLEEP IN SOCIALLY ISOLATED AND PAIRED MICE

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ABSTRACT

Background and objective: Sleep fragmentation (SF) is an important constituent of many sleep disorders. Sleep rebound following sleep disruption is regulated by homeostatic processes that also are influenced by stress and social isolation stress has not been studied in context of sleep disruption. We investigated interactions between social isolation and SF on sleep-wakefulness and delta EEG power during SWS in mice.

Methods: C57/BLJ adult male mice were exposed to 6 h SF using a custom-designed apparatus that elicits minimal stress, along with telemetric polygraphic recordings for 24h. In paired or isolated mice, baseline recordings were followed by SF (every 2 min), for 6h.

Results and conclusions: In contrast with other published methods that induce sleep disruption, SF procedures were void of increased serum corticosterone. SF in both paired and socially isolated mice elicited an increase in slow wave sleep (SWS) and REM, and a decrease in wake during the dark period. However, there was no change in total time (24 h) in wake or SWS in both the groups. SF also induced reduced sleep latencies following arousal. EEG delta power during SWS was significantly attenuated in isolated animals when compared to the paired group. Social interactions exert important effects on sleep structure and homeostasis, as evidenced by sleep latency and delta power of the EEG, the latter serving as a surrogate indicator of sleepiness. Social isolation may negatively affect the quality of sleep, even when total sleep time is unaffected, and experimental paradigms that induce sleep restriction should take into consideration the underlying effects of isolation on sleep.

Keywords: Sleep, Sleep fragmentation, Social isolation stress, Delta power, Sleep homeostasis, mice

INTRODUCTION

Sleep fragmentation (SF), unlike prolonged sleep deprivation, is a notable consequence of many diseases in adults and children, including obstructive sleep apnea (OSA) (1,2), narcolepsy (3,4), depression (5,6) and post-traumatic stress disorder (7,8). It has been postulated that uninterrupted sleep for a minimum length of time is required for optimal daytime vigilance and neurocognitive function (9-11). As a corollary to this assumption, experimentally-induced SF resulted in excessive daytime sleepiness and cognitive impairments in humans (9,11) and in animals (12). In sleep-disordered breathing, especially OSA, the neurocognitive impairments observed are most likely due to intermittent hypoxia (13,14) and to SF, rather than sleep deprivation, because in these patients total sleep time is not markedly compromised (9,11,15).

Although, there are many studies in animals that have examined the effects of sleep deprivation on sleep-wakefulness (16-18), there are only a selected few that have looked into the effects of SF (19). Multiple methodological approaches have been employed to restrict sleep, including the slow rotating wheel (20,21), disk over water (22), small platform (23), treadmill (19,24) and gentle handling (18,25). Even though the stress levels may alleviate after long-time adaptations to such methodologies, they do not simulate disease conditions, especially OSA. Moreover, the stress induced by the cable required for recording of electroencephalogram (EEG) and electromyogram (EMG) may persist. Indeed, recording cables introduce another set of stressors (limited climbing on water bottles and cage covers), especially in small animals, such as mice. A recent study concluded that cable weight and flexibility could affect amount and patterns of sleep in mice (26).

In this paper, we report on a newly designed and validated device to elicit SF in rodents. This approach entails relatively minimal stress, particularly when combined with telemetric recordings, thereby providing an improved and desirable methodological approach for the study of the effects of intermittent sleep disruption, which ideally mimic the SF that occurs in disease conditions, such as OSA. Thus, concurrent with the recent developments in transgenic technologies, the methods described herein should allow for examination of unaltered physiological responses to sleep disruptors, and their corresponding mechanisms. Furthermore, the absence of tethering in a telemetric sleep recording set-up provides the opportunity to study the effect of social interaction on sleep. Many recent studies have successfully demonstrated the efficacy of telemetric sleep recordings (11,27).

Multiple studies have conclusively identified social isolation as inducing behavioral abnormalities, such as increased aggressiveness, anxiety-related behaviors, cognitive deficits, and hyper locomotion (28,29). However, how social isolation affects sleep, and how it affects the response to sleep disruption has never been explored. We therefore examined whether intermittent sleep interruption leads to increases in 'sleep pressure', and also whether social isolation differentially modulates natural sleep patterns and the 'sleep pressure' responses to SF.

MATERIALS AND METHODS

Animals

Male C57BL/6J mice (20-25 g) were purchased from Jackson Laboratories, (Bar Harbor, Maine), were housed in a 12 hr light/dark cycle (light on 7:00 am to 7:00 pm) at a constant temperature (26 ±1°C) and were allowed access to food and water ad libitum. The experimental protocols were approved by the Institutional Animal Use and Care Committee and are in close agreement with the National Institutes of Health Guide in the Care and Use of Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Surgical procedure and implantation of telemetric transmitter and electrodes

All surgical procedures were performed under sterile conditions and general anesthesia (i.p. injection of pentobarbital at a dose of 50 mg/kg body weight). First, the animals were positioned in sternal recumbency, and a dorsal neck incision of 2-3 cm was made through the skin along the dorsal midline, covered with a sterile bandage, after which, a 1.5 - 2 cm incision was performed through the skin and abdominal wall along the ventral midline. A telemetric transmitter weighing 3.5 g, F20-EET (DSI, Minnesota, USA), which allows simultaneous monitoring of two biopotential channels, temperature and locomotor activity was inserted, biopotential leads were exteriorized, and the abdominal wall was closed using 4-0 non-absorbable suture with a simple interrupted pattern. The 2 pairs of biopotential leads were then advanced subcutaneously from the ventral abdomen incision to the dorsal neck incision using a trocar. Animals were then fixed in a stereotaxic apparatus for implantation of EEG electrodes, with the first pair of biopotential leads being fixed to the skull above the frontal area (1mm anterior to bregma and 2mm lateral to mid sagittal suture for one of the leads, and 1mm anterior to lambda and 2.5 mm lateral to mid sagittal suture for the other lead). The other pair of biopotential leads was placed within the same bundle of dorsal neck muscles for the recording of nuchal EMG.

Design and fabrication of a novel sleep fragmenter device for sleep deprivation / sleep fragmentation

The sleep fragmenter device used to induce SF in rodents has been previously presented in abstract form (30) and employs intermittent tactile stimulation of freely behaving mice in a standard mouse laboratory cage, using a near-silent motorized mechanical device. However, mice can hear higher frequencies than humans, and this factor has to be taken into consideration. In brief, tactile stimulation is achieved with a horizontal bar sweeping just above the cage floor from one side to the other side of the mouse cage, the sweeper being powered by an electrical motor system in which the speed, torque, and interval of the intermittent functioning mode (2 min) are controlled (Fig. 1A), eliminating error induced by human intervention. On the other hand SD was performed by switching on the sweeper in the cage to continuous functioning mode. In this mode, the sweeper required around 9 sec to sweep the floor of the cage one way. When it reached to the end of the cage, a relay engaged the sweeper to move in the opposite direction.

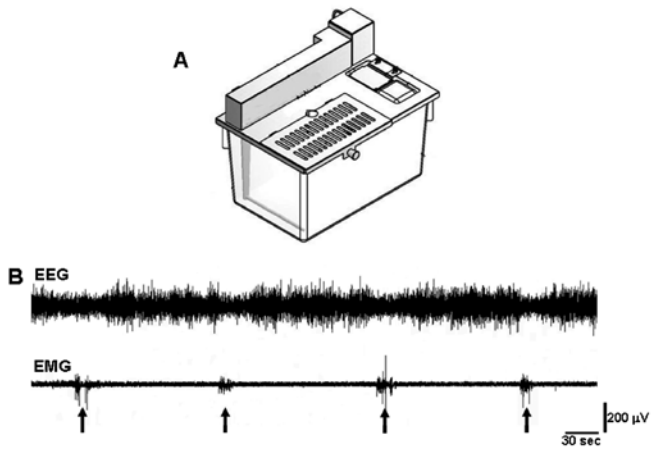

Fig. 1: A. Illustration of the new SF device. B. A representative recording of the polysomnogram during sleep fragmentation showing periodic arousal at 2 min intervals. Please note during each arousal event (arrows), there is a transitional desynchronized EEG waves corresponding to the EEG muscle artifacts. This methodology gently aroused the mice and did not appear to induce obvious stress. EEG, electroencephalogram; EMG, electromyogram.

Assay of corticosterone plasma levels

The fabrication of the sleep fragmenter device was designed to induce minimal stress to the animals, while effectively eliciting the desired frequency of sleep fragmentation. To verify this assumption, initial experiments were conducted to determine corticosterone (CT) plasma levels, as a surrogate indicator of stress. SF and sleep deprivation were carried out for 6 hours, starting at 7:00 am in C57BL/6J mice. Control mice were sacrificed at 1.00 pm (no intervention; n=12). SF using the novel sleep fragmenter device was conducted in 12 mice, sleep deprivation with the same device was completed in 11 animals, sleep deprivation using the disk over water method was completed in 7 mice, and REM sleep deprivation using the inverted flower pot technique was performed in 9 mice. Mice were rapidly decapitated immediately after their respective experimental procedure at 1.00 pm, and blood collected in EDTA-containing tubes, immediately centrifuged, and frozen at -80oC. Plasma levels for CT were then determined using a commercially available ELISA kit following the manufacturer recommendations (Immunodiagnostic Systems Ltd, Boldon, England, AC-14F1). This method has a detection level of 0.75 ng/ml, and exhibits linear behavior up to 200 ng/ml, with intra-assay and inter-assay coefficients of variability of 7.2% and 9.3%. The results were tabulated and statistics carried out by Student's t tests or ANOVA as appropriate.

Acclimatization, sleep recording and sleep fragmentation

After complete recovery from surgery, mice were transferred to the new sleep fragmenter device for habituation of the cage and the sweeper. The recording cages were mounted on a DSI telemetry receiver (RPC-1), which was in turn connected to an acquisition computer through a data exchange matrix. After at least one week of acclimatization in the cages, the magnetic switch of the transmitter was activated, and polygraphic recordings were begun at 7.00 am. Physiological data were continuously acquired for 24h using Dataquest ART acquisition software (DSI, Minnesota, USA; version 3.1), at a sampling rate of 500 Hz. Data were first scored automatically using Sleepsign software (Kissei Comtec, Japan), and records were visually confirmed or corrected as needed. Many researchers have adopted and successfully applied this software for sleep-wake analyses (31,32).

Behavior was classified into 3 different states: wake, slow wave sleep (SWS) and rapid eye movement (REM) sleep. EEG during W had low-amplitude, high-frequency (desynchronized) waves. During wake, EMG records showed gross body movement artifacts and behaviorally, animals had grooming, scratching and orienting activity. The SWS stage was characterized by low-frequency, high-amplitude (synchronized) EEG with a considerable reduction in EMG amplitude. The mice assumed a curled recumbent posture during this period. REM sleep was characterized by desynchronized EEG, and a drastic reduction in EMG (muscle atonia). Sleep-related low frequency (delta) activity was also derived from the records using bandpass filtering of 1– 4.0 Hz. Delta power was computed by using SleepSign software by Fast Fourier Transform (FFT), which was based on 512 points corresponding to 10 sec epochs, at a sampling rate of 250 Hz with Hanning as the window filter of FFT. Those SWS epoch which showed movement artifacts were excluded when computing delta power, since EEG signals are especially sensitive to movement, with the resulting artifact specifically enhancing signals in the delta band.

SF was performed by switching on the sweeper to a timer mode in the cage. In this mode, the sweeper required around 9 sec to sweep the floor of the cage one way. When it reached to the end of the cage, a relay engaged the timer which paused for 2 min before enabling the sweeper to move in the opposite direction. Between the 2 intervals, the animal remained undisturbed. During sweeper motion, animals would need to step over the sweeper, and continue with their unrestrained behavior. If the mouse was asleep, a brief tactile stimulation elicited intermittent brief arousal by the sweeper motion. This method prevents the need for human contact and intervention, and minimizes physical activity during the entire sleep disruption procedure, and closely mimicked the best methodological approach to study sleep disorders such as OSA. Since on average, 30 episodes of arousal per hour occur in patients with severe OSA (i.e, every 2 min), our aim was to mimic closely the severe disease condition, and thus, chose the interval of 2 min in our SF paradigm.

Experimental design

The various phases of the experimental paradigm are illustrated in Fig. 2.

Group 1: Social isolation

Part 1: During the 7-day acclimatization period and prior to recordings, implanted animals (n=5) were paired with another male mouse with which they had previously been housed. On day 8, baseline sleep recordings were carried out for 24h from 7.00 am

to 7.00 am next day (Fig. 2). The animals were left undisturbed on day 9. On day 10, animals were subjected to SF for 6 h during the light period from 7.00 am to 1.00 pm, and recovery sleep recordings were continued for the subsequent 18 h until 7.00 am next day.

Part 2: Following the above experiment, the companion mice were removed from the cages, and the experimental mice were placed in social isolation for 5 weeks. On day 45, baseline sleep recordings were conducted for 24h from 7.00 am to 7.00 am next day. The animals were left undisturbed on day 46. On day 47, the animals were subjected to SF for 6 h during the light period from 7.00 am to 1.00 pm, and recovery sleep recordings were continued for the subsequent 18 h until 7.00 am next day.

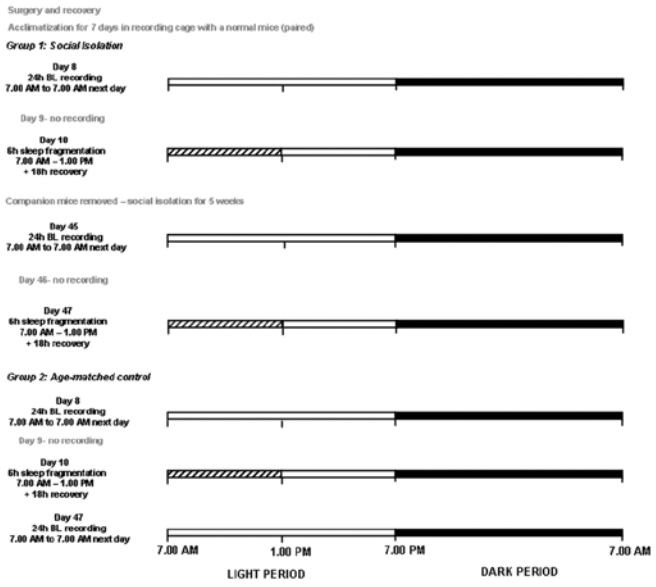

Fig. 2: Experimental protocol diagram. Open and dark portions of the bar represent light and dark periods of the 12:12-h light: dark cycle respectively. Hatched portion of the bar (within the light period) indicates the time of sleep fragmentation.

Group 2: Age-matched control

Part 1: During the 7-day acclimatization period and prior to recordings, implanted animals (n=5) were paired with another male mouse with which they had previously been housed. On day 8, baseline sleep recordings were carried out for 24h from 7.00 am to 7.00 am next day (Fig. 2). The animals were left undisturbed on day 9. On day 10, animals were subjected to SF for 6 h during the light period from 7.00 am to 1.00 pm, and recovery sleep recordings were continued for the subsequent 18 h until 7.00 am next day.

Part 2: Following the above experiment, the companion mice continued to stay in the cages. On day 47, baseline sleep recordings were conducted for 24h from 7.00 am to 7.00 am next day.

Sleep latency measurement:

To determine the time elapsed following a wake episode to initiation of SWS, the latency in seconds was calculated for each arousal during the first two hours (7.00 am to 9.00 am) and the last two hours (11.00 am to 1.00 pm) during baseline conditions and during SF recordings in both paired and socially isolated conditions. The time was measured from the beginning of each wake episode to the beginning of the next SWS episode and the mean calculated.

Temperature and activity:

Body temperature and gross motor activity were acquired every 10 sec through out all experiments. To increase the precision of recording, the lower limit of temperature records was set at 34°C and the upper limit at 41°C, while in the activity record the lower limit was set at 0 counts (no gross activity) and upper limit was set at 3840 counts (a high level of activity) at the polling rate of 64 Hz. The transmitter underwent 3 point calibration at 35 °C, 37 °C and 39 °C.

Data analysis:

In all the experimental conditions, the sleep-wake data were divided into 10 sec epochs and scored. They were then divided into 2-h bins. EEG delta power (1–4 Hz) during SWS was calculated as percentage of each animal's baseline recording. We used multivariate MANOVA model (SPSS 11) to allow full assessment whether different conditions on three different behavioral states were present. The MANOVA model had: Two hr time bins as within factors (12 time points) and Two between factors: (1) Condition (four levels): BL (paired), SF (paired), BL (socially isolation) and SF (socially isolation) (2) State (three levels): wakefulness, SWS, and REM sleep. All F statistics are reported using Pillai's Trace. The interaction of three different factors, i.e., time, condition and state were determined using this mixed model repeated measures MANOVA.

To further elucidate the nature of identified interactions for the paired and socially isolated conditions, the data were analyzed by one way ANOVA. Firstly, overall statistical significance was determined for the 24-h period between the treatment groups (baseline and sleep fragmentation). In addition, statistical significance for 2 h bins for 24 h was assessed, followed by post-hoc Holm-Sidak analyses, as needed. Similar statistical approaches were used to compare delta power during SWS and the latency of SWS after each episode of wake. Repeated measures one-way ANOVA were used to analyze body temperature and gross activity in the paired and socially isolated conditions. For all comparisons, a p value <0.05 was considered to achieve statistical significance.

RESULTS

The main objectives of the present study were to assess changes in sleep architecture during and after SF under socially paired and isolated conditions. The novel technique used to fragment sleep was remarkably efficient in eliciting periodic arousals at the desired intervals (Fig. 1B), and did not appear to induce obvious stress in the animals. As shown in Fig. 3, animals subjected to SF spent more time in the awake state during the initial hours of SF, but subsequently manifested the same duration of wake as controls during the last 2 hours of SF.

		PAIRED		SOCIALLY ISOLATED	
State	Time of day	Significance	Percentage time spent	Significance	Percentage time spent
Wakefulness	7:00 AM - 9.00 AM	F=5.44, p<0.04	74.6 ±6.3	F=64.68, p<0.001	75.5 ±1.2
	9:00 AM - 11.00 AM	F=17.41, p<0.003	52.7 ±3.3	NS	55.9 ±4.8
	11:00 AM - 1.00 PM	NS	46.2 ±5.2	NS	48.3 ±3.9
	1:00 PM - 3.00 PM	NS	47.1 ±7.1	F=8.08, p<0.04	36.9 ±3.5
	3:00 PM - 5.00 PM	NS	33.9 ±6.4	NS	26.1 ±2.5
	5:00 PM - 7.00 PM	NS	37.8 ±2.4	NS	56.3 ±4.7
	7:00 PM - 9.00 PM	NS	78.3 ±7.0	NS	84.4 ±2.8
	9:00 PM - 11.00 PM	F=7.61, p<0.025	63.5 ±6.7	NS	71.4 ±2.0
	11:00 PM - 1.00 AM	F=5.34, p<0.05	60.4 ±8.0	F=42.74, p<0.003	47.4 ±7.4
	1:00 AM - 3.00 AM	F=18.90, p<0.002	45.4 ±6.0	NS	50.9 ±4.0
	3:00 AM - 5.00 AM	NS	60.7 ±6.0	F=26.70, p<0.007	80.5 ±1.6
	5:00 AM - 7.00 AM	NS	60.5 ±3.7	F=9.83, p<0.03	54.5 ±4.2
SWS	7:00 AM - 9.00 AM	NS	24.7 ±6.2	F=35.04, p<0.004	24.5 ±1.2
	9:00 AM - 11.00 AM	F=12.05, p<0.008	44.2 ±3.2	NS	40.7 ±4.1
	11:00 AM - 1.00 PM	NS	48.2 ±4.4	NS	47.3 ±2.6
	1:00 PM - 3.00 PM	NS	45.0 ±5.8	NS	49.5 ±1.7
	3:00 PM - 5.00 PM	NS	54.2 ±4.1	NS	58.6 ±2.6
	5:00 PM - 7.00 PM	NS	52.9 ±2.2	NS	34.9 ±3.7
	7:00 PM - 9.00 PM	NS	18.8 ±5.9	F=8.71, p<0.042	14.6 ±2.7
	9:00 PM - 11.00 PM	F=7.851, p<0.023	34.1 ±5.9	NS	25.5 ±1.8
	11:00 PM - 1.00 AM	F=5.258, p<0.051	35.1 ±6.3	F=37.36, p<0.004	44.5 ±5.8
	1:00 AM - 3.00 AM	F=16.69, p<0.004	47.9 ±5.1	F=17.94, p<0.013	41.2 ±3.6
	3:00 AM - 5.00 AM	NS	35.3 ±5.1	F=277.60, p<0.001	18.4 ±1.5
	5:00 AM - 7.00 AM	NS	36.8 ±3.6	F=8.63, p<0.04	40.0 ±3.2
REM sleep	7:00 AM - 9.00 AM	F=8.70, p<0.018	0.7 ±0.5	F=149.06, p<0.001	0.0 ±0.0
	9:00 AM - 11.00 AM	F=15.75, p<0.004	3.1 ±0.6	F=30.04, p<0.005	3.5 ±0.7
	11:00 AM - 1.00 PM	NS	5.6 ±1.0	NS	4.4 ±1.3
	1:00 PM - 3.00 PM	NS	7.9 ±1.5	NS	13.7 ±1.8
	3:00 PM - 5.00 PM	NS	11.9 ±2.5	NS	15.3 ±1.1
	5:00 PM - 7.00 PM	NS	9.3 ±0.9	NS	8.8 ±2.0
	7:00 PM - 9.00 PM	NS	3.0 ±1.3	NS	1.1 ±0.3
	9:00 PM - 11.00 PM	NS	2.4 ±0.8	F=9.66, p<0.036	3.1 ±0.3
	11:00 PM - 1.00 AM	NS	4.5 ±1.7	F=24.45, p<0.008	8.2 ±1.8
	1:00 AM - 3.00 AM	F=15.27, p<0.004	6.7 ±1.4	F=11.40, p<0.028	7.9 ±0.7
	3:00 AM - 5.00 AM	NS	4.0 ±1.3	NS	1.1 ±0.3
	5:00 AM - 7.00 AM	F=17.70, p<0.003	2.6 ±0.3	F=11.56, p<0.027	5.5 ±1.1

Table 1: The percentage time spent in wakefulness, slow wave sleep (SWS) and rapid eye movement (REM) sleep for paired and socially isolated groups. Data are expressed mean ± SEM.

Corticosterone plasma levels:

CT plasma levels did not increase in mice subjected to SF and sleep deprivation using the new SF technique reported herein, when compared to control animals. Indeed, control mice CT levels were 89.5 ±7.3 ng/ml, while in SF mice CT concentrations were 92.5 ±8.1 ng/ml (p-not significant), and were also similar to sleep deprivation mice (95.2 ±8.3 ng/ml; p-not significant). However in animals undergoing sleep deprivation using the disk over water technique, CT levels were significantly higher (198.5±14.3 ng/ml; p<0.001 vs. controls, SF, and sleep deprivation). Similarly, animals exposed to the inverted flower pot approach also showed increased CT levels (178.9±11.7 ng/ml; p<0.002 vs. controls, SF, and sleep deprivation).

MANOVA analysis:

Multivariate analysis showed that behavioral state was found to vary with time, state and condition, as reflected in a significant two-way interaction of time×state ($F=12.33$, $p<0.0001$) and condition×state ($F=3.02$, $p<0.0001$). Furthermore, the significant three-way interaction of time×condition×state showed that the experimental manipulations did have an influence on state and across 24 h recordings ($F=2.81$, $p<0.0001$).

Sleep-wakefulness and EEG delta power in socially paired mice:

Wakefulness: Overall analysis of the polygraphic data for a period of 24 h revealed significant changes between baseline and SF, ($p<0.001$) indicating that SF had influenced state. EEG monitoring during 6 h SF showed that the mice were awake 57.8 ±8.6 % of the time, while the undisturbed control animals were awake 37.4 ±6.6 % of the time. The SF group showed an initial increase in wake which was statistically significant compared to controls. However, SF-exposed animals showed decreased wake thereafter, indicating that they could easily resume sleep in the presence of the SF procedure from 7.00 am to 9.00 am, mice were awake 74.6 ±6.3 % of the time ($F=5.44$, $p<0.04$), during 9.00 am to 11 am, they were awake 52.7 ±3.3 % of the time ($F=17.41$, $p<0.003$), and during 11.00 am to 1.00 pm they were awake 46.2 ±5.2 % of the time which were comparable to baseline (Fig. 3A). While there were no significant differences between controls and SF animals for the 6 hours of the light period immediately following cessation of SF, SF-exposed mice showed a significant decrease in wakefulness, 9.00 pm to 11.00 pm, ($F=7.61$, $p<0.025$), 11.00 pm to 1.00 am, ($F=5.34$, $p<0.05$) and 1.00 am to 3.00 am, ($F=18.90$, $p<0.002$) (Fig. 3A; Table 1).

Slow wave sleep: Overall analysis of the polygraphic data for a period of 24 h showed a significant change between baseline and SF, ($p<0.001$) indicating that SF influenced state. SF mice were in SWS 39.1 ±7.3 % of the time while control animals were in SWS 55.3 ±5.5 % of the time. The SF group showed an initial decrease in SWS during the first 2 h of the SF procedure (Fig. 3B), there were no significant differences thereafter till cessation of SF and even during the last 6 h of the light period. However, the SF group showed significant increases in SWS from 9.00 pm to 11.00 pm, ($F=7.85$, $p<0.023$), 11.00 pm to 1.00 am, ($F=5.25$, $p<0.05$) and 1.00 am to 3.00 am, ($F=16.69$, $p<0.004$) (Fig. 3B; Table 1).

REM sleep: As with other states, similar results were obtained with REM sleep for SF procedures ($p<0.001$). EEG monitoring during the 6 h of SF showed that the animals were in REM sleep 3.1 ±1.4 % of the time and the undisturbed sleeping control animals were in REM sleep 7.2 ±1.1 % of the time. There was a significant decrease in REM during SF, 7.00 am to 9.00 am ($F=8.70$, $p<0.018$) and 9.00 am to 11.00 am ($F=15.75$, $p<0.004$). However, as the SF progressed, animals showed a gradual increase in REM sleep towards control values (Fig. 3C). No significant differences were seen in REM sleep between controls and SF animals during the latter 6h of the light period. However, the SF group showed significant increases in REM sleep from 1.00 am to 3.00 am ($F=15.27$, $p<0.004$) and from 5.00 am to 7.00 am ($F=17.70$, $p<0.003$) (Fig. 3C; Table 1).

EEG delta power during SWS: Overall analysis of the data for a period of 24 h showed significant changes between baseline and SF ($p<0.001$), indicating the experimental condition had significant effects on global EEG delta power. SF animals showed a slight increase in delta power immediately after SF procedure, which was significantly greater throughout the dark period, 9.00 pm to 11.00 pm ($F=7.975$, $p<0.04$), 11.00 pm to 1.00 am ($F=7.984$, $p<0.04$), and 5.00 am to 7.00 am ($F=14.50$, $p<0.019$) (Fig. 3D).

Sleep-wakefulness and EEG delta power in socially isolated group:

Wakefulness: Overall analysis of the data for a period of 24h showed a significant change between treatments (control and SF) ($p<0.001$). EEG monitoring during 6 h SF showed that the mice were awake 59.9 ±8.1 % and the undisturbed sleeping control animals were awake only 36.5 ±2.3 % of time. SF animals exhibited a gradual decrease in wakefulness after the initial peak. The animals undergoing SF were awake (during 7.00 am to 9.00 am, 75.5 ±1.2 %, during 9.00 am to 11 am, 55.9 ±4.8 % and during 11.00 am to 1.00 pm, 48.3 ±3.9 %) of the time (Fig. 4A). The SF group showed a significant increase in wakefulness only for the first 2 h during the SF period (7.00 am to 9.00 am ($F=64.688$, $p<0.001$) (Fig. 4A). Immediately after SF the SF group showed no significant changes in the percent time of wake. However during the dark period, the SF group showed a significant decrease in wake (11.00 pm to 1.00 am, ($F=42.74$, $p<0.003$)), (3.00 am to 5.00 am, ($F=26.70$, $p<0.007$)) and during (5.00 am to 7.00 am, ($F=9.83$, $p<0.035$) (Fig. 4A; Table 1).

Slow wave sleep: The polygraphic data analysed for a period of 24h showed a significant change between treatments (control and SF) ($p<0.001$). EEG monitoring during 6 h SF showed that the mice were in SWS 37.5 ±6.8 % and the undisturbed sleeping control animals were in SWS only 52.7 ±1.8 % of time. Both SF animals exhibited a gradual increase in SWS after the initial dip. The animals undergoing SF were in SWS (during 7.00 am to 9.00 am, 24.4 ±1.2%, during 9.00 am to 11 am, 40.6 ±4.1 % and during 11.00 am to 1.00 pm, 47.3 ±2.6%) of the time (Fig. 4B). The SF group showed a significant decrease in SWS only for the first 2 h ($F=35.04$, $p<0.004$) during the SF intervention respectively when compared to the sleeping controls (Fig. 4B). Immediately after SF the SF and SF-A groups showed no significant change in SWS. However, during the dark period, SF group showed a significant increase in SWS [7.00 pm to 9.00 pm, ($F=8.718$, $p<0.042$); 11.00 pm to 1.00 am, ($F=37.36$, $p<0.004$); 1.00 am to 3.00 am, ($F=17.94$, $p<0.013$) and 5.00 am to 7.00 am, ($F=8.63$, $p<0.042$)] and a significant decrease in SWS during [3.00 am to 5.00 am, ($F=277.60$, $p<0.001$) (Fig. 4B; Table 1)].

REM sleep: Similar results were obtained as with other states for a period. 24h data showed a significant change between treatments (control and SF) ($p<0.001$). EEG monitoring during 6 h SF showed that the mice were in REM sleep 2.6 ±1.3 % and the undisturbed sleeping control animals were in REM sleep 10.7 ±1.1 % of time. SF animals exhibited a gradual increase in REM sleep during the intervention, yet REM sleep remained significantly low throughout the 6 h SF period [7.00

am to 9.00 am, 0.03 ±0.02 %, (F=149.06, p<0.001); 9.00 am to 11.00 am, 3.5 ±0.7 %, (F=30.04, p<0.005)] (Fig. 4C). There were no significant differences between controls and SF animals during the latter 6 h of the light period. However, SF group showed a significant increase in REM sleep during the dark period from 9.00 pm to 11.00 pm, (F=9.66, p<0.036), 11.00 pm to 1.00 am (F=24.45, p<0.008), 1.00 am to 3.00 am, (F=11.40, p<0.028) and from 5.00 am to 7.00 am (F=11.56, p<0.027) (Fig. 4C; Table 1).

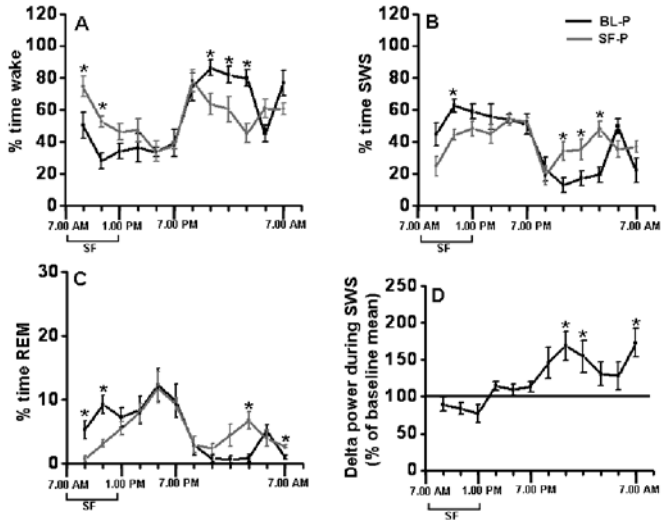

Fig. 3: Sleep-wakefulness and EEG delta power in paired mice. All graphs are plotted per 2 h for a 24 h period. A. Percent time waking during baseline (black line) and sleep fragmentation (SF; gray line). There was no significant difference in wake immediately following SF, but showed a significant decrease in wake during the dark period (9.00 pm to 3.00 am). B. Percent time in SWS during baseline (black line) and SF (gray line). There was no significant difference in SWS immediately following SF, but showed a significant increase in SWS during the dark period (9.00 pm to 3.00 am). C. Percent time in REM sleep during baseline (black line) and SF (gray line). There was no significant difference in REM sleep immediately following SF, but showed a significant increase during the latter part of the dark period. D. There was a significant increase in EEG delta power during the post-SF period. The black line indicates SF period (7.00 am to 1.00 pm). BL-P, baseline-paired; SF-P, sleep fragmentation- paired; SF, sleep fragmentation (7.00 am to 1.00 pm). * p<0.05. See text for more details.

EEG delta power during SWS: Overall analysis of the delta power for a period of 24h showed a significant change between treatments (control and SF) (p<0.001) (Fig. 4D). There were no significant changes in the EEG delta power in SF group during the 6 h of SF procedure (7.00 am to 1.00 pm). Immediately following SF, there was an increase in delta power only for the first 2 h (1.00 pm to 3.00 pm, 28.26%) (Fig. 4D). During the post fragmentation period there was a significant increase in delta power during 11.00 pm to 1.00 am (29.8%, (F=11.31, p<0.028)) (Fig. 4D).

Sleep-wakefulness and EEG delta power in age-matched control group:

Overall analysis and pair wise comparison showed there was no significant change in wake, SWS, REM and delta power in age matched control (Fig. 5 A-D).

Delta power during SWS is attenuated in socially isolated groups:

Comparison between baselines: Overall data for the period of 24 h showed a significant reduction in delta power between baseline (paired) and baseline (socially isolated) mice (F=2.50, p<0.001). In the light period, there was a significant decrease in delta power in socially isolated animals during 11.00 am to 3.00 pm, (11.00am to 1.00 pm, -31.3%, (F=7.12, p<0.028); 1.00 pm to 3.00 pm, -42.1% (F=9.31, p<0.016). During the dark period, significant decreases emerged from 11.00 pm to 3.00 am (11.00pm to 1.00 am, -28.2%, (F=6.08, p<0.039); 1.00 am to 3.00 am, -14.6% (F=5.46, p<0.048) (Fig. 6A).

Comparisons between sleep fragmentation: The 24h period data showed no homeostatic increase in delta power between SF (paired) and SF (socially isolated) mice (F=2.98, p<0.001). There was no significant decrease in delta power in socially isolated animals during the light period, except during 5.00 pm to 7.00 pm, (-43.8%, (F=6.88, p<0.030). However, the dark period showed a significant decrease throughout (7.00 pm to 9.00 pm, -49.1%, (F=12.80, p<0.007); 9.00 pm to 11.00 pm, -51.4%, (F=8.72, p<0.018); 11.00 pm to 1.00 am, -41.1%, (F=5.47, p<0.047); 1.00 am to 3.00 am, -45.7% (F=7.26, p<0.027); 5.00 am to 7.00 am, -46.3%, (F=6.35, p<0.036). No significant change during 3.00 am to 5.00 am was noted (Fig. 6B).

Comparisons between sleep fragmentation and age matched control: There was no significant change in delta power between SF and age matched control.

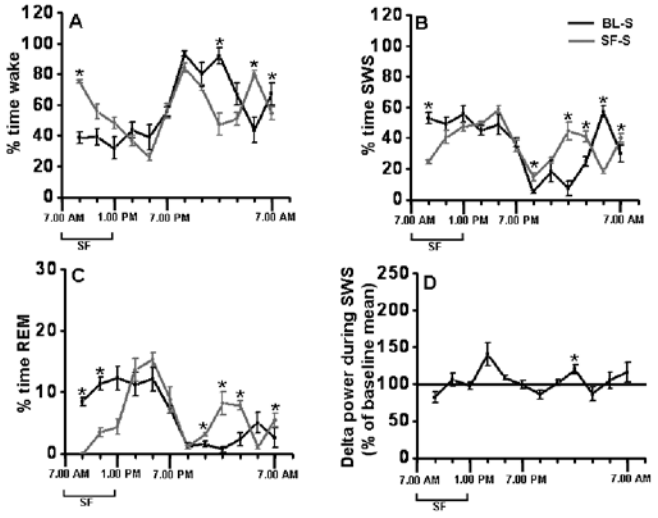

Fig. 4: Sleep-wakefulness and EEG delta power in socially isolated mice. All graphs are plotted per 2 h for a 24 h period. A. Percent time waking during baseline (black line) and sleep fragmentation (SF; gray line). There was no significant difference in wake immediately following SF,

but showed a significant decrease in wake during the latter half of the dark period (11.00 pm to 1.00 am). B. Percent time in SWS during baseline (black line) and SF (gray line). There was no significant difference in SWS immediately following SF, but showed a significant increase in SWS during the latter part of the dark period (11.00 pm to 1.00 am). C. Percent time in REM sleep during baseline (black line) and SF (gray line). There was no significant difference in REM sleep immediately following SF, but showed a significant increase in REM sleep during the latter part of the dark period. D. There was no significant increase in delta power after SF in isolated group). The black line indicates SF period (7.00 am to 1.00 pm). BL-S, baseline- single; SF-S, sleep fragmentation- single; SF, sleep fragmentation (7.00 am to 1.00 pm). *p<0.05. See text for more details.

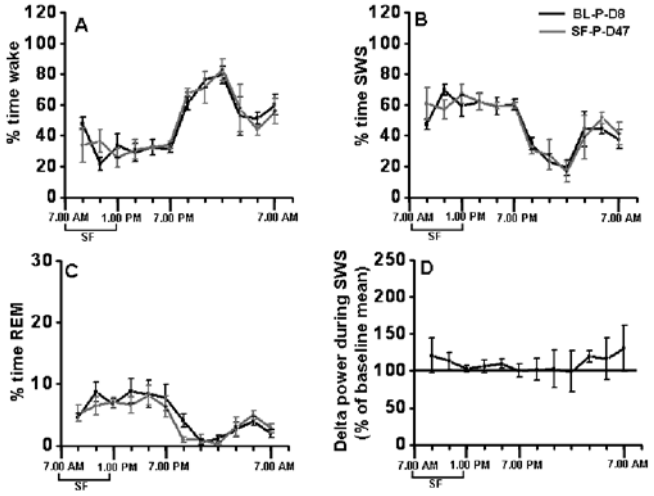

Fig. 5: Sleep-wakefulness and EEG delta power in age-matched control mice. All graphs are plotted per 2 h for a 24 h period. A. Percent time waking during baseline – paired Day 8 (black line) and baseline – paired Day 47 (gray line). There was no significant difference in wake (A), SWS (B), REM (C) and delta power (D). The black line indicates SF period (7.00 am to 1.00 pm). BL-P-D8, baseline- paired- day8; BL-P-D47, baseline- paired- day 47).

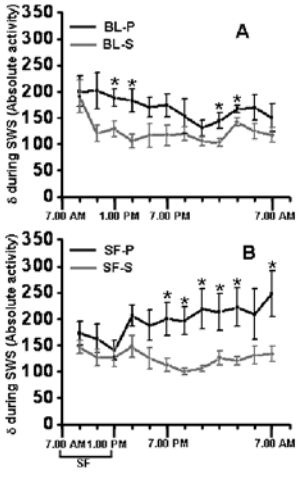

Fig. 6: EEG delta power during SWS is attenuated in socially isolated mice. A. Baseline recording showed a trend level decrease in EEG delta power through out the 24 period in socially isolated mice (gray line) as compared to the paired mice (black line). B. After sleep fragmentation, the socially isolated mice showed a significant decrease in delta power through out the dark period (gray line). The black line indicates SF period (7.00 am to 1.00 pm). BL-P, baseline- paired; BL-S; baseline- single; SF-P, sleep fragmentation- paired; SF-S, sleep fragmentation- single; SF, sleep fragmentation (7.00 am to 1.00 pm); * p <0.05.

Latency from wake to sleep is greatly reduced in SF animals:

The average latency of SWS after every episode of wake was calculated during the first 2 h (7.00 am to 9.00 am) and during the final 2 h (11.00 am to 1.00 pm) to determine the sleep propensity during sleep fragmentation. During the first 2 h, the mean latency to sleep was comparable to the corresponding baseline in both paired and socially isolated mice. However as the time progressed, the latency to sleep was significantly reduced in both the paired and socially isolated mice indicating development of sleep pressure. During the final 2 h, the paired mice had a latency of 25.0 ±3.0 sec ($p < 0.05$) compared to the same circadian time of paired sleeping controls, 145.6 ±45.9 sec (Fig 6). Similarly, during the final 2h of SF, the socially isolated mice had a latency of 14.5 ±0.9 sec ($p < 0.04$) compared to the same circadian time of socially isolated sleeping controls, 115.7 ±34.2 sec (Fig. 7).

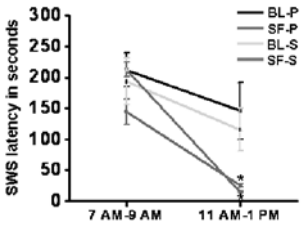

Fig. 7: Latency from wake to sleep is greatly reduced in sleep fragmented mice. The mean latency from wake to sleep was comparable to baseline recording during the first hour of recording. However during the last 2 hour of sleep fragmentation, both the paired and socially isolated mice showed a significant decrease in mean latency from wake to sleep indicating mounting sleep pressure. * p <0.05. BL-P, baseline- paired; BL-S; baseline- single; SF-P, sleep fragmentation- paired; SF-S, sleep fragmentation- single; SF, sleep fragmentation (7.00 am to 1.00 pm); * p <0.05.

Temperature and activity

To determine the overall statistical significance of the effect of baseline, SF (paired) and SF (isolated) body temperature and gross motor activity, repeated measures ANOVA was performed. There was a significant effect on body temperature across the 24 h period between the experimental groups ($F=14.26$, $p < 0.001$). Post hoc analyses comparing the baseline with SF (paired) and SF (isolated) for each 2 h bin showed a significant increase in body temperature in SF-isolated group during 11.00 am to 1.00 am ($F=5.68$, $p<0.029$), and decrease during 3.00 pm to 5.00 pm ($F=5.88$, $p<0.027$) and during 5.00 am to 7.00 am ($F=6.37$, $p<0.022$) (Fig. 8A). Gross activity also showed a significant effect across the 24 h period between the

experimental groups (F=7.97, p <0.001). Further, post-hoc analysis showed a significant increase in activity in both SF-paired and SF-isolated during the initial 2 h of SF procedure (F=6.37, p<0.022) but gradually decreased for the latter part of the SF procedure. The SF-isolated animals showed a marked decrease in activity through most of the post-SF period, 3.00 pm to 5.00 pm (F=5.30, p<0.034) and 7.00 pm to 9.00 pm (F=5.14, p<0.037) (Fig. 8B).

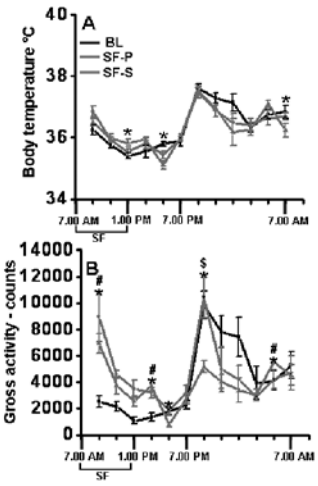

Fig. 8: Body temperature and gross activity in paired and single mice. A. 24 h recording showed no significant change in body temperature in socially isolated mice (red line) and paired mice (gray line) when compared to the baseline recording (black line) after SF. B. Similarly, 24 h recording showed no significant change in gross activity in socially isolated mice (red line) and paired mice (gray line) when compared to the baseline recording (black line) after SF, although the post-SF period showed a trend level decrease in activity in isolated animals. The black line indicates SF period (7.00 am to 1.00 pm). BL, baseline; SF-P, sleep fragmentation-paired; SF-S, sleep fragmentation-single; SF, sleep fragmentation (7.00 am to 1.00 pm). p<0.05. * = comparison between baseline and SF-S; $ = comparison between SF-P and SF-S; # = comparison between baseline and SF-P. See text for more details.

DISCUSSION

We developed an animal model of SF in which mice were aroused periodically, to mimic the SF that occurs in many disease conditions, particularly in OSA. Using this model, we studied the effect of social isolation on sleep and EEG delta power during SWS. Six hours of SF in both socially paired and socially isolated group did not elicit an immediate change in sleep-wakefulness. There was an increase in SWS and REM and a decrease in wake during the dark period. However there were no significant differences in total time spent in wake or SWS. In fact, the most prominent changes emerged in the delta spectral power of the EEG, whereby socially isolated animals did not exhibit homeostatic increases in delta power as compared to the socially paired groups, both during baseline and also after SF. Furthermore, there was no increase in delta power during the post-SF period. Taken together, these findings suggest that the sleep homeostatic responses seen in acute sleep deprivation (SD) cannot be generalized to the responses elicited by acute SF, in which sleep loss is markedly smaller.

Sleep in mammals is a complex phenomenon, generally alternating between two distinct states, SWS and REM sleep. The exact oscillatory mechanisms underlying the periodic cycling between these states are still largely unknown. Nevertheless, it is well documented that the duration of the prior wake period may directly influence the succeeding sleep bouts, especially with a sleep rebound and an increase in EEG delta power during SWS, a relationship that has now been firmly established in a variety of species (33-36), including humans (37,38). However, most of the observations indicate there is no consistent relationship between the duration of prior wakefulness and the duration of subsequent SWS, since the duration of the latter can be compensated by a shorter sleep period that exhibits higher slow-wave intensity. Notwithstanding these observations, how EEG delta power during SWS is affected by different kinds of stressors remains unknown. A previous study reported that rats showed a sharp increase in slow-wave activity during SWS after a social conflict with aggressive and dominant rats (39,40). This type of fear-awakening which elicits intense social stress will increase the ensuing slow-wave activity. However, the impact on recovery sleep following imposition of other stressors, such as those inherent to the disruption of sleep was never ascertained.

SF method is relatively stress-free

A plethora of studies supports the notion that acute sleep loss induces stress and the release of stress-related hormones. One of the major limitations of existing methods aiming to induce sleep loss is that they are stressors per se, and as such, the intrinsic stress effect of sleep loss can not be ascertained. Sleep deprivation induced either by the 'inverted flower pot' method, gentle manual handling, or by the forced locomotion method has been shown to increase levels of ACTH and corticosterone (41-43). Since our main interest was to mimic the patterns of activity seen in sleep disorders as closely as possible, these methods were not ideal for our experiments. In addition, these methods are either labor intensive (e.g., manual handling) or impose forced locomotion (12,19,41-43). In contrast, when mice were subjected to SF and SD using the novel procedure described herein, the resultant CT levels were comparable to the levels measured in control mice, indicating that this methodology does not appear to modify the circulating levels of stress hormones. Even though SF procedure did not result in increased CT levels at the time of euthanasia (i.e., the end of 6 h SF), this measurement does not conclusively demonstrate whether CT levels are increased earlier, during the initial phase of SF procedure. Future additional studies are needed to determine whether the absence of a systemic elevation in CT levels as seen in our novel SF/SD technique, when applied for a 6-hour period, is also confirmed with more extended exposures and not accompanied by changes in other hypothalamic-pituitary-adrenal response markers. Another surrogate indicator of stress is alterations in body temperature. Although restraint stress has shown to increase body temperature in mice (44), some studies have reported no change in body temperature in rats (45,46). Social stress in rats, on the other hand, has been associated with an increase in body temperature (47). In our study the SF procedure did not

cause significant changes in body temperature, suggesting a stress-free nature of the procedure itself. It is worth noting that the body temperature decreased after the initial 2 h SF period, indicating the mice could return to sleep in between arousals due to SF.

An additional comment pertaining to the social context in which SD or SF is applied deserves comment (see also below). The stressor response associated with the SD procedure may also be modulated by the social isolation that traditionally accompanies this type of experiments. Indeed, Suchecki and Tufik (2000) have shown that adrenocortical responses and eating behaviors were improved when rats exposed to SD using the multiple platform technique were allowed the presence of stable cagemates as opposed to unknown rats (48). Our ability to induce sleep disruption in either social isolation or socially paired conditions should permit improved delineation of the role played by the contextual social situation in the regulation of sleep homeostatic responses.

Further evidence attesting to the efficacy of our new SD/SF approach resides in the finding that the latency to enter SWS from a wake episode was significantly reduced over time in all the mice that underwent SF. Thus, the momentary arousal elicited by the device was not sufficiently stressful to maintain the vigilance of the animals for long periods of time.

Social isolation is accompanied by decreased EEG delta power during SWS compared to social pairing under natural sleep conditions and sleep fragmentation

Socially isolated mice showed a dramatic decrease in EEG delta power, even during the basal conditions, even though the total time spent in sleep-wakefulness did not change. Furthermore, socially isolated animals did not show a rebound increase in delta power when compared to the socially paired group. Recent studies have shown the role of NF-κB and TNF-α in modulating SWA and EEG delta power. In particular, when NF-κB activity is blocked by the inhibitor peptide, SN50, there was a significant reduction in the relative delta power (49). Such reduction in delta power, without gross change in sleep-wake activity was also reported when NF-κB activity is blocked by the inhibitor peptide, SN50 in rats, particularly during the first 2 h of recovery sleep following 6h SD (18). TNF-2R KO mice also showed reduced delta power in response to viral challenge (50). In addition to sleep, social isolation dramatically affects many physiological functions, including level of aggressiveness, anxiety-related behaviors, cognitive deficits, and hyperlocomotion (51-53).

In the present study, SWS showed no immediate rebound in delta power in the socially paired group after SF, but instead showed a considerable increase in delta power in the dark period, which may have been partly due to enhanced SWS pressure, as reflected by such increased delta activity. Such a trend was not seen in socially isolated animals. However, these animals showed significantly reduced EEG delta activity when compared to the socially paired group.

The immediate increase in REM sleep following SF in the socially isolated group could be related to a compensatory mechanism for the reduced SWS activity seen in parallel with the light period homeostatic drive. If this is indeed the case, then we should expect increased REM sleep during the following light period. Stress may affect REM sleep but the effect is thus far controversial. On the one hand, when restraint stress is used REM sleep was suppressed (54,55) and yet on the other hand, stress was associated with an increase in REM sleep in other studies (56,57). It is possible that different types of stressors and the underlying conditions in which the particular stressors are applied (i.e., time of day, intensity of stimulus applied and duration of stressor) may all account for the opposite effects of stress on REM sleep reported to date.

After SD, sleep rebound during recovery period is a well established phenomenon that is tightly regulated by homeostatic processes (58-62). The homeostatic drives that influence sleep rebound are in turn influenced by stress. However, this influence depends on the type and duration of the stressor and shows a dual-effect pattern. Acute stressors lead to a subsequent sleep rebound (54,63), while prolonged and/or chronic stress reduce the time spent in SWS, thereby affecting sleep quality (64,65). Previous studies have shown that short-lasting immobilization or restraint stress increase REM sleep duration (57), whereas chronic immobilization leads to a decrease in REM sleep rebound (54,55) and induces hippocampal atrophy (66), further confirming the hypothesis that stress actively modulates behavioral state. The absence of dramatic changes in sleep-wake patterns as observed in the mice subjected to SF, may therefore reflect the relatively stress-free nature of this novel intervention, and therefore allow conducting chronic SF procedures.

We should emphasize that the SF procedure used herein induced increases in sleep pressure but also acutely disrupted the amount of total sleep, particularly during the initial hours after onset of the device activity. While this is an undesired consequence of the procedure, the reduction in overall sleep progressively abated as the SF procedure is continued, such that global sleep duration returns to the pre-intervention baseline levels. Thus, the normalization of sleep duration using this new SF technique opens the way for long lasting studies on the effect of fragmented sleep independent from the effect of sleep restriction or deprivation.

The effect of SF in social isolation differs from the effect of SF in socially paired conditions

Social bonding in animals plays a pivotal role in modulating many physiological functions, including sleep-wake patterns. Previous studies have shown that REM sleep plays an important role in social bonding in mammals (67,68). In several species including humans, maternal deprivation is associated with disrupted and decreased REM sleep during separation followed by a REM sleep rebound after reunion (69,70). This "bonding hypothesis" suggests that, in addition to other physiological functions, REM sleep is fundamental to promote attachment between parent and siblings (and vice versa) and also between adult mating partners. In a recent study, rats subjected to 6h SD using a modified multiple platform method, who had their bonding renovated everyday for the remainder 18 h, showed marked rebounds in REM sleep compared to socially isolated rats (23). Thus, the social contextual setting needs to be controlled for in future experiments involving sleep manipulations and their subsequent recovery responses.

In summary, we present evidence supporting the use of a novel approach to induce either SD or SF in a murine model that is void of some of the major limitations of previous reported techniques, namely elevated stress responses or forced locomotion. Further-

more, we show that social interactions in the context of SF play an important role in modulating the quality of sleep and its recovery from SF, thereby emphasizing the need to incorporate the contextual social setting in future experiments aiming to determine the regulation of sleep homeostatic responses.

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STUDY OF METABOLIC CHANGES IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA SYNDROME BEFORE AND AFTER USE OF CONTINUOUS POSITIVE AIRWAY PRESSURE

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ABSTRACT

Background and objective: Obstructive Sleep Apnea Syndrome (OSAS) is the most common sleep-disordered breathing (SDB) syndrome and is characterized by repetitive, total, or partial pharyngeal collapse during sleep. These symptoms induce both hypoxemia and brief arousals from sleep. As a result, daytime sleepiness, reduction in cognitive performance, an increase in the incidence of cardiovascular diseases and traffic accidents have been observed. To date, the most effective treatment for OSAS has been the use of Continuous Positive Airway Pressure (CPAP). Studies confirming the reversibility of alterations such as hypertension, hyperleptinemia, and an increase in inflammatory cytokines after therapy with CPAP in patients with OSAS are controversial. The purpose of this study was to evaluate the metabolic markers present in the blood of patients with OSAS before and after use of nasal CPAP treatment.

Methods: Thirteen patients with moderate to severe OSAS were selected for the current study. After submitting their informed consent to participate in the study, the selected patients answered a sleep questionnaire and were submitted to a physical examination. They were also asked to perform a polysomnography throughout an entire night for diagnosis, CPAP pressure titration, and blood collection. All patients were submitted to treatment with CPAP for six months, after which all evaluations were repeated.

Results: When we compared patients with OSAS with healthy control individuals, we found increased levels of ghrelin and triglycerides and reduced levels of HDL in patients with OSAS ($p < 0.05$). In addition, no difference in leptin levels was observed. After a six-month treatment period with CPAP, a significant drop in ghrelin levels could be observed ($p < 0.05$). No changes in the patients' body weight was observed during the treatment period (29 ± 4 x 29 ± 3 Kg/m^2).

Conclusion: We thus concluded that OSAS can be considered an independent factor for increasing ghrelin levels and that a reduction in the levels of this hormone occurs after six months of CPAP treatment.

Keywords: Obstructive Sleep Apnea Syndrome, CPAP, leptin, ghrelin, obesity.

INTRODUCTION

Obstructive Sleep Apnea Syndrome (OSAS) is the most common chronic sleep-disordered breathing (SDB) syndrome among the adult population. Approximately 4% to 24% of male adults and 1% to 9% of female adults in the general population suffer from OSAS (1,2).

OSAS is characterized by repetitive episodes of partial upper airway obstruction (hypopnea) or total upper airway obstruction (apnea), causing oxyhemoglobin desaturation and, in persistent cases, hypercapnia, which are frequently reverted by arousals, thus inducing sleep fragmentation (3).

Recent prospective and controlled studies with prolonged follow-ups demonstrated that OSAS is an independent risk factor for hypertension (4). In addition, OSAS contributes to other comorbidities, such as cardiac arrhythmias (5), coronary insufficiency (6), heart failure (7), and stroke (8).

Currently, researchers are searching for physiopathogenic explanations that concur to the increase in cardiovascular abnormalities associated with OSAS. Two main lines of research that aim at assessing the two major OSAS consequences, hypoxia-hypercapnia and sleep fragmentation (9-11), have been established.

It has been suggested that obesity (proinflammatory syndrome), which is frequently associated with OSAS, would be a determinant or predisposing agent for increasing cardiac risk in this syndrome (12). In early studies in the field, it was hypothesized that resistance to insulin in patients with OSAS was thoroughly dependent on obesity (13). Nevertheless, in 2000, Vgontzas and collaborators detected higher resistance to insulin and hypercytokinemia in obese patients with OSAS when compared with obese patients without OSAS, and patients of normal weight, thus suggesting that such alterations might contribute to a high cardiovascular risk when this syndrome is present (14). Another study found higher levels of leptin, insulin, and triglycerides in obese patients with OSAS compared with obese patients without OSAS (15). After treatment with CPAP, a reduction in leptin and triglyceride levels was observed, but not in serum insulin levels. This evidence suggests that resistance to insulin may be involved in the genesis of OSAS (15).

Previous studies demonstrated that in both OSAS and in obesity, in addition to resistance to insulin, patients showed an independent increase in inflammatory cytokines levels (TNF-α and IL-6). In 1997, Vgontzas and collaborators reported an increase of tumor necrosis factor-alpha (TNF-α) and in interleukin-6 (both related to sleepiness), not only in the presence of OSAS, but also in obese patients. These results were independent of each other. Nevertheless, it still remains to be established whether the increase in such cytokines and resistance to insulin are primary OSAS events or events secondary to OSAS (16,17).

The vast majority of obese people show high levels of circulating leptin. The strong correlation between obesity and OSAS in humans and the establishment of leptin as a ventilatory stimulant and appetite suppressor in rats has raised the possibility that apnea is associated with a leptin deficiency. However, recent studies demonstrate that individuals with OSAS show an increase in leptin levels, thus suggesting that there may be a relative resistance to circulating leptin (15).

Phipps and collaborators have recently reported hyperleptinemia associated with a collapse of hypercapnic breathing in obese people. In such cases, treatment with leptin might reverse the hypoventilatory syndrome in obese patients who show a ventilatory response to hypercapnia (18).

Shimizu and collaborators have recently evaluated 21 patients with an apnea/hypopnea index (AHI) above 20 after one-day of treatment with CPAP. They detected reduction in serum leptin levels and in cardiac sympathetic activation. It still remains to be elucidated whether the reduction in leptin levels after treatment with CPAP has influenced sympathetic activity or whether the reduction in sympathetic activity has contributed to the reduction in leptin levels (19).

Ghrelin is an appetite-stimulating peptide derived predominantly in the stomach. Ghrelin levels increase before meals and decrease after meals. The mechanism of this hormone can be considered opposed to that of the leptin hormone, which is produced in fatty tissues and controls appetite (20). In obese people, plasma ghrelin concentrations are lower than those found in people with average weights. (21). Yildiz and collaborators have demonstrated that, in average weight subjects, ghrelin concentrations show an increase during the night, which exceeds the increase after meals, although this type of increase was not observed in obese subjects (22).

Most studies conducted with patients who have OSAS and measurements of metabolic hormones are controversial, as obesity represents a confounding factor. Obese patients with OSAS compared with obese patients who do not have OSAS showed high levels of ghrelin and leptin, which decreased after treatment with CPAP. No changes in the body mass index (BMI) of such patients could be observed (23).

We could thus conclude that leptin and ghrelin hormones play key roles in the metabolism of patients who have OSAS, regardless of obesity.

The purpose of this study was to verify whether obesity in the presence of OSAS determines metabolic alterations or aggravates them and to verify the clinical response and the metabolic markers before and after the use of CPAP equipment.

MATERIALS AND METHODS

Casuistics

Thirteen patients who underwent ambulatory treatment for breathing disorders at the Universidade Federal de São Paulo (UNIFESP) in 2004 and who were clinically and polysomnographically diagnosed as having OSAS, as per the American Academy of Sleep Medicine (24) task force, were selected for study. After receiving orientation regarding the study, patients agreed to participate in the study and signed an informed consent term.

The study was approved by UNIFESP Ethics Research Committee (number 1266/03).

Inclusion Criteria

- Patients who met the polysomnographic criteria for OSAS

proposed by American Academy of Sleep Medicine in 2005 included:

- Adults between 30 and 60 years of age.
- Both male and female.
- BMI < 35 Kg/m^2.
- AHI higher or equal to 15 events per hour (OSAS from mild to severe)
- Normal hemogram, hepatic function, renal function and electrocardiogram.
- Patient's consent after explanation of the protocol and signature of the informed consent term.
- Availability for answering questionnaires, visiting the sleep laboratory for undergoing polysomnography (for diagnosis, CPAP titration, and control after a six-month treatment and use of CPAP equipment for a minimum period of 6 months).

Exclusion Criteria

- Endocrine disorders, such as hypothyroidism and diabetes.
- Lung diseases.
- Cardiovascular diseases.
- Infectious diseases.
- Neurological and psychiatric diseases.
- Other sleep disorders.
- Individuals using sleep inductors, neuroleptic drugs, or beta blockers.
- Tobacco smokers, alcohol addicts and shift workers.
- Patients who have already undergone previous OSAS treatment.

Controls

Thirteen healthy volunteers matched for BMI, age, and sex were selected and studied using the same clinical, polysomnographic, and laboratorial methods.

Study Design

Anamnesis, a physical examination, and filling out the Epworth Sleepiness Scale (ESS) were performed both at the beginning and the end of the research period (25). Patients were also submitted to routine lab tests for selection and exclusion of other pathologies not related to the study.

Three polysomnography PSGs were accomplished during the research period. The first aimed at assigning a diagnosis, the second was for CPAP titration, and the third was performed six months after the use of CPAP equipment. During the research period, the subjects under evaluation visited the lab once a month in order to clarify any possible doubt regarding diagnosis and treatment for the syndrome. They also visited for analysis of adherence to treatment through the CPAP built-in compliance meter. Blood collection for measuring any metabolic changes was performed at the beginning and end of the treatment. At the end of treatment with CPAP equipment, the clinical tests and ESS were also repeated.

Study development

Clinical analyses of the patients

Anamnesis and a detailed physical test including ectoscopy, oropharyngeal evaluation, arterial pressure, pulse, weight, and height, as well as neck, hip and waist circumferences were accomplished. ESS evaluates eight routine situations, ranging from those which do not require great attention to those which require a great level of attention. The Epworth Sleepiness Scale ranges from 0 to 3 (0, 1, 2, 3), where 0 corresponds to no possibility of sleeping, 1 to a slight possibility of sleeping, 2 to a relative possibility of sleeping, and 4 to a great possibility of sleeping (25).

Lab Analysis

Blood samples for the assessment of metabolic changes were collected at two independent times (at the beginning of the study and six months after treatment with CPAP). On the evening before blood was to be collected for detection of any metabolic change, patients were admitted in the Sleep Institute and were instructed to fast from 08:00 pm to 06:00 am the following day; blood was collected early in the morning. Serum ghrelin and leptin levels, as well as blood glucose levels at fasting, insulin, total cholesterol, triglycerides, HDL, LDL, VLDL, fibrinogen and CRP (C-reactive protein) were tested.

Polysomnographic Study

PSG equipment used for data processing, collection, analysis, and compilation of the elements necessary for a polysomnographic study was utilized. This equipment was contained in a Sonolab computerized system, version 2003-A.

The study utilized fifteen channels, including three for electroencephalography (EEG - C3/A2, C4/A1, O1/A2, O2/A1), two for right and left electrooculography (EOG), one for submentonian electromyography (EMG), one for anterior tibial electromyography, one for electrocardiography (ECG), one for airflow (thermistor) and nasal pressure cannula, two for thoracic-abdominal movements, one for registering tracheal vibration, one for measuring oxyhemoglobin saturation by pulse oximeter (Oxycap, Ohmeda, Denver, CO or Model N1000, Nelcor Inc, Hayward CA), and one for registering corporeal position.

All polysomnographic tests were performed at night, in a dark and quiet room specifically designed for this procedure. Patients arrived at the lab approximately two hours before their usual bedtime to become familiar with the space and prepare to sleep. After that, the polysomnographic equipment was assembled. On average, it took nine hours to accomplish all tests (10:00 pm to 07:00 am). Sleep staging followed patterns established by Rechtschaffen and Kales (1968) (26). For breathing events, the analysis followed AASM patterns (1999) (27), and, for arousals, it followed ASDA patterns (1992) (28). For lower limb movements, ASDA patterns were followed (1993) (29).

Statistical Analysis

The parametric results were expressed through mean and standard deviation. For comparison of parametric variables between groups, we applied the t test for independent samples.

RESULTS

Ages and BMIs for the control group and the OSAS group were

similar to each other (Table 1). When comparing data referring to the control group and the OSAS group, ESS scores showed an increase in the group with OSAS ($p<0.001$). In addition, increases in AHI and arousal index ($p<0.01$), and a lower SpO_2 when compared with the control group ($p<0.001$) were observed (Table 1).

Table 1: General data and PSG data from controls and patients with OSAS.

	Controls (n = 13) Mean ± SD	Patients with OSAS (n = 13) Mean ± SD	p
Age	36 ± 6	37 ± 7	NS
BMI (Kg/m²)	27 ± 3	29 ± 3	NS
ESS	5 ± 4	15 ± 4	0.001
AHI	2 ± 1	41 ± 29	0.001
SpO_2 min	89 ± 3	77 ± 13	0.001
SE	87 ± 8	88 ± 9	NS
S1	3 ± 2	7 ± 5	0.04
S2	59 ± 6	64 ± 12	NS
S3+4	15 ± 4	11 ± 7	NS
REM	22 ± 3	18 ± 9	NS
ARI	8 ± 3	38 ± 37	0.01

BMI: Body Mass Index, ESS: Epworth Sleepiness Scale; AHI: Apnea/Hypopnea Index; SpO_2 min: minimal oxygen saturation; SE: Sleep Efficiency; ARI: Arousal Index.

During their basal evaluations, patients with OSAS showed increased ghrelin and triglyceride levels and reduced HDL levels when compared with the control group. In addition, no significant difference in leptin levels could be detected (Table 2).

Table 2: Metabolic parameters of to controls and patients with OSAS.

	Controls (n = 13) Mean ± SD	Patients with OSAS (n = 13) Mean ± SD	p
Ghrelin (pg/ml)	1010 ± 262	1642 ± 942	0.04
Leptin (ng/ml)	15 ± 6	20 ± 11	NS
Glucose (mg/dl)	90.5 ± 7.5	93.63 ± 8.95	NS
Insulin (μIU/ml)	6.28 ± 4.85	9.11 ± 4.65	NS
Triglycerides (mg/dl)	120.5 ± 55.6	164 ± 47	0.04
Total cholesterol (mg/dl)	203 ± 43	213 ± 42.84	NS
LDL (mg/dl)	123 ± 33	137 ± 39	NS
VLDL (mg/dl)	24.5 ± 11	33 ± 9	NS
HDL (mg/dl)	56.5 ± 10.44	44 ± 11	0.01
CRP (mg/dl)	0.13±0.1	1.6±5	NS
Fibrinogen (g/L)	3.2 ± 0.7	3.6 ± 1.3	NS

LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; HDL: high-density lipoprotein; CRP: C-Reactive Protein.

When comparing overweight and average weight patients (BMI between 24 and 29.9 Kg/m²) with Class I obese patients (BMI between 30 and 35 Kg/m²), no difference was observed in either ghrelin or leptin levels (Table 3).

Table 3: Ghrelin and leptin values in patients with OSAS: normal weight/overweight versus obesity Class I.

	Normal Weight/ Overweight (n=7) Mean ± SD	Obesity Class I (n=6) Mean ± SD	p
BMI (Kg/m²)	26 ± 2	32 ± 1	0.0002
Ghrelin (pg/ml)	1597 ± 919	1705 ± 1078	NS
Leptin (ng/ml)	17 ± 8	27 ± 12	NS

BMI: Body Mass Index.

After treatment with CPAP, a significant drop in ghrelin levels was observed, while no difference could be observed in the leptin levels of patients with OSAS (Table 4). No change in patients' weight during the treatment period was observed (29±4 x 29±3 Kg/m²). In addition, no significant difference in the parameters evaluated was observed after the treatment period.

Table 4: Basal data from patients with OSAS after 6 months using CPAP equipment.

	Basal (n = 13) Mean ± SD	After CPAP (n = 13) Mean ± SD	p
BMI (Kg/m2)	29 ± 4	29 ± 3	NS
AHI	41 ± 29	4 ± 2	0.0001
Ghrelin (pg/ml)	1642 ± 942	1032 ± 444	0.04
Leptin (ng/ml)	20 ± 11	22 ± 10	NS
Glucose (mg/dl)	93.63 ± 8.95	94.63 ± 9.90	NS
Insulin (μIU/ml)	9.11 ± 4.65	13.11 ± 4.69	NS
Triglycerides (mg/dl)	164 ± 47	168 ± 69	NS
Total Cholesterol (mg/dl)	213 ± 42.84	220.45 ± 43.89	NS
LDL (mg/dl)	137 ± 39	142 ± 40	NS
VLDL (mg/dl)	33 ± 9	34 ± 14	NS
HDL (mg/dl)	44 ± 11	44 ± 8	NS
CRP (mg/dl)	1.6 ± 5	0.3 ± 0.2	NS
Fibrinogen (g/L)	3.6 ± 1.3	3.6 ± 0.6	NS

BMI: Body Mass Index; AHI: Apnea/Hypopnea Index; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; HDL: high-density lipoprotein; CRP: C-Reactive Protein.

DISCUSSION

Our study has established that patients with OSAS show increased ghrelin and triglycerides levels in addition to reduced HDL levels. We also established that these patients did not show any increase in leptin and glucose levels or resistance to insulin when compared with control subjects. When we compared average weight and overweight OSAS patients with the more obese OSAS patients, we could not find any difference in metabolic measurements among these patients. This leads to the conclusion that obesity is not a determining factor for the results obtained from this study. The use of CPAP for reverting ghrelin levels reinforces the role of OSAS as the responsible agent for this alteration.

The age and the absence of other comorbidities associated with OSAS in our sample justify the mild metabolic alterations in patients with OSAS who participated. The time elapsed from illness onset, which was not estimated in this study, may be another determining factor.

Ulukavak and collaborators analyzed 30 obese subjects with moderate to severe OSAS and 22 obese control subjects without OSAS. They reported a significant increase in leptin levels in the group of obese subjects with OSAS when compared with the group of obese subjects without OSAS. In view of these results, Ulukavak and collaborators suggest that leptin is a hormonal factor affected by OSAS and is not determined by obesity alone (30). Our results differed, perhaps due to ethnic or environmental differences existing in the sample population studied.

We observed that, in our sample, regardless of obesity, patients with OSAS showed increased levels of ghrelin, which decreased after six months of CPAP treatment. However, patients did not show any difference in leptin levels. The fact that we have selected patients without severe obesity (BMI > 35 Kg/m^2) may have interfered with our results. Interesting enough, we observed that, when comparing our data with data collected by Ulukavak and collaborators (2005), the leptin results we found in our subjects were quite similar to those found in their groups of obese people without OSAS. This finding points to the possibility that differences in leptin levels in patients with OSAS occur only when such patients are severely obese.

In a recent study, 30 patients with severe OSAS were selected. In this study, leptin and basal ghrelin levels were evaluated two days and two months after treatment with CPAP. Patients with OSAS showed high ghrelin levels in the basal analysis when compared with the control group. In most cases, patients with OSAS showed a decrease in these levels after a two-day treatment with CPAP. Leptin values did not show a significant difference in the two first days of treatment, although patients with OSAS showed higher values than those in the control group. After a two-month treatment period, leptin levels were significantly reduced and BMI levels showed no alteration. The most significant drop in this study was observed in patients with a BMI <30 Kg/m^2 (23).

In addition, patients with OSAS, although they had fragmented sleep in view of arousals, usually maintained their total sleep period. Recent studies have demonstrated that a reduction in sleep period is associated with a reduction in leptin levels, an increase in ghrelin levels, and an increase in weight (31,32).

As for insulin, a previous study demonstrated that patients with moderate to severe OSAS showed an increase in insulin sensitivity after a two-day treatment period with CPAP and remained stable for three months after treatment. The improvement in insulin sensitivity after two days was much greater in patients with a body mass index less than 30 Kg/m^2 than in more obese patients. These results may reflect a reduction in sympathetic activity, indicating that OSAS is an independent risk factor for increasing insulin resistance (33).

OSAS contributes to unbalanced ghrelin levels, even in patients without severe obesity. Thus, our data suggest that OSAS has an isolated effect for determining increases in the levels of this hormone. In view of this, CPAP therapy has resulted in a significant reduction in the levels of this hormone without any association with a reduction in weight, confirming an OSAS-independent effect. Despite the limited size of the sample utilized in this study, our data point to the importance of metabolic alterations for the development of comorbidities associated with OSAS. In addition, our results illustrate the importance of controlling these alterations via adequate treatment of the syndrome.

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THE EFFECTS OF SLEEP DEPRIVATION AND SLEEP RECOVERY ON PAIN THRESHOLDS OF RATS WITH CHRONIC PAIN

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ABSTRACT

Background and objective: The aim of this study was to compare the effects of different paradoxical sleep deprivation methods on the pain threshold in rats submitted to inflammatory and neuropathic pain models. We also investigated whether changes in pain threshold could be reverted by sleep recovery period.

Methods: Wistar rats were randomly assigned in arthritis-induced by adjuvant (AIA), chronic constrictive injury (CCI) of sciatic nerve and non-handled control group. Paradoxical sleep deprivation was performed using small or large platforms in the water tank technique. Grid and home-cage groups were also evaluated. Pain threshold was determined in dry environment using the hot plate test, before, during and after (recovery) paradoxical sleep deprivation.

Results and conclusion: The data showed that AIA and CCI differ from control groups from the second day on after pain inducing-procedures and lasted until the third day of sleep recovery. Paradoxical sleep deprivation reduced the pain threshold in all groups studied, independently the method used. Sleep recovery did not restore the baseline pain threshold in arthritis-induced animals, but it was restored in CCI group submitted to both paradoxical sleep deprivation methods.

Keywords: sleep deprivation, pain, adjuvant-induced arthritis, sciatic nerve constriction, hot plate, rats.

INTRODUCTION

Acute and chronic pain are closely associated with sleep disturbances. Pain has been reported to be an important cause of sleeplessness (1) and, conversely, interrupted sleep has often been associated with increased pain (1-8) and a leading cause of insomnia in medical conditions (3,9). Clinical trials using rheumatic and fibromyalgia patients (10,11) and animal studies with experimental models of polyarthritic rats (8,12-17) confirm the association between painful manifestations and sleep disruption.

Sleep constitutes a dynamic form of homeostasis restoration and it is pertinent to assume that its abolishment would lead to different behavioral alterations, such as increasing pain sensitivity. In fact, some studies report the influence of sleep disturbances on pain sensitivity. However, such influence is not completely understood. Ukponmwan et al. (18) report reduction of antinociceptive

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property in enkephalinases, morphine and swimming in paradoxical sleep deprived (PSD) rats. Onen et al. (5) described that threshold of vocalization response to pressure nociceptive stimuli in rats is not reduced by PSD, but it is augmented during the recovery period.

Chronically painful conditions are associated with sleep disturbances such as sleep continuity changes, decreased sleep efficiency and fragmentation of sleep pattern (10,13,15-17,19-24). A recent in-depth review by Lautenbacher et al. (21) highlighted the fact that basic mechanisms governing sleep-induced alterations in pain sensitivity are still unknown, and the difficulty in defining the mechanism by which altered sleep affects pain thresholds suggests a fairly complex chain of events. Among these, sleep-deprived animals might have alterations in the opioidergic receptor system, as hypothesized previously (7,25,26).

The reciprocal influence between pain and sleep-deficit does not seem to be a problem in normal individuals as it vanishes with the cessation of pain. Chronic pain sufferers, however, may develop a positive feed-back relationship and aggravate their problems. Some studies (2,4,9) suggest that non-efficient sleep produces an increase in pain as well as fatigue in rheumatoid arthritis and fibromyalgia patients. If such increase in pain worsens sleep again and enhances pain much more, or if there are adaptative processes, are some of the still unanswered issues. Animal models of chronic pain seem suitable to investigate these questions. Among them, the inflammatory chronic pain of the experimental arthritis induced by adjuvant and the neuropathic chronic pain of the sciatic nerve constriction are valuable tools. To use them for such purpose, it is heuristically necessary to demonstrate that they reproduce the clinical observation of increased pain after non-efficient sleep.

The aim of this study was to investigate the effects of PSD methods as well as sleep recovery on the pain threshold of rats submitted to inflammatory and neuropathic pain models.

METHODS

Animals

Adult, male Wistar rats, aged approximately 90 days at the beginning of the study were used. The whole study was conducted under a controlled 12:12h light/dark cycle (light on at 7:00h) and room temperature (23 ± 2°C). The animals were kept in a quiet room inside plastic cages covered with soft sawdust, with rat chow and water available ad libitum. Seven days were allowed for adaptation to housing environment before baseline nociceptive testing.

Ethical Standard

All animal procedures were approved by the University Ethics Committee (Protocol #065/99). The rats were randomly assigned to three groups: Adjuvant-induced arthritis (AIA), Chronic Constrictive injury (CCI) and non-manipulated controls (CTRL).

Adjuvant-induced arthritis

After administration of the anesthetic (140 mg/kg de ketamine, i.p.), arthritis was induced in 40 animals by a s.c. injection of 0.1 ml of Freund adjuvant (complete fraction of denatured Mycobacterium butyricum suspended in mineral oil, Sigma Chemical Co., St. Louis, USA) in the right hind limb.

Chronic Constrictive injury

After onset of ketamine anesthesia (140mg/kg of body weight, i.p.), CCI was produced in 40 rats. The sciatic nerve was exposed to the level of the lateral face of the right posterior limb and 4 ligatures (4.0 chromic catgut) were tied around the common sciatic nerve, so that circulation through the epineural vasculature was not totally interrupted. The procedure was comparable to the original description (27).

Study design

The experiment was performed throughout a 9-day period: baseline in dry environment (day 1 and 2), paradoxical sleep deprivation (day 3, 4, 5 and 6) and recovery in dry environment (day 7, 8 and 9). Following the first test, the animals were randomly distributed into three groups (AIA, CCI or CTRL) and chronic pain inducing procedures were performed. Two days after (test 2), the pain threshold was measured and the animals were placed in the tank or remained in the home-cages. Daily, during the 4 days of PSD (Tests 3 to 6) and during the 3 days of recovery (Tests 7 to 9) the hot plate test was performed. The investigator was blind to the type of manipulation used to induce sleep deprivation.

Paradoxical Sleep Deprivation Procedures

Two methods of PSD procedures were employed using small (6.5cm in diameter) and large (14cm in diameter) platforms. The PSD technique consists in placing ten rats for 96 h in a tiled water tank (123 x 44 x 44cm), containing 14 platforms, dipped in water until 1cm of their upper surface. In this method, the animals are capable of moving inside the tank, jumping from one platform to the other. When the animal enters stage of paradoxical sleep, it falls into the water, due to muscle atonia, and wakes up. Since the large platforms also produce sleep deprivation, a new proposed control group (28), in which animals are placed onto a grid, was used. The grid group was placed on a stainless steel wire grid, which segments spaced 2.5cm from each other. The grid was fixed horizontally and 1cm above the water surface in the deprivation tank. The other (CTRL) group was housed in plastic cages and allowed to sleep normally.

Assessment of nociception

Pain sensitivity to noxious thermal stimuli was assessed between 9:00h and 11:00h. The hot-plate apparatus to test pain threshold consists of a 20-cm diameter metal hot-plate surface set at 50° C with a Plexiglas cage that fits onto the hot metal surface, and a foot-switch operated timer. Pain threshold was measured by the latency to nociceptive response (licking of any paw) with a maximum cutoff time of 90 seconds.

Statistical analysis

The data were analyzed using two-way ANOVA for repeated measures with behavioral test and group as main factors, followed by Dunnett as post hoc test. The level of significance was set at $p<0.05$.

Table 1: Means (±SEM) of pain threshold of normal-pain control (CTRL) animals.

	Cage	Grid	Large Platform	Small Platform
Baseline	53.2 ± 3.7	53.5 ± 2.7	53.1 ± 4.4	53.2 ± 4.6
Pre-PSD	53.1 ± 4.8	53.6 ± 4.6	52.9 ± 3.2	53.0 ± 3.6
PSD-24h	53.4 ± 1.4	48.6 ± 2.5 *	47.3 ± 2.5 *	37.3 ± 1.9 *
PSD-48h	53.4 ± 1.7	46.5 ± 2.7 *	43.3 ± 2.9 *	35.6 ± 2.5 *
PSD-72h	53.2 ± 1.6	43.3 ± 2.9 *	40.0 ± 1.1 *	32.6 ± 3.1 *
PSD-96h	53.1 ± 1.9	40.6 ± 3.2 *	37.1 ± 2.4 *	29.7 ± 2.8 *
R-24h	52.9 ± 2.0	51.4 ± 2.1	48.3 ± 3.5 *	44.1 ± 4.4 *
R-48h	52.8 ± 1.9	53.0 ± 3.5	51.3 ± 3.0	50.6 ± 6.1
R-72h	53.0 ± 1.8	52.9 ± 3.0	53.0 ± 2.7	53.0 ± 6.6

***Values significantly different from those of the cage group, p<0.05 (two-way ANOVA followed by post hoc Dunnett test). (PSD: paradoxical sleep deprivation; R: rebound).**

Table 2: Means (±SEM) of pain threshold in arthritic rats (AIA).

	Cage	Grid	Large Platform	Small Platform
Baseline	53.7 ± 2.3	53.8 ± 6.7	53 ± 3.1	54.1 ± 1.3
Pre-PSD	42.5 ± 2.3	42.5 ± 3.0	42 ± 1.7	42.9 ± 1.3
PSD-24h	36.2 ± 1.8	40.3 ± 3.2	36.3 ± 6.9	25.1 ± 3.6 *
PSD-48h	40.2 ± 1.3	37.8 ± 5.0	29.4 ± 5.7 *	24.9 ± 3.3 *
PSD-72h	39.0 ± 2.8	34.8 ± 4.0	23.5 ± 4.7 *	17.6 ± 9.4 *
PSD-96h	38.3 ± 3.2	34.9 ± 4.4	21.2 ± 1.7 *	17.0 ± 7.8 *
R-24h	38.1 ± 2.5	42.5 ± 2.1	31.2 ± 8.6 *	31.1 ± 2.0 *
R-48h	39.9 ± 2.0	45.1 ± 2.9 *	35.0 ± 7.2 *	32.6 ± 2.2 *
R-72h	42.7 ± 3.6	46.2 ± 2.6 *	37.8 ± 3.9 *	32.8 ± 1.9 *

***Values significantly different from those of the cage group, p<0.05.**

Table 3: Means (±SEM) of pain threshold of rats with chronic constrictive injury of the sciatic nerve.

	Cage	Grid	Large Platform	Small Platform
Baseline	53.7 ± 2.7	53.3 ± 3.0	53.5 ± 3.2	54.0 ± 5.5
Pre-PSD	39.4 ± 2.9	39.6 ± 2.3	39.8 ± 1.5	39.0 ± 2.5
PSD-24h	30.7 ± 2.1	34.8 ± 7.9	22.9 ± 8.1 *	20.8 ± 6.7 *
PSD-48h	33.9 ± 1.7	31.3 ± 5.1	19.8 ± 1.3 *	15.7 ± 8.6 *
PSD-72h	32.7 ± 1.6	25.0 ± 2.9 *	19.0 ± 1.0 *	10.8 ± 2.7 *
PSD-96h	31.9 ± 1.1	24.2 ± 4.7 *	18.8 ± 1.1 *	10.3 ± 4.3 *
R-24h	30.7 ± 1.3	39.0 ± 5.9 *	27.5 ± 2.5	26.0 ± 6.3
R-48h	26.0 ± 1.5	37.0 ± 4.8 *	28.7 ± 2.4	30.1 ± 5.5
R-72h	29.5 ± 1.0	36.4 ± 5.7	32.6 ± 3.0	35.0 ± 11.4

***Values significantly different from those of the cage group, p<0.05.**

RESULTS

The effect of experimental pain models on pain threshold

Two-way ANOVA followed by Dunnett test revealed that AIA and CCI differed from cage control groups from the second day on after pain inducing-procedures (test 2) and lasted until the third day of sleep recovery (test 9).

The effect of PSD methods on induced experimental pain models

The CTRL group (non-manipulated animals) showed decreased latency on the hot plate test during the 4 days of PSD and on the first day of rebound using both small and large platforms. The grid group presented also reduction of pain threshold during the PSD period (Table 1).

Regarding the AIA group (Table 2), PSD induced a conspicuous alteration in pain thresholds when sleep-deprived by the small and large platforms methods. The latency to paw withdrawal was lowered in small platforms from the first day of PSD on and remained lower even during the rebound period compared to CTRL group. The large platforms group presented reduction of pain withdrawal on the second day after the PSD and also remained low until the third day of rebound period. Regarding the grid group, the pain threshold was significantly higher in the second and third days of rebound sleep compared to CTRL animals.

In regard to CCI animals (Table 3), we observed that the exposure to both small and large platforms methods resulted in a decrease of the pain threshold during the 96 h of PSD. When placed on the grid, animals exhibited a reduced latency to paw withdrawal on days 3 and 4 of PSD and on the two first days of rebound.

DISCUSSION

Regarding the relevance of sleep and pain, several studies have described sleep disturbances in patients suffering from different pain disorders, and although it seems logical that pain can disturb sleep, sleep disturbances per se may also exacerbate pain (8,21,22,29). In the present study, we observed that pain thresholds to thermal noxious stimulation were reduced during PSD in all groups studied, independently of which deprivation method was used. Beside the confirmation that sleep disturbance increases sensibility to pain in experimental animals, this result indicated that chronic pain models may be used as a valuable paradigm to study the reciprocal influences between non-efficient sleep and pain. The results disclosed also some new aspects for investigation. Sleep recovery did not restore the baseline pain threshold in arthritic rats, but it did so in CCI group placed on both small and large platforms. Additionally, the second day of rebound was sufficient to restore pain threshold to baseline values in control animals. The grid method proposed as control environmental to PSD induced an increase of pain threshold latency in AIA animals (test 8 and 9) and a decrease in CCI (test 5 and 6) and control (test 3 to 6) groups, leading to the understanding that this method also interferes with pain sensitivity.

The small platform method of PSD induced a greater increase in pain sensitivity in all groups studied, comparatively to the large platform. It is known that large platform does not deprive sleep as much as the small one (30). The correlation found between the magnitude of PSD promoted by small and large platforms and the level of increase in pain sensitivity indicates the linearity of the effect studied. On the other hand, both models of chronic-pain seem to offer a valuable way to study the pain-sleep relationships. A choice between them may be determined by the differences observed in the rebound period or other details as the observed in the grid method.

The mechanism by which PSD and sleep recovery modifies the pain thresholds has not been completely established. Neurotransmission systems, such as serotonergic and opioidergic pathways have been investigated in respect to the participation of pain and sleep manipulation (1,5,7). Concerning sleep manipulations, an inverse relationship between brain serotonergic activity and pain has been reported in several animal studies. Therefore, PSD appears to increase the rate of serotonin metabolism in the rat brain (31).

Ukponmwan et al. (18) reported that 96 hours of PSD abolish the antinociceptive effects of analgesic compounds such as phosphoramidon (an enkephalinase inhibitor) and morphine in the rat brain. These findings are consistent with the hypothesis that animals deprived of paradoxical sleep might have smaller responsiveness of opioid receptors to endogenous enkephalines (5). Earlier, Kay (32) demonstrated that during chronic administration of morphine, paradoxical sleep time persistently decreases, suggesting that the chronic use of this analgesic produces PSD. Thus, the tolerance that takes place with chronic use of some analgesics may be mediated, in part, by PSD-induced reductions in pain threshold (26).

If pain remains unrelieved for several days, then patients would suffer of anger and depression, which also contribute to the vicious circle as patients become demoralized and lose confidence in the ability of their medical attendants to relieve their pain. Moreover, the sleep disturbance participates in the problem (33). The psychological component has the potential to interact with both pain and sleep further complicating the situation. In addition to this etiological link between depression, chronic pain, and sleep disturbances, depressive patients seems to have greater necessity for paradoxical sleep, since they manifest shorter latencies than normal controls do (34), as well as increased paradoxical sleep time (35,36). The demonstration that rats under chronic pain and submitted to PSD may be used to approach this vicious circle seems to be a promising idea. Recently, Andersen, Hoshino and Tufik (37) reported that animal models for chronic neuropathy exhibit reduced sucrose ingestion. Accordingly, this anhedonic condition that constitutes the core manifestation of depressive states does not occur in response to a single episode of total PSD.

Sleep disturbances attributable to pain suggest a bidirectional relationship between sleep disturbance and pain (29,38,39); both may interact in complex ways that ultimately impact biological and behavioral activity (40). It has been proposed that recognition of disturbed or unrefreshing sleep influences the management of painful medical disorders (29). Specifically, our group has investigated this complex association in animal models as well as clinical conditions (7,10,14-17,19,20,24,39). For instance, it has been demonstrated that arthritis induced by Freund's adjuvant injected into rat hind paws causes reduced sleep efficiency, slow wave sleep and paradoxical sleep, as well as increased latency to the first sleep episode and arousal events (14). Previous studies have also shown similar findings in arthritic rats that were characterized by increased wakefulness, decreased slow wave sleep and paradoxical sleep, and increased sleep fragmentation (13).

We have previously reported that a dose of substance P insufficient to depolarize spinal cord motoneurons of mice tested on a hot plate test was still able to impair the sleep pattern (20). The sleep disturbance found in the group injected with substance P is most likely due to its direct action on the sleep-wake system. Collectively, these findings indicate a strong association between the reduction of the pain threshold and sleep disruption during osteoarthritis, where reduction of the pain threshold is probably

responsible for sleep disturbances that in turn cause further lowering of the pain threshold (8).

It has been proposed that fragmentation of the sleep pattern may promote sleep deprivation (26), which, in turn, increases behavioral responses to mechanical (14), thermal (7), and painful electrical stimuli (5). Sleep deprivation might affect sleep homeostasis and may play an important role in nociception. Indeed, Nascimento el al. (7) showed that the threshold of paw withdrawal after thermal noxious stimulation was reduced by 96 h of PSD and that this effect persisted after 24 hours of sleep recovery. Collectively, our data suggest that chronic pain models may present a valuable framework for the study of the reciprocal influences between non-efficient sleep and pain.

Finally, one may considerer that the reciprocal relationship of pain sensitivity and sleep is not fortuitous. Pain is an important evolutionary acquisition that granted survival by its role to warn the occurrence of some dangerous or noxious process in the organism. To be awake in such situations seems adaptive. Inversely, as sleep deprivation induces somnolence and lowers attention, an increase in pain sensitivity seems to compensate them and grant wakefulness. Such considerations indicate that the search for an efficient help for chronic pain patients will not be easy. However, whatever the amount of work needed, the result seems undoubtedly rewarding.

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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

This article is based on a study first reported in the Hypnos in 15, Jan 2004

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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CHANGES IN SLEEP HABITS OF MEDICAL STUDENTS ACCORDING TO CLASS STARTING TIME: A LONGITUDINAL STUDY

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ABSTRACT

Background and objective: Good quality sleep and adequate amount of sleep are important in order to have better cognitive performance and avoid health problems and psychiatric disorders. Sleep-related disturbances affect a large percentage of university students and may cause impairments in their academic performance. Among the wide range of factors that can influence the sleep habits, university schedules are strongly related with sleep deprivation in students. This longitudinal study aims to investigate the effect of university schedules on medical students' sleep-wake cycle.

Methods: We evaluated the Pittsburgh Sleep Quality Index (PSQI), a Sleep Habits Questionnaire and a sleep diary in three university semesters with different class starting times.

Results and Conclusion: The results demonstrated that when classes started earlier in the morning, the students had shorter sleep duration during weekdays, greater difference between weekday and weekend sleep duration (restriction-extension pattern) and worse sleep quality, showing the influence of class starting time on students´ sleep habits.

Keywords: sleep habits, class starting time, sleep deprivation, sleep quality.

INTRODUCTION

Sleep is a physiological process essential to life. Its quality is strongly related to psychological and physical health and other measures of well-being (1). Individuals who report poor sleep quality and other sleep-related disturbances may be at higher risk for depression and other psychiatric disorders throughout their lifetime (2,3).

The amount of sleep is also very important and is positively correlated with alertness and psychomotor vigilance (4). A number of studies were conducted to measure the consequences of insufficient sleep. It is known that one night of sleep loss impairs innovative thinking, flexible decision-making, and several forms of cognitive performance (5,6). One of the major consequences

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of sleep deprivation is daytime somnolence and its inevitable outcomes. One author observed excessive sleepiness prevalence of 93.2% in a population of medical students (7). Daytime sleepiness may result in mood disturbances and increased vulnerability to substance use (8). Another study showed that employees with subjective daytime sleepiness lose more working days due to health reasons than their more alert colleagues (9).

Sleep-related disturbances affect a huge part of the population, regardless of age, gender and ethnic group. However, there are certain population groups that are more susceptible to sleep disorders. University students, for instance, usually exhibit irregular sleep-wake cycles, with short sleep duration on weekdays and bedtime delays on weekends, which can lead to daytime sleepiness, depressive mood, and sleep-wake behavior problems (10). Medical students, in particular, are often overloaded with classes and hospital activities. In addition, they have to cope with anxiety and stress due to academic demands and the constant contact with patients' suffering and death.

Academic performance is one of the major goals of university students. One study reported the relationship between sleep habits, particularly wake-up times, and grade point average in university students (11). Another study demonstrated that students with irregular sleep-wake cycles and sleep deprivation show worse academic performance than those with regular sleep-wake cycles and sufficient sleep duration (12). And, most alarming of all, university students are not aware of the extent to which sleep deprivation negatively affects their cognitive performance (13).

Multiple factors influence the sleep-wake cycle. Along with endogenous factors (chronotype, circadian timing system, core body temperature, and hormones), there is a wide range of environmental cues, including the light-dark cycle, professional activities, and social interaction, which can affect sleep habits. In a study with university students, for example, about one third of the sample that reported insufficient sleep indicated visual media, particularly computers, as the primary reason (14). Several other researchers have pointed out to study and work schedules as the underlying causes of sleep deprivation (15-17).

Aiming to investigate the effect of university schedules on medical students' sleep-wake cycle, we chose to carry out a longitudinal study and analyze sleep quality and sleep-wake habits of the same group of students during three school semesters. In addition to class starting time differences between the semesters, during the last analyzed period most of the students were involved in extra-curricular night work in hospitals. Thus, we also analyzed the interference of this factor in the sleep-wake cycle of the future physicians.

METHODS

Participants

Our subjects were 31 medical students (19 male and 12 female) from Universidade Federal do Rio Grande do Norte, Brazil. The mean age was 20.54 ± 2 years at the beginning of the study and they attended classes according to a regular schedule from Monday through Friday. Informed consent was obtained from all volunteers.

Instruments

During the survey, we used the following protocols: an identification form with questions about personal information, health status and schedule of their curricular and extra-curricular activities; a Portuguese version of the Horne and Östberg Morningness/Eveningness Questionnaire to identify the subjects' chronotype (18); the Pittsburgh Sleep Quality Index (PSQI) (19); a sleep diary, in which the students recorded their bedtime and wake-up time for two consecutive weeks; and a questionnaire about sleep habits, also including, among other issues, their average bedtime, wake-up time, and sleep duration, on weekdays and weekends.

Procedure

In our University, the medical course is completed in six years. The first two years are part of the basic cycle. After that, the students go to the professional cycle and have classes at the university hospital, being in contact with patients, and most of them engage in volunteer night work in hospitals. Data collection took place in three different periods. The first one occurred during the third semester of the medical course, when classes started at 07:00h on Tuesdays and Thursdays and 08:00h on Mondays, Wednesdays and Fridays. The students filled out the identification form, the Morningness/Eveningness Questionnaire, the PSQI and the sleep diary. On the following semester, with classes starting at 10:00h from Monday through Friday, the participants completed the same protocols of the previous semester plus the Sleep Habits Questionnaire. The last part of the research was carried out during the professional cycle, on the seventh semester of the course, when classes started at 07:00h everyday of the week. Once more, the students filled out all protocols, except for the Morningness/Eveningness Questionnaire and the sleep diary.

Data analyses

With the results from the Morningness/Eveningness Questionnaire, we built a normal distribution curve, and, in order to correlate the chronotype scores with the sleep onset, a linear regression test with ANOVA was used. The bedtime, sleep duration, and PSQI scores were compared between the semesters with the One-way Within-Subjects (repeated measures) ANOVA. The level of significance was established at $p<0.05$.

RESULTS

According to the Morningness/Eveningness Questionnaire, 71.4% of the students were classified as indifferent type, 14.3% as moderate morning type, 11.4% as moderate evening type, and 2.9% as extreme evening type. The linear correlation between bedtime (based on sleep diary) and chronotype scores ($p<0.04$) is evidence that the subjects' answers were coherent on both questionnaires.

Based on the sleep diary, the students' average bedtime during the first period was 23:47h ± 57min on weekdays and 00:18h ± 77min on weekends. Sleep duration was 397min ± 52min and 459 min ± 59min, on weekdays and weekends, respectively. During the second period, the students delayed their bedtime to

00:25h ± 58min on weekdays and 01:14h ± 65min on weekends. During the weekdays, the sleep duration increased to 437min ± 50min, but the weekend duration remained the same. In the third data collection, the bedtime and sleep duration data were obtained from the Sleep Habits Questionnaire. We had already found a strong correlation between the data from the sleep diary and the Sleep Habits Questionnaire during the second period and chose not to use the sleep diary during the third period. The results revealed that weekdays and weekend bedtimes were 23:38h ± 53min and 00:29h ± 88min, respectively, and sleep duration of 385min ± 56min and 519min ± 91min (Figure 1).

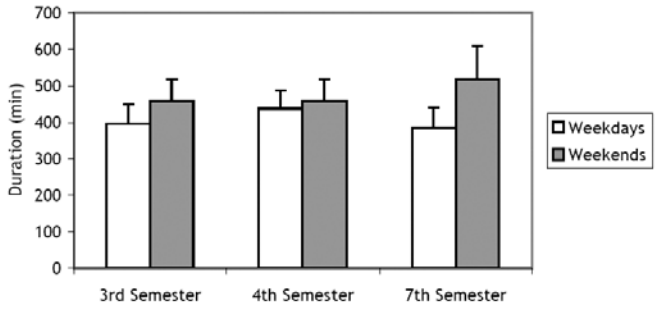

Figure 1. Sleep duration on weekdays and weekends, showing the "restriction-extension" pattern. Data are presented as mean ± s.d.

PSQI scores range from 0 to 20 and a value above 5 indicates bad sleep quality. During the first period analyzed, the average PSQI score was 5.00 ± 1.5, with 42.3% of the students presenting bad sleep quality. During the second period, this percentage decreased to 11.5% and PSQI mean score was 3.86 ± 1.5. During the third period, PSQI increased to 5.57 ± 2.8 and bad sleep quality was detected in 60% of the students (Figure 2). ANOVA comparison between the PSQI scores revealed a statistically significant difference between the first and second periods (p=0.044) and between the second and third periods (p=0.002), but no significant difference was found between the first and third periods.

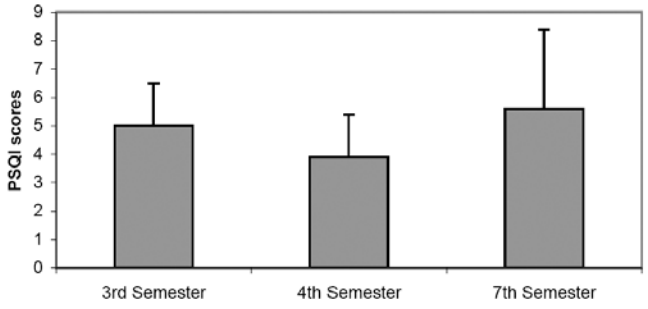

Figure 2. Pittsburgh Sleep Quality Index on the 3rd, 4th and 7th semesters

DISCUSSION

The normal distribution of chronotype frequencies revealed that the studied group is a homogeneous representation sample of the general population, with no significant predominance of morning or evening types.

Evaluation of the same group of students throughout the entire experimental period allowed us to use One-way Within-Subjects (repeated measures) ANOVA, and thus, we were able to consider the subjects' individual characteristics, such as personality and chronotype, as constant variables with little effect on the comparisons.

When the students went from the first analyzed period to the second, their morning classes starting time was delayed by 2 hours on Mondays, Wednesday and Fridays and by 3 hours on Tuesdays and Thursdays. This allowed them to delay their wake-up time by about 78min during college mornings. But they also delayed their bedtime, which seems to be associated with a tendency of the human circadian system to maintain a delayed phase (17). Despite this delay in bedtime, the students were able to increase their sleep duration by 40 minutes. During the third period, their class starting time went back to 07:00h and the students were forced to adopt a wake-up time, as well as a bedtime and sleep duration, similar to the first period. These results suggest that the class starting time affected the sleep-wake cycle and that the students slept less when classes started earlier in the morning.

When we compared the sleep onset on weekdays with that of weekends, we observed a delay of 31, 49 and 51 minutes on weekends for the third, fourth and seventh semesters, respectively. This was probably due to the already mentioned tendency of human beings to delay their sleep wake cycle (17) in addition to their engagement in social activities, such as parties.

We also found an increase in sleep duration on weekends compared to weekdays. During the third semester, the students slept approximately one hour more on the weekends and on the seventh semester, the difference between weekdays and weekends exceeded two hours. During the fourth semester, on the other hand, sleep duration during the weekend increased only 22 minutes. The reduced sleep length during weekdays and extended sleep length during weekends is denominated restriction-extension pattern and indicates partial sleep deprivation (Figure 1). During the third semester, 88.9% of the students presented this pattern. This percentage decreased to 66.7% during the fourth semester and reached 93.5% during the seventh semester. These results confirm the link between class starting time and sleep deprivation.

Even though the difference in weekdays sleep duration between the third and seventh semesters was subtle, the greater percentage of restriction-extension pattern found for the seventh semester indicated that the students were more sleep deprived at this time, which suggests that night work in hospitals during the professional cycle of the medical course could be another interfering factor on the sleep-wake cycle of students. Studies have demonstrated that fatigue in medical students and professionals is due to long hours of study and work and the associated sleep deprivation is the main factor that influences performance as reviewed by Gaspar et al. (20).

Sleep quality was also affected by class starting time. When classes started later in the morning, i.e., during the fourth semester, the students reported better sleep quality than during the third and seventh semesters, when classes started earlier. But, once again, we cannot rule out the possible influence of hospital night work on sleep quality since it was slightly worse on the seventh

semester compared to the third.

It is essential for students and health professionals to understand the importance of sleep deprivation and other sleep disorders and their consequences. Loayza and colleagues (3) showed an association between sleep disturbance and suspicion of psychiatric disorders in medical students. As demonstrated, sleep-related disorders affect not only the individuals' health and well-being (1), but also their performance. The effect of sleep loss on cognitive performance of resident physicians is well documented by Jacques and co-workers (6) in a study that demonstrated a decline in composite test score with decreasing sleep on the night before the examination.

Sleep medicine is an important field in the medical study and allows medical students and professional to diagnose their own sleep disorders as well as their patients'. Despite the numerous publications regarding the subject, students and professionals tend to ignore the sleep disorders and their possible consequences (13,21).

Medical students suffer high levels of stress due to academic demands, particularly during examination periods, and the constant contact with patients' suffering. Stress, associated with insufficient sleep and excessive daytime sleepiness (7) can lead to difficulties in interpersonal relationship, depression, anxiety (22), and alcohol and drug abuse (8). Even though a number of studies have supported the need of stress-management programs for medical trainees (23), most of these programs do not take sleep habits into account. One way to improve sleep quality, avoid sleep disorders, and decrease stress is to have good sleep habits, which include regular bedtimes and wake-up times, sufficient sleep duration, appropriate sleep environment, and, particularly for students, better organization of their study schedule.

In this study we investigated the influence of class starting time on sleep deprivation and the effect of hospital night work on sleep. These are only two of the numerous causes of disruption of sleep habits. The identification and analyses of these factors contribute to an increased understanding of their relationship with the sleep-wake cycle and, most importantly, to the elaboration of intervention methods to avoid sleep-related disorders and their consequences.

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REVIEW ARTICLE
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TYPE 1 DIABETES ASSOCIATED WITH SLEEP DISORDERS

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ABSTRACT

Although the number of published studies regarding the interaction between T1DM and the sleep-wake cycle is limited, the findings on the subject are nevertheless significant. Glycemic variation, as well as poor glycemic control, in T1DM seems to affect the sleep-wake cycle as a whole. Some important evidences available in the literature, comparing sleep in subjects with T1DM with control subjects, are: a trend towards increased total sleep time, decreased sleep latency, increased sleep efficiency, higher blood levels of growth hormone, epinephrine and ACTH throughout the night, and higher cortisol levels during the first half of the night. It is likely that not only the long-term consequences of hyperglycemia affect nocturnal sleep, but also the acute responses to hypoglycemia. In our view, the latter affects sleep in individuals with T1DM regardless of whether the hypoglycemic event occurred during the day or night. On the other hand, there are evidences that a poor night's sleep and sleep disorders may not only result in diurnal sleepiness, but could also worsen diabetes control, revealing a vicious circle. We suggest that improved glycemic control and avoidance of hypo- and hyperglycemia would minimize the impact of T1DM on circadian rhythms, lessening sleep disorders and improving both sleep duration and quality. At the same time, it would favor the maintenance of a good metabolic control.

Keywords: type 1 diabetes, glycemia, sleep, sleep-wake cycle, sleep quality.

Several authors have reported an association between type 2 diabetes and sleep impairment. Previous studies have clearly established that sleep quality influences glucose utilization (1-3), and have implicated sleep debt as a cause for decreases in glucose tolerance (4). Furthermore, sleep-disordered breathing has been associated with glucose intolerance and insulin resistance, which may lead to type 2 diabetes mellitus (T2DM) (5-7). Other authors have argued that T2DM in adults is associated with higher rates of sleep-related disorders such as insomnia, excessive sleepiness, breathing pauses, snoring, daytime sleepiness, restless legs or sleep debt (8-10). A study conducted by Knutson et al in individuals with T2DM determined not only that low sleep quality and high scores for perceived sleep debt are associated with poor glycemic control, but that poor diabetes control can then contribute to a higher perceived sleep debt and lower sleep quality (11). These results reveal a vicious circle, in which sleep debt and sleep quality impact glucose metabolism; in turn, glucose intolerance, insulin resistance or T2DM influence sleep length and quality, thereby

creating a feedback loop.

The serum level of glycosylated hemoglobin (HbA1c) is known to reflect average glycemia over the preceding 2–3 months (12). Studies that have evaluated HbA1c levels offer further substantiation for the link between glucose metabolism and perceived sleep debt or quality in people with T2DM (11). In a cohort of non-obese and non-diabetic children, HbA1c levels strongly correlated with the severity of sleep-disordered breathing (SDB) (13).

Unlike T2DM, the interaction between type 1 diabetes mellitus (T1DM) and the sleep-wake cycle has not been extensively studied. Although the number of published studies regarding this association is limited, the findings on the subject are nevertheless significant. Villa *et al.* showed that children with T1DM have more frequent and longer lasting apneas than those without diabetes (14). They also demonstrated that apnea events during sleep correlate significantly with poor glycemic control and with the duration of diabetes (14). Moreover, rapid changes in glucose levels that are independent of absolute glucose values may affect night sleep, resulting in awakening (15). Considering these and other data, along with complaints of excessive daytime sleepiness (16) and low sleep quality among people with T1DM, we hypothesize that glycemic variation in T1DM might affect the sleep-wake cycle as a whole.

Interestingly, Pillar et al have reported a trend toward increased total sleep time, decreased sleep latency, and increased sleep efficiency in children with T1DM (15). Furthermore, they indicate an association between hypoglycemia during night sleep and an increase in sleep efficiency (15). In light of these findings, we are led to conclude that the tendency towards increased night sleep duration in T1DM individuals is potentially a result of a higher frequency of hypoglycemia during the night. In our view, it is likely that hypoglycemia affects night sleep duration and efficiency in individuals with T1DM, regardless of whether the hypoglycemic event occurred during the day or night.

The physiological path that links exposure to intermittent or prolonged hypoglycemia with sleep disorders is unknown. We do know, however, that acute or chronic hypoglycemia may act as a stressor that elicits physiological responses from the sympathoadrenal and sympathoneuronal systems as well as the hypothalamo-pituitary-adrenocortical (HPA) axis (18). Both humans and animals with poorly controlled or uncontrolled diabetes display diurnal hypersecretion of glucocorticoids, as well as altered regulation of the HPA axis (19). These effects could be related to the deterioration of sleep quality, as seen in patients with depression (20).

Jauch-Chara et al recently reported that 14 individuals with T1DM displayed higher blood levels of growth hormone, epinephrine and ACTH throughout the night, as well as a tendency toward higher cortisol levels during the first night-half, compared with healthy control subjects (21). Even though their blood glucose had been monitored throughout the experimental night, these patients spent slightly less time in slow wave sleep during the first night-half and reported less restorative sleep than the healthy subjects. Nocturnal hypoglycemia was prevented in this study, which indicates that the increased level of counterregulatory hormones detected was not a result of hypoglycemia during the experimental night. The authors suggest that the slightly, but persistently, elevated concentrations of glucose and insulin observed might be responsible for stimulating HPA activity and the release of epinephrine (21).

Although T1DM subjects were monitored during a single night, it is possible that changes in hormone levels and sleep architecture (21) were associated with disruption of the sleep-wake cycle and other circadian rhythms, leading to a phase shift of daily glucose tolerance (2). Taking into account all presented data, we suggest that improved glycemic control and avoidance of hypo- and hyperglycemia would minimize the impact of T1DM on circadian rhythms, improving both sleep duration and quality.

Further investigation is necessary in order to clarify the pathways through which T1DM and glycemic excursions may affect the sleep-wake cycle, and vice versa. We understand that it will be difficult to establish a cause-effect interaction, given the reciprocal nature of the influence between sleep and glucose metabolism. Glycemic excursions or poor glycemic control can lead to a poor night's sleep in terms of quality and/or length (11). In turn, a poor night's sleep and sleep disorders can not only result in diurnal sleepiness, but could also worsen diabetes control by raising pro-inflammatory cytokines. These cytokines inhibit glucose uptake by fat and muscle tissue as well as increase the secretion and plasmatic concentrations of counterregulatory hormones (22-24), which can lead to insulin resistance and glucose intolerance (25), thereby worsening the glycemic control.

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PHYSIOLOGY OF DREAMING

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Dreaming has been a subject of cogitation since remote Antiquity. In ancient Greece, Socrates, Plato and Aristotle discussed about the meaning of dreams, concluding that the prevailing mistic and mythic concepts about them were incorrect. Instead, they thought that dreams were not provoked by spirits, ghosts or gods, which took over the mind to express themselves through dreaming. Aristotle (1), who had carefully observed several animal species while asleep, noticed that movements of several of their body parts were quite similar to those performed by humans during dreaming. Some of his statements, hereby reproduced in a simplified form from his book on sleep and dreams, briefly illustrate his contribution to the study of this subject:

"All creatures that have four limbs and are sanguine (mammals) *display signs that they dream while asleep. It seems that not only humans but also dogs, cows, sheep and goats and the entire family of four-legged viviparous animals do dream."*

"As to the oviparous creatures, it is obvious that they sleep but it is impossible to state that they dream. The same holds true for animals that live in water, such as fishes, molusks, crustacea and other similar animals; it is impossible to invoke as a proof that they do sleep the shutting of their eyes, inasmuch as they do not have eyelids but it is obvious that they periodically do rest, immobile, what perhaps does explain why at night their predators attack them heavily and devour them. When they sleep, fishes keep quiet, with no apparent movements, and then they can be easily fished with a hand."

"Insects are also creatures that do sleep, so much so that they can be seen resting with no movements whatsoever. This is specially true as to bees, that at night do interrupt their hum, "even if they are exposed to the light of a lantern".

"Dreams are not ghosts (phantasmata), since they are closely related to the events of the previous day".

In Greece dreams were called *oneiros*, a word that originated the adjective *oniric* but that meant not exactly what was dreamed about neither the dreaming process, which was not rated as something important, but the *phantasmata*, i.e. the apparitions. As a prevailing concept even today, dreams were considered premonitory, messages from the dead and mystical warnings. Herodotus, in his *Histories*, the first textbook on History ever written, tells that the Persian King Xerxes dreamed quite often about the war he was about to fight against Athens. He properly related such dreams to his concern with that important war. His personal oracle, however, disagreed and convinced him that his dreams were warnings from the gods. Xerxes, in fact, had discovered an important aspect of dreams but his oracle discarded such an explanation, in favor of the mystic one.

In the past, most civilizations boasted having wise people who could tell the meaning of dreams if conveniently paid for that, a fancy profession that still has its counterparts in modern nations. Psychoanalysis considers dreams as an important window to the unconscious world, what makes dream interpretation a crucial factor in psychonalytic diagnosis and treatment. However, psychoanalysts take into account only a few dreams that are occasionally

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recalled, despite the fact that we dream four or five episodes every night, what means that the fraction of dreams we can recall is a small portion of what we in fact do experience as dreams. Psychoanalysis also considers dreams as the expression of repressed wishes; this is undoubtly true as to only a few dreams, whereas several studies reveal, instead, that most dreams are closely related to the events of the previous day, as Aristotle had already demonstrated.

Socrates, Plato, Aristotle and Xenophanes, nearly 2,400 years ago, were opposed to the prevailing view of the *phantastikon,* that is, mystic apparitions, and to the premonitory character of dreams as their main characteristics. However, they ignored that the dreams were produced by the brain. Hippocrates and Alkmaeon, who discovered that the mind is in the brain, not in the heart, knew that dreams were originated in the brain. Later, the Roman writer Lucretius, the first popularizer of science, in his book *De Rerum Natura* (1978) credited these Greek philosophers for the discovery of the characteristics of sleep and dreams (2). Plato, despite his logical view of dreams, antecipated by 24 centuries one of the dogmas of psychoanalysis, stating that the dreams with a sexual background, mainly those with an incestuous content, and those in which the dreamer attacked or even killed someone, did, in fact, represent occult wishes that only could be fulfilled without punishment as an oniric experience. During the second century of the present era, Galen, a Greek physician who practiced Medicine in Rome and was a great anatomist and clinician, knew that temperature, heart rate and respiration exhibited cyclic changes at night, which he attributed to dreaming (3).

During the medieval era in Spain, by then the very cultural center of Europe (probably of the entire world), and mainly in the 13th century, some Muslim Arabs and Jewish rabis, centered in Cordoba rediscovered the Greek literature, that had been concealed by early Christianism, and translated all that important work into Latin, Arabic and Hebraic. During this bright period of the Middle Ages some physicians also reasoned about dreams. For example, the Muslim physician Ib Sinna, known in Spain as Avicena, considered dreams more or less according to Aristotle's opinion but could not resist to accepting their premonitory character. The ancient Chinese scientific inquiry tried to understand dreaming but usually also considered them mistically.

During the nineteenth century several physiologists and neuropsychiatrists tried to understand the mechanisms and meaning of dreams. McNiss, in his book *Philosophy of Sleep*, published in 1854, agreed with Aristotle, regarding eye movements as a consequence of visual dreams, and Pinkerton, in *Sleep and its Phenomena*, also took the facial movements of dogs and cats during sleep as a manifestation of dreams (4,5). An important contemporary of these authors, Charles Darwin, in his landmarking book *Emotions in Man and Animals*, published in 1872 and reedited several times in the twentieth century (6), states that "*at least* birds and mammals do dream", a concept that still remains unchallenged, despite which most researchers that carry out studies on sleep still hold that dreaming is specifically human.

At the end of the 19th century several authors published on oniric activity. Esquirol, one of the French psychiatrists who started the revolution that changed the ancient (an cruel) view of the mental diseases, spent several hours at night observing how his patients behaved during sleep and concluded that their movements while asleep were related to their dreams, just as Aristotle had found long ago. The American psychologist Mary Whiton Calkins published in 1893 an important, although entirely unknwown, article under the title *Statistics of Dreams*, wherein she introduced the technique of arousing people when they moved parts of the body during sleep and asking them to report their dreams (4,7). Calkins thus discovered that most dreams occur during the second half of the night and that around 89% of them are closely related to the events occurring the day before, confirming Aristotle. Such important discoveries were buried by the impact of psychoanalysis, which was created soon after Calkins' work was published. Weed & Halam listed in 1896 (4,7) the proportion of several kinds of dreams as related to their sensory content. Their data do not depart from modern studies of the same kind.

De Sanctis, in 1899, in his book *I Sogni, Studi Clinici ed Psicologici di un Alienista* (*Dreams, Clinical and Psychological Studies of a Psychiatrist*), cites no less than 323 articles and books dealing with dreams, which proves that the objective study of dreams did not start during the middle of the 20th century, as is usually taken for granted (4). De Sanctis, whose main research on sleep was the incorporation of sensory stimulation into dreams, states in his book that "by measuring the pulse and observing the movements in humans and other animals during sleep it is possible to detect the occurrence of dreaming and sometimes even to guess the dream content". Inasmuch as all this relevant knowledge is entirely ignored, we hope the present review may help in rescuing it (4).

Around 1860, Kohlschütter, a young medical student in Germany, showed that the threshold to awake humans by auditory stimulation oscillates along the night (4,8). In 1867, Michelson, a physiologist who was a relative to Kohlschütter, replicated his study and obtained the curve shown in figure 1 (4,8). The oscillation of the sleep depth as cycles, as is well known presently, is quite clear in this figure. The first oscillation lasts around two hours, when sleep attains its deepest level; the ensuing cycles last less and their depth tends to decrease until arousal finally occurs, a sequence that recent research has fully confirmed.

During the first half of the twentieth century, despite the heavy influence of psychoanalysis, dreaming was again but sporadically studied scientifically. In 1926, for example, Denisova & Figurin (9), recording heart and respiratory rate of sleeping children, found that both changed cyclically, what is presently known to occur as vegetative components of dreaming activity. In 1944 Obhlmeyer, Brilmayer & Uhlstrung (10) observed that in humans penile erection occurs during sleep at intervals of 85 minutes, which is the average duration of a sleep cycle. Penile erection, that also occurs in monkeys, is present during *desynchronized* (*paradoxical* or *REM-sleep*) but it is not necessarily linked to erotic dreams. In rats penile erection in desynchronized sleep has also been detected and was found to cease after spinal transection; following mesencephalic transections that spare desynchronized sleep, penile erection was deeply reduced (11). However, reflex penile erection is facilitated after spinal transection whereas mesencephalic transections significantly increase the latency to its reflex induction, without affecting the percentage of tests eliciting an erectile event. The authors suggest that structures rostral to the midbrain are essential for

the maintenance and integrity of the erection that occurs during desynchronized sleep.

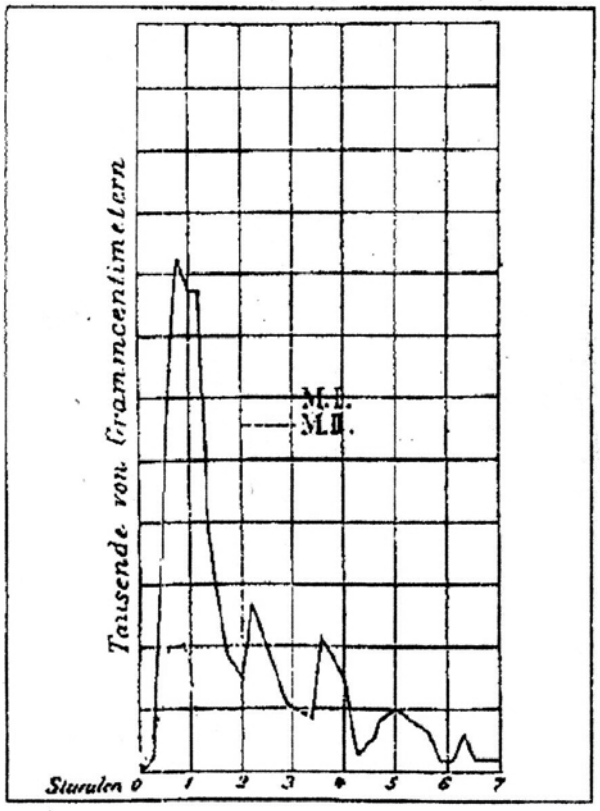

Figure 1. Depth of sleep, as originally expressed by Eduard Michelson in 1897 and evaluated through the intensity of sound able to produce an arousal. Sound was produced by a bang and measured as pressure exerted on a plate. In ordinates, pressure in thousands of grams x centimeter, in abscissae, hours. (*Reproduced from Kluger 1997*).

It is well known that during desynchronized sleep the pupil undergoes an increase in diameter (midriasis), which is not produced by direct sympathetic activation but rather to parasympathetic inactivation, that overcomes the tonic pupillary constrictor activity of the parasympathetic system during synchronized sleep.

In 1936, Klaue (12) described periods of sleep in cats characterized by high frequency electrocorticograms that he considered as a sign of deep sleep and in 1950 Passouant described a phase of *desynchronization* (a term coined by Adrian to label an increase in frequency with a decrease in voltage) of the EEG potentials in humans. Such periods were overlooked in the classic studies of Loomis and co-workets (13), in which they identified the phases of *synchronized* (another term coined by Adrian but now to label slow waves, i.e., potentials with a low frequency and a high voltage) sleep. Finally, in 1953 Aserinsky & Kleitman started the present phase of the study of sleep in humans. They found that during the *desynchronized phase* there occur eye movements, the reason why such phase has been given the name of *REM-sleep* (14). Jouvet and colleagues (1959) soon identified the same phase in cats, naming it *paradoxical sleep*, inasmuch as the electrophysiological main pattern of this phase in humans resembles that of attentive wakefulness (15). Moruzzi's coined the name *desynchronized sleep*, which we prefer, because in humans desynchronization is the main electrophysiological marker of this phase. However, considering the high prevalence of dreams during this phase it should be more appropriately named *oniric phase of sleep*.

Animal experimentation, by making it possible to implant electrodes in any part of the nervous system and to lesion and stimulate (electrically or chemically) also any nucleus or pathway, has been of the utmost relevance for the understanding of the mechanisms causing not only sleep but also the manifestations of dreaming. Unfortunately, despite the opinion of great scientists of the past, most researchers that deal with sleep and dreaming, probably moved by philosophical, religious prejudice and a faulty reasoning, do not accept the idea that non-human animals do dream. With Darwin (1965), we are fully convinced that "*at least birds* and mammals do dream" (6). As a matter of fact, manifestations of dreaming have been identified in many species, including chickens, chimpanzees, cats, rats and in some birds. While humans dream around 100 minutes every night, cats exhibit signs of dreaming during nearly 200 minutes per day. Desynchronized sleep has been identified in many mammals and birds (16) but below the birds only in crocodiles brief periods of an equivalent phase (eye movements, low voltage electro-oscillograms and cervical hypotonia) seem to occur (17). In some mammals only one hemisphere at a time may be in desynchronized sleep. In cats, Thomas & Benoit (18) have found oniric activity during synchronized sleep, similar to what we described in rats as *pre-paradoxical sleep* (19,20) as *intermediate phase*.

What is a dream?

A dream is a conscious experience that occurs during sleep. Although it may happen in any sleep phase, it prevails during the desynchronized phase.

The very essence of dreams is, certainly, memorized information. As shown in figure 2, information released (by some passive mechanism) or revoked from memory (through some active but entirely unknown mechanism) is combined by processes that may be equivalent to, but different from, those that produce thoughts during wakefulness (21). As any neural information, it has to be analyzed, so that the nervous impulses, which carry it be decoded and integrated as a specific neural configuration, that contains all the information released (or revoked) from the mnemonic archives. Such a configuration is subsequently compared to memorized patterns and then, and only then, it can be identified by means of the conscious process. *The result of such conscious identification is a dream*. As any information consciously identified, a dream triggers a specific behavior, that we call an *oniric behavior*.

In humans a dream may be reported and its content can thus be analyzed. Recall of dreams is much greater and the report is much more detailed when one is awakened *during* desynchronized sleep and the stage I of synchronized sleep, right after alpha waves disappear and are replaced by a lower frequency and lower voltage electro-oscillographic pattern (22,23). A correlation has been proposed between the development of desynchronized sleep in

children and their waking cognitive maturation (24). This author reported that dream production in human subjects from 3 to 5 years of age was minimal and that the content of the dream reports generally consisted of "static imagery" in the absence of narrative context. Consequently, Foulkes concludes that they do not dream but this conclusion is probably incorrect, inasmuch as at this age children have a highly limited narrating capacity and their poor reports about dreams are certainly linked to such a limitation, not their absence. At the age between 7 and 9 years Foulkes' subjects produced much more consistent narrations of the dream content, as should be expected (24).

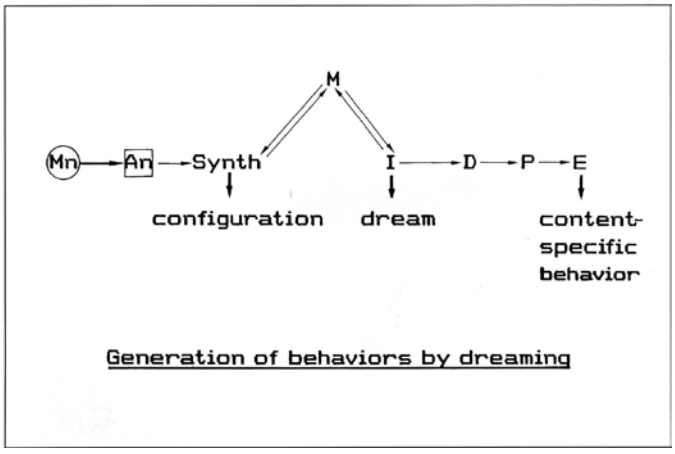

Figure 2. Flowchart of steps that probably generate a dream and the consequent oniric behaviors. *Mn*: memorized information in mnemonic archives. *An*: analysis of such information. *Synth*: specific synthesis as a neural configuration of the information released (or retrieved) from memory archives after it has been analyzed. *M*: memory (expressed as *M* instead of *Mn* to mean memorized patterns). *I*: identification of the synthetized pattern after its matching with memorized patterns, the result of such identification is the dream. *D*: decision. *P*: programming. *E*: execution of the oniric behavior. (*Modified from Timo-Iaria & Valle 1995*).

Researchers working on dream usually do not believe that dreaming may occur in non-human animals, probably due to religious and philosophical reasons but also to a great mistake, i.e., that dreaming is a high level mental activity, such as doing mathematics, but it is not. It is most likely an elementary brain activity in homeotherms and thus, if dreaming has a function, it probably plays a similar role in the human brain and in non-human brains as well.

In non-human animals the report regarding dreams is obviously impossible but, fortunately, a dream can be detected in both humans and other species by analyzing its motor, vegetative and electrophysiological manifestations, as will be described below.

Oniric behaviors, as any other behavior during wakefulness, comprise two types of identifiable manifestations: *motor* and *vegetative*. The *motor components* are usually weak and poorly expressed movements during a dream, mainly if it occurs during desynchronized sleep; when a dream takes place during synchronized sleep phase I, near wakefulness, not only movements are more faithful to the dream content but also the latter is much more logic. The *vegetative components*, that are phasic increases of heart rate, blood pressure, respiration, pupillary diameter, and most probably metabolic adjustments as well, are expressed more consistently during a dream, as they are during attentive wakefulness. The reason for such vegetative adjustments is obviously that the nervous tissue is metabolically very demanding, so much so that 20% of the inspired oxygen goes to the nervous system. Therefore, any neural event, be it running or just thinking, or dreaming, requires a large amount of oxygen, which is carried to the nervous system by the blood through powerful hemodynamic adjustments, such as increase in blood pressure, heart rate and central blood flow (21,25,26). When a dream is a nightmare, both motor and vegetative events may be very intense. In some animals, however, a reduction of heart rate and respiration may occur, what also happens during an attentive wakefulness if they are threatened. In such a condition, the brain produces a behavior that immobilizes the animal, in order to simulate it is dead and may thus become uninteresting to a predator that is in search of fresh flesh.

Recordings of the electrical activity of the brain, which we will refer to as *electro-oscillograms*, reveal specific patterns that express the phases of sleep in several central regions of the brain, including the phase during which most oniric activity takes place, the *desynchronized* or *paradoxical sleep*. Desynchronization is the rule, during this phase, in all cortical electro-oscillograms in humans and other primates. In cats, cortical electro-oscillograms are also desynchronized but in the hippocampus *theta waves* (that will be later described) predominate. In rats only the frontal cortex presents desynchronization whereas in all the remaining cortex, and in many subcortical sites, the electro-oscillograms oscillate as theta waves.

Analysis of the electro-oscillograms yields extremely relevant information that can be correlated with movements and changes in heart rate, blood pressure and respiration. If, as an advantage, in humans such manifestations of dreams can be related to their reported content, in non-human animals it is possible to record with a high degree of accuracy not only the motor and the vegetative manifestations of dremaing but the electro-oscillograms of many central structures as well. Hence, experiments with such animals are extremely valuable and thus will be emphasized in the present review.

Motor components of dreaming

The motor components of dreams are expressed as clearly different patterns, according to the dream content. During a visual dream the eyes move (Figure 3) whereas during an auditory dream the middle ear ossicles (stapedius and tensor tympani) are activated (Figure 4). When a dream has a verbal content the tongue, lips and other facial muscles do contract and if the dream is deambulatory several lower limb muscles do contract, expressing the behavior triggered by the imagined walking. Visual dreams provoke eye movements. Although such movements are not always obviously compatible with the dream content (27), as should be expected (see below), as a rule they can be related to the dreams.

In 1937, Fenn & Bursh, recording the eye movements while their subjects closed and opened the eyes, found that the voltage (*V*) of the potentials that expressed the movements were propor-

tional to the angle of rotation [V=k.2.senα] in which V is the voltage of the recorded potentials, k is a factor of proportionality and α is the angle of rotation (28). Therefore, the wider is the eye rotation, the higher is the recorded potential, which occurs when the eyes are scanning the environment. The narrower is the angle of rotation, the lower is the recorded potential, which happens when attention is being directed to a very small part of the object or when the object is very near. By measuring the voltage of the potential generated by the rotation it is possible to know if the object is near or far. Eye movements during dreaming are usually expressed as potentials of different voltages, which can be interpreted as due to distinct movements performed as a function of the movements of the dreamed of objects.

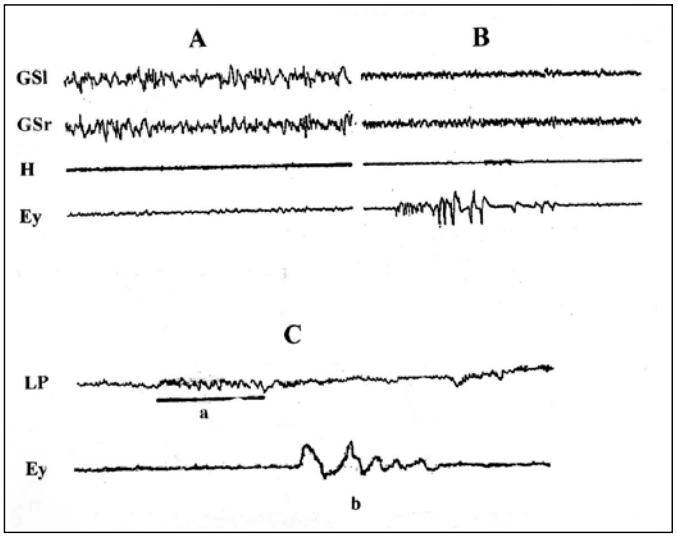

Figure 3. *A*: **synchronized sleep of a cat. Notice spindling and delta waves that characterize phase SII and absence of movements.** *B*: **desynchronized sleep a few minutes after the previous phase, showing light motor activity of the neck muscles (trapezius) but intense eye movements.** *C*: **saw-thooth waves in the electroencephalogram from the right parietal cortex (human), followed by eye movements.** *GSl*: **left sigmoid girus.** *GSr*: **right sigmoid gyrus.** *H*: **electromyography of the trapezius muscles, expressing head movements.** *EM*: **eye movements.**

Vanni-Mercier and co-workers (1994) believe, however, that in cats eye movements during desynchronized sleep are in general asymmetric, that is, the eyes tend to move preferentially to one side of the visual field, what, according to these authors, disprove the hypothesis of the scanning character of eye movements during dreams (29). Our experience with eye movements in rats (30-32) and cats (33) shows, however, that eye movements are sometimes asymmetric but in other occasions they tend to be of the scanning kind. The preferential eye movements direction may be related to the dream content and, perhaps, as such also to hemispheric dominance but it should always be taken into consideration that *any movement originated by a dream is always faulty, otherwise we would perform normal behaviors during a dream, what does not happen due to the inhibition of motoneurons*. If we dream we are walking, the electromyographic recordings from muscles involved in such behavior show quite clearly that they are not able to produce normal movements. In humans, Hansotia and colleagues (34) found in humans, in accordance with our own observations in rats and cats, that oniric eye movements may be directed to one side or the other, not exclusively to one side, as stated by Vanni-Mercier and co-workers (29).

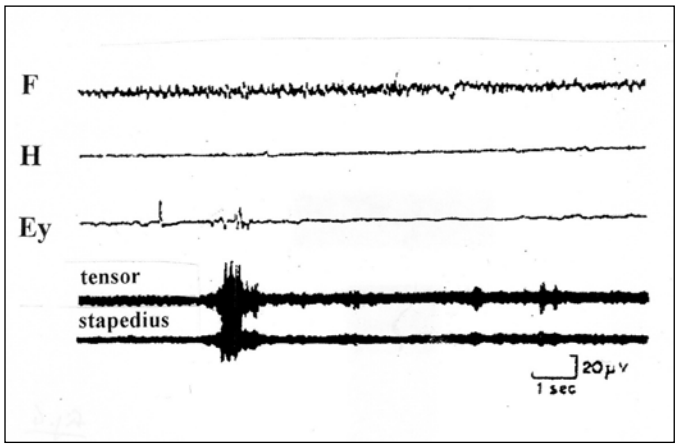

Figure 4. Episode of desynchronized sleep of a cat. The frontal electro-oscillogram (*F*) is desynchronized, the neck electromyogram (*H*), that expresses head movements, shows a very weak activity whereas eye movements (*Ey*) are intense. Concomitantly with the eye movements the tympanic muscles (tensor and stapedius) exhibit a powerful activity, which is suggestive of a dream with auditory components. (*Baust 1971*.)

Eye movements in humans predominate because vision is our main sensory channel and our visual memory is overwhelmingly predominant, resulting in preponderance of visual dreams. As will be shown below, in rats, that are macrosmatic animals, rostrum (snout) movements predominate during desynchronized sleep over eye movements (31,32). Miyauchi et al. (1987) suggested the occurrence of two kinds of eye movements during dreams, one associated to the very dream content, another of reflex nature, that may be involved in those occurring in children and in blind people but such a hypothesis is unlikely to be valid (35). Eye movements in born-blinds are probably due to a quite different reason. Vision is our predominant sensory channel, so much so that if we hear a sound we immediately convey the eyes to the source of the sound, trying to identify its origin, even if vision is absent. Similarly, in rats any kind of sensory stimulation does immediately mobilize sniffing and vibrissal scanning movements. No wonder that most dreams in humans have a visual component, explaining the reason why eye movements occur in any kind of dream, alone or as part of non-visual dreams. In nocturnal macrosmatic animals, olfaction is the predominant sensory channel and their vibrissae are usually very long, to detect the presence of objects at relatively large distances. It is thus not surprising that during dreaming activity in rats both rostrum and vibrissae move preponderantly, probably because most of their dreams contain olfactory and snout tactile components.

As commented upon concerning visual movements, the span of rostrum movements does probably reflect the distance of the

olfactory source. If the animal is trying to identify the source of an odor that is located at a large distance, snout movements are expected to span wide angles at low frequencies, whereas when the source is near such movements are expected to span narrow angles, at high frequencies, just as during wakefulness.

Roffwarg et al. (36,37) have recorded contraction of the tympanic muscles (stapedius and tensor tympani) during human sleep. Around 80 per cent of such motor activity was found to occur during desynchronized sleep, what points to its participation in dreaming activity. In blind people, whose auditory and somesthetic sensitivity is enhanced, auditory dreams predominate, as expected from their high auditory sensibility. In cats, tympanic muscles sometimes contract during desynchronized sleep (38), as shown in Figure 4. This may well reflect auditory dreams, as has been found in humans (36,37). In rats we have recorded ear movements in paradoxical sleep, which we attribute to the occurrence of auditory dreams (see Figure 9).

Head jerky movements may reflect vestibular dreams. Doneshka & Kehaiyov (1978) reported dreams with striking vestibular sensations. In normal humans they found that around 20% of the dreams contain a vestibular component (vertigo, sensation of head drop) but in people with a vestibular illness the proportion of such dreams increased to over 70%, as expected from the close relationship between dreams and the events occurring in the previous day (39).

Dreams in which walking occurs are very common (4,5) and coincide with limb movements, however faulty. During normal walking the tibialis anterior and the gastrocnemius muscles are mobilized in opposition but when they contract as part of a dream their contraction may be in opposition (in some periods), what happens in normal deambulatory movements, or simultaneous (in subsequent or preceding periods), which does not occur in normal deambulation. Such patterns mimic oniric eye movements, which may occur in functional coincidence or not with the visual scenes that are dreamed of.

The correlation between dream content and the oniric movements was first studied by Aristotle, who *identified lip, eye and limb movements and correctly related them to what was being dreamed of.* Many studies performed during the eighteenth century confirmed such statement (4,7). Several authors also quantified the kinds of dreams as related to their sensory content. In 1896 Weed & Halam (4) published the first quantification of dreams content. During the past two decades several authors also did quantify the kinds of dreams. Table 1 shows the results of some of such studies, including our data concerning nearly 2,000 dreaming episodes recorded from rats. Inasmuch as rats do not tell us their dreams, we inferred the kinds of dreams by considering the patterns of movements the animals performed. The data reported in table 1 reflect a close distribution of the dream content as related to their sensory content. Its is noteworthy that Weed & Halam's data, published in 1896, are close to those reported by Rechtschaffen & Buchignani in 1992, which was calculated as the mean of the average of seven different studies published by other authors (40).

It should be recalled here that, comparing the dream content in humans with events of the previous day, Calkins found in 1876 that nearly 89% of the reported dreams were closely related to such events.

The reason why when we dream we are walking we do not get out of the bed and really walk, or when we dream we are talking to someone we do not really talk, is that neural circuits located in the neighborhood of locus coeruleus, in the pontine tegmentum, inhibit the motoneurons and do not allow the real movements to occur. However, we still do not know why most motor units are inactivated while a few ones are mobilized, *causing real but incoherent and non-efficient movements*. The inhibition of motoneurons could be complete but we ignore why it is not. Fortunately, thanks to this peculiar incomplete motoneuron inhibition we are able to record movements occurring in both humans and non-human animals and thus infer the presence of dreams. Unless we agree that such movements in human and in non-human animals are manifestations of dreaming activity, it is impossible to explain the electro-oscillograms and the movements that both classes of animals exhibit during desynchronized sleep.

Table 1. Proportion of the types of dream, as a fraction (percentage) of the total, according to their sensory (in humans) or motor (in rats) manifestations.

	Proportion of the dream patterns according to their sensory or motor content			
	Weed & Halam (human, 1986)	McCarley & Hoffman (human, 1981)	Rechtschaffen & Buchignani (human, 1992)	Valle, Pellarin & Timo-Iaria (rat, 2002)
	Mean %	Mean %	Mean % of the mean of 7 studies	Mean %
Visual	84.4	100	100	18.3
Auditory	67	64	69	1.6
Tatctile	10.8	1	11.5	-
Olfactory	-	1	1	48.8
Gustatory	6.7	1	<1	-
Thermal	-	4	-	-
Vestibular	-	8	-	1.1
Forelimb	-	-	-	3.1
Hindlimb	-	-	-	0.7

Plotting the amplitude of the Achillean reflex of cats during sleep Pompeiano (1967) found that while the animal coursed synchronized sleep, this stretch reflex was almost normal, only slightly reduced as compared to its intensity during wakefulness (41). However, during desynchronized sleep it was drastically reduced, being entirely inhibited for most of the time. A powerful defensive behavior, the withdrawal "reflex" (or *retraction behavior*, as we prefer to call it), is also completely inhibited during this phase of sleep. In humans, both reflex activities are also deeply inhibited during desynchronized sleep. Yet, it is well known since Kohlschütter and Michelson (4,8) that the threshold to awaken a human being during desynchronized sleep is much lower than the one to produce wakefulness during synchronized sleep. It seems that a systematic investigation regarding the threshold to different types of stimulation is still lacking and should be performed, in order to establish which kinds of stimulation and effective thresholds are able to awaken humans and non-humans during sleep. It is likely that even strong stimuli may be ineffective in producing an arousal during sleep if they are trivial, whereas light stimulation containing relevant information may be highly efficient. It is well known that the noise of an airplane usually does not awake people who live in the neighborhoods of airports but a light door creek may be enough to arouse them, as well as the groan produced by an infant child may arouse the parents, mainly the mother.

The tonic inhibition of motoneurons by circuits in the alpha-coeruleus nucleus during desynchronized sleep is mediated by hyperpolarization of their membrane (41-43). This hyperpolarization is due to an increased motoneuronal membrane permeability to chloride ions, which suggests that glycine or γ-GABA are released on the motoneuronal membrane during desynchronized sleep (44). In cats, during movements related to dreams such hyperpolarization is reinforced by presynaptic inhibition of afferents to motoneurons.

Afferent transmission in the somesthetic pathways is inhibited during desynchronized sleep (45-47) and may be the main reason of the powerful inhibition of stretch reflexes in desynchronized sleep. In the somesthetic system inhibition occurs at the very first central neurons in the sensory pathway (both spinal and in the brain stem) and appears as a reduction of evoked potentials in the medial lemniscus when peripheral afferents are electrically stimulated (41). Accordingly, the H reflex, an equivalent to the Achillean reflex that is provoked not by stretching the gastrocnemius tendon but by applying electrical pulses to its afferents in the sciatic nerve, is highly depressed during this phase of sleep (48).

Muscle atonia during desynchronized sleep is, as stated above, generated in the alpha-coeruleus nucleus and involves both direct and indirect pathways that inhibit the motoneurons. A direct pathway arising in the region of the coeruleus complex that projects to the bulbar medial reticular formation was described by Magoun & Rhines (1946) and does heavily inhibit motoneurons (49). Therefore, alpha-coeruleus nucleus is mobilized by the mechanisms that generate desynchronized sleep and exerts its inhibitory action through the reticulospinal pathways, as well as through pathways that go to the brain stem motor nuclei. By lesioning the alpha-coeruleus nuclei such an inhibitory effect is prevented and during oniric activity the movements generated by the dream itself can be expressed, as was clearly demonstrated in Jouvet's Laboratory (50,51) in cats; the animal suddenly gets up, walks, miews and strikes with the paws, as if the animal were awake.

Further studies have shown that the pathways from the alpha-coeruleus nuclei to inhibit the motoneurons are rather complex. Electrical stimulation of the nucleus reticularis pontis oralis evokes bilateral muscle atonia in decerebrate cats (52). Neurons from the nucleus reticularis pontis oralis send fibers to nucleus reticularis gigantocellularis in the medulla, a part of which passes through the dorsal tegmental field of the pons, and electrical stimulation of both nuclei also produces inhibition of muscle tone (53,54). Axons from neurons of the nucleus reticularis gigantocellularis descend along the ventral and ventrolateral funiculi and connect with inhibitory interneurons in the spinal cord (55,56). These interneurons inhibit motoneurons by means of glycinergic synapses (glycine is a powerful inhibitory neurotransmitter), as shown by Soja et al. (57). Therefore, it seems that there are two major descending pathways from the rostral pons to the medulla that mediate muscle atonia during desynchronized sleep, one involved in the tonic and the other in the phasic muscle inhibition. Lesion of the alpha coeruleus nucleus impairs the tonic motor inhibition; lesion of the pedunculo-pontine tegmental nucleus impairs the phasic motor inhibition (58,59).

It is interesting to consider that while muscles all over the body are paralyzed during sleep, respiration is little affected, except that some muscles in the upper respiratory airways are inhibited during sleep (44). Respiratory frequency decreases during the entire sleep cycle but is phasically activated during dreaming because it is a vegetative function that has to be increased in any behavior, including the oniric ones.

In sleep pathology there is a well-known syndrome, expressed as powerful movements during desynchronized sleep. Such movements may take the sleeper to fall off the bed. Although it has not been shown that the alpha-coeruleus nuclei are lesioned in these patients, it is tempting to consider that their lesion underlies such sleep disturbance.

The motoneuron inhibition, responsible for the sleep atonia and abolition of movements, is not complete in infancy (figure 5). Motoneuron inhibition is mild in the early post-birth days and increases according to a saturation curve (60). From a very weak inhibition in early infancy, it goes up rapidly up to 15 years of age, evolving asymptotically from this period on.

Vegetative components

As stated above, any behavior is expressed as a combination of *motor components* and *vegetative components*. The latter are absolutely necessary for any neural activity to occur, inasmuch as the oxygen required by the nervous system amounts to 20% of the total oxygen consumption (near ten times as much as the average of the body as a whole). There are two kinds of vegetative components: *1*. Those that are common to all behaviors (increase in heart rate, blood pressure, blood flow to the nervous system and muscles, ventilation, pupil diameter and palmar and plantar electrical conductance) and are intended to increase the supply of blood, oxygen, glucose etc. to the nervous tissue and muscles during the activation

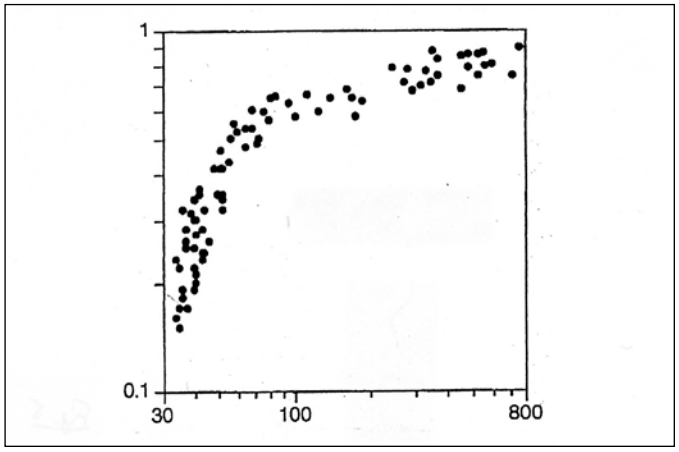

Figure 5. Relative degree of motor inhibiton during desynchronized sleep as a function of age. Maximal inhibition (which is not complete, thus allowing eye, limb, lips, tongue etc. movements to occur during dreaming) is reached around 15 years of age. (*Kobyama, Shimomira & Iwaka 1997.*)

of the circuits that program and execute a particular behavior; and *2*. Those that are specific to certain behaviors. Salivary, gastric, enteric, pancreatic and billiary secretion and contraction of the smooth muscle of the gastrointestinal viscera are specific vegetative components of feeding behavior, as well as secretion of luteotropic hormone, increase in cavernous blood pressure and vaginal blood flow and several other endocrine adjustments are part and characteristic of sexual behavior.

Some authors have not been able to find changes in heart rate and respiration during desynchronized sleep (61) but there are striking demonstrations that blood pressure is reduced (figure 6), attaining values as low as 60 mmHg of systolic pressure; heart rate is also reduced and ventilation decreases (38,62). Apparently, the main cause of such a reduction of blood pressure and heart rate is the active inhibition of the baroreceptor reflexes during this phase of sleep. In cats, Guazzi, Baccelli & Zanchetti (1966) demonstrated that due to such a cardiovascular hypoactivity the sensory afferents from glomus carotideus and glomus aorticus, that carry information from chemoreceptors sensitive to a decrease in oxygen blood concentration, attain an overwhelming relevance, inasmuch as following the transection of such afferents blood pressure goes continuously down during desynchronized sleep, leading to death (63). It is not known if such a mechanism does exist in humans; if it exists, what is highly possible, we can reason that it is the activity of the chemoreceptor system that senses pO_2 that keeps us alive during desynchronized sleep.

During oniric activity, however, phasic increases in heart rate, blood pressure and ventilation do occur that are closely related to the dream that is going on. Usually such increases in blood pressure are not enough to lead it to attain normal levels but during a nightmare blood pressure may go up to 200 mmHg. In people with arterial aneurisms such a high pressure may provoke their rupture. In cats, Baust (1971) recorded tachycardia starting 1 or 2 seconds before eye movements appear (38). Figure 6 shows an increase in heart rate from 150 bpm to 180 bpm (the latter is the normal heart rate during resting wakefulness in this species), coinciding with the peak of eye movements. Heart rate decreases down to nearly 150 bpm 1 or 2 seconds following the cessation of eye movements. The lag between tachycardia and eye movements may be related to all the neural processes that are involved in the phases preceding adn succeeding the oniric behavior, including the very identification of the dream content.

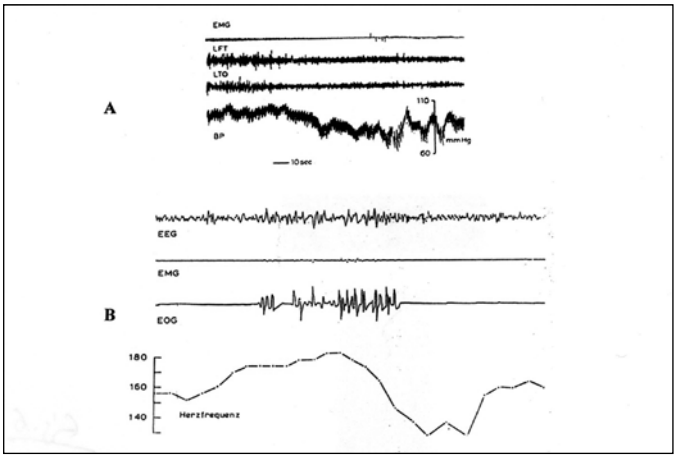

Figure 6. *A*: period of synchronized sleep of a cat (notice the slow waves in the left fronto-temporal lead, *LFT,* and in the left temporo-occipital lead, *LT0)* is followed by desynchronized sleep (low voltage, high frequency EEG), when blood pressure (*BP*) decreases steeply to very low levels. (*Candia, Favale, Guissani & Rossi 1962.*) *B*: desynchronized sleep (see desynchronized *EEG*), with mild movements of the head (*EMG*) but very active eye movements (*E0G*) while heart rate increases, attaining levels prevalent during wakefulness. (*Baust 1971.*)

In rats, heart rate is clearly accelerated during the periods of oniric activity, expressed as rostrum+vibrissae, eye, head, ear and limb movements. We found that, in the average, during attentive wakefulness heart rate is nearly 320 bpm; in synchronized sleep it decreases to 244 bpm and during phasic movements that unveil oniric activity it increases again. When the rat moves the head, for example (which may indicate a vestibular dream), heart rate goes up and may be as high as 330 bpm, similar to that occurring during attentive behavior.

Despite such facts, some physiologists do not agree that heart rate and blood pressure decrease during desynchronized sleep. During dreaming, however, it is well known that both heart rate and blood pressure undergo short duration increases (as related to the decreased values), which are most likely linked to the oniric behavior. Candia et al. (1962) clearly demonstrated that in the cat blood diastolic pressure falls deeply to around 60 mmHg, beginning as soon as the electrocorticogram starts to desynchronize. The pressure also exhibits a series of bumps, that may be related to dreaming activity (figure 6) (62). Our data with rats are quite consistent as to the variation of blood pressure and heart rate during oniric activity. Baust's data regarding the cat are also evident (38).

Thermoregulation is impaired in desynchronized sleep (64) but it is unlikely that body temperature changes due to dream-

ing activity, inasmuch as variations of temperature are slow while dreaming is a fast pace phenomenon. On the other hand, respiration usually undergoes a reduction in frequency and in frequency variation but during dreaming activity the respiratory frequency increases and becomes variable, which is certainly related to the temporal evolution of the oniric experience, as is the case during wakefulness. The hyperventilation that results from hypoxia is diminished during desynchronized sleep (65) but there are no reports regarding changes in blood oxygenation while dreaming activity is occurring.

Electrophysiological characterization

In humans the electro-oscillograms during desynchronized sleep are expressed as overall cortical desynchronization, whence the adequacy of the name created by Moruzzi, *desynchronized sleep.* Generally, in humans oniric activity is expressed as eye movements, what is obviously linked to vision as the main human sensory channel. Often eye movements are preceded in the electroencephalogram by small sawthoot-waves that superimpose on desynchronized potentials (figure 3). However, human oniric behaviors are also expressed as lips, tongue and facial movements, as well as fingers, toes and whole limbs jerks, as described above.

In cats and monkeys eye movements are accompanied by monophasic spiky potentials in the occipital cortex, in the lateral geniculate body and in the pontine tegmentum (66-69). Accordingly, they are known as PGO (*p*ontine, *o*ccipital cortex and lateral *g*eniculate nucleus) potentials. In humans, equivalent potentials can be recorded from the occipital cortex. In rats we found similar potentials in the amygdala as related to olfactory dreams, expressed as rostrum movements (32). No PGO potentials have been found in rats (70). Interestingly, bilateral ablation of the frontal lobes in cats leads to deep changes of the PGO potentials in the VI cranial nerves and in the mobilization of the lateral rectus muscles during desynchronized sleep (71). The number of PGO potentials undergoes a high increase after the frontal ablation, which is suggestive of a tonic inhibition of these potentials by the frontal cortex.

It is usually taken for granted that PGO potentials are essential manifestations for the electrophysiological identification of dreaming activity but such view is not well founded. Essential manifestations of dreaming are the conscious experience, the electrophysiological, the motor and the vegetative expression of oniric behaviors in humans as well as in other animals. The PGO potentials are correlates of dreams. Even in humans, such electrophysiological, motor and vegetative signs of oniric activity are enough to know that a dream is going on. Considering that most dreams in rats (31,32) are related to olfaction, not to vision, potentials that resemble PGOs in the amygdala of this animal species should also be taken as signs of dreaming rather than PGOs.

In cats desynchronized sleep appears also as tonic cortical desynchronization (figure 3) but in the hippocampus, septal area and amygdala theta waves predominate, as in rats and rabbits. Theta waves, discovered by Jung and Kornmüller in 1938 (72), were extensively studied by Green & Arduini (73), who proved they are related to arousal. Later, theta waves were also found in rats during both attentive wakefulness and desynchronized sleep (19,30,31,74-76). Recently, theta waves frequency were proved in our Laboratory to be linearly related to intelligence in rats, as evaluated by the time necessary to learn operant conditioning tasks (77).

Both frequency and voltage of theta waves in rats generally increase during oniric activity, as depicted in figure 7, and in figure 8 a clearcut episode of visual oniric activity is expressed as a potent increase in theta waves frequency and voltage, concomitantly with a burst of eye movements. Figure 9 illustrates an episode of olfactory and vibrissal movements.

By visually examining the amplitude of theta waves in these examples it seems they vary at random but when the instant variation of voltage is plotted as a function of time, a regular variation appears during the phasic movements (figure 10). A regular oscillation modulates the amplitude of the potentials. Frequency clearly increases and becomes regular, as compared with the trend before oniric activity. Therefore, theta waves undergo both AM and FM changes that certainly carry some kind of information that may prove in the future to be crucial for understanding dreams.

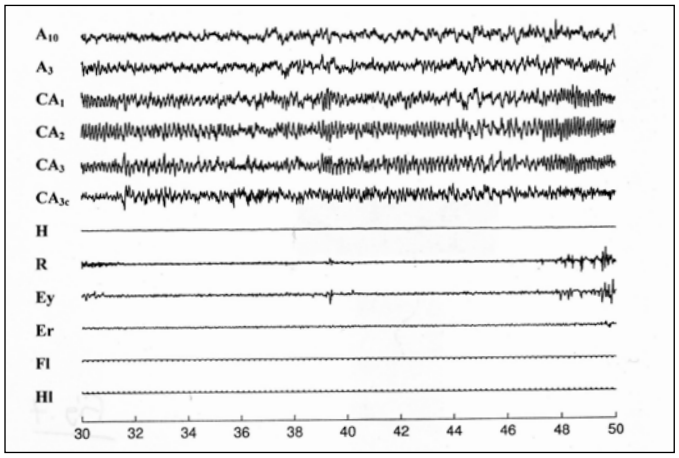

Figure 7. Desynchronized sleep and oniric activity of a rat. This is a typical figure in which a correlation between theta waves and oniric movements is quite clear. During the first episode the oniric activity, expressed as rostrum (*R*) and eye (*Ey*) movements, occurs when theta waves oscillate at a high frequency (8 Hz). It is then followed by a period in which theta frequency decreases to 5.7 Hz and no movements are detected in the recording. In the middle, a short burst of rostrum and eye movements do occur in coincidence with a short duration increase in voltage and frequency of theta waves. Finally, theta waves are again highly activated and rostrum, eye and ear movements occur, revealing oniric activity. Preceding the last episode theta waves frequency suddenly increases from 6.1 Hz to 9 Hz and then oniric activity starts again. A_{10}, frontal area 10. A_3, fronto-parietal area 3. CA_1, CA_2, CA_3 and CA_{3c}, corresponding hippocampal fields. *H*, head movements. *R*, rostrum+vibrissae movements. *Ey*, eye movements. *Er*, ear movements. *Fl*, forelimb movements. *Hl*, hindlimb movements. Time in seconds.

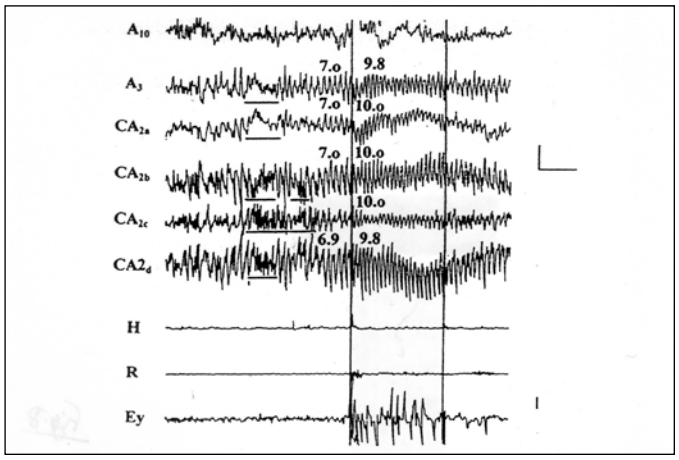

Figure 8. An oniric episode in a rat, expressed as very intense eye movements and a large increment in theta waves frequency (frequencies, in Hz, are indicated above the electro-oscillograms), brief head and rostrum movements are present only during the earliest 300 milliseconds of the oniric episode. During the first half of the figure synchronized sleep suddenly becomes desynchronized sleep and a short period of intense desynchronization lasting nearly one second breaks through synchronization. Such a period may correspond to a brief but intense arousal or to a brief but intense dreaming activity, in both cases without any detectable movement. A_{10}, frontal area 4. A_3, fronto-parietal area 3. CA_{2a}, CA_{2b}, CA_{2c} and CA_{2d}, four sites within hippocampal field 2. *H*, head movements. *R*, rostrum+vibrissae movements. *Ey*, eye movements.

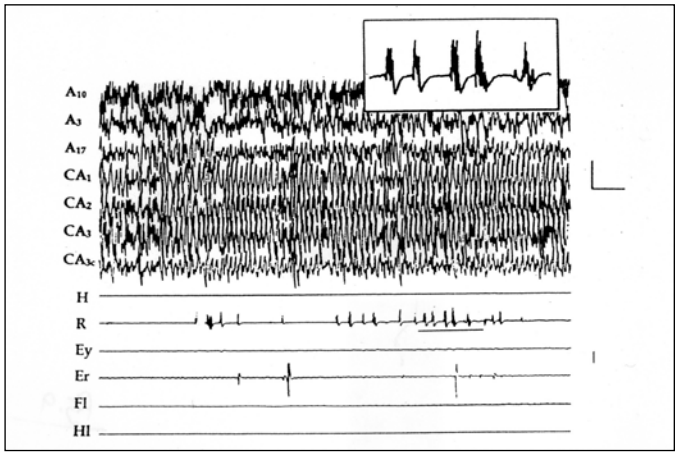

Figure 9. Oniric episode expressed as intense rostrum+vibrissae and ear movements, whereas eye movements are very weak. The underlined portion in *R* (rostrum+vibrissae lead) is magnified in the inset. The high frequency (around 7 Hz) of the *R* movements points to the prevalence of vibrissae mobilization, similar to what occurs during wakefulness when the rat is exploring the environment with its vibrissae. Ear movements are common as twitches of the ear. A_{10}, frontal area 4. A_3, fronto-parietal area 3. A_{17}, area 17 in the occipital cortex. CA_1, CA_2, CA_3 and CA_{3c}, corresponding hippocampal fields. *H, R, Ey, Er, Fl* and *Hl*, head, rostrum+vibrissae, eye, ear, forelimb and hindlimb movements, respectively. Calibration: 100 μV and 1 second.

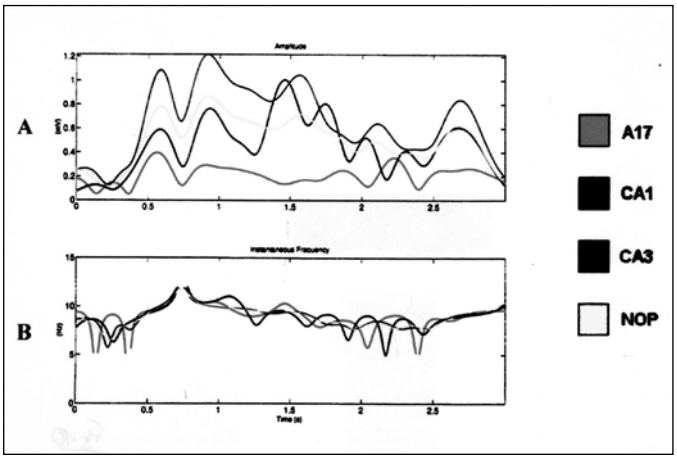

Figure 10. *A*: time course of the change in peak voltage of theta waves occurring in area 17, in CA_1 and CA_3 hippocampal fields and in nucleus reticularis pontis oralis (*NOP*) of a rat during a brief oniric episode. At right, colors correspond to the different curves. When the oniric episode begins, voltage of the theta potentials starts to oscillate regularly. *B*: frequency of the same potentials in *A*. It is very interesting that during a mere 0.5 period theta frequency went up quite steeply and in phase in all sites and then started to occur at a different rate in each site. Time: seconds.

Another change of the electro-oscillograms we disclosed in rats by carefully analyzing their time-course while a dream is on (as well as during attentive wakefulness) is the presence of short periods of desynchronization that interrupt or superimpose on theta waves. Considering that desynchronization is predominant all over the cortex in humans and in the frontal cortex of both cats and rats, we consider it to be a phylogenetically more recent functional acquisition. Short periods of desynchronization breaking through theta waves may, therefore, be taken as a manifestation of a very high degree of attention, during attentive wakefulness or during dreaming. In fact, it does frequently occur when movements are expressed as high frequency potentials. During wakefulness such periods in rats are concomitant with short but complete immobilization, which is well known to occur when a high degree of attention is being directed to some external object. In humans it has been shown that not only EEG desynchronization but also increase in vegetative functions, such as heart rate and ventilation (27), accompany mental activity.

We hypothetize that theta waves are commanding signals that recruit in due sequence the circuits that generate wakefulness and desynchronized sleep and their components; their frequency and voltage generally increase in parallel with heart rate and intensity of movements (Valle & Timo-Iaria, unpublished results).

The command character of theta waves is probably the reason why such potentials occur almost simultaneously in different brain structures. In fact, when the voltage of each theta wave in one site is compared with the voltage in another site it is possible to assess the degree of coincidence or phase shift between the two sites. For instance, during desynchronized sleep theta waves, in rats, are highly coherent in nucleus reticularis pontis oralis and in the fronto-parietal cortex, as well as with the hippocampus (78).

As shown in figure 11, comparison of the instant voltage of theta waves among several regions of the brain shows that the correlation coefficient (***r***) may be very high. The value of ***r*** is as high as 0.9618 when theta waves in the hippocampal CA_1 field of one side are matched with those in the nucleus reticularis pontis oralis, what points to a close temporal relationship between theta waves in hippocampus and in the nucleus reticularis pontis oralis. Also, correlation is high when theta waves in the thalamic reticular nucleus are matched to those occurring in the nucleus reticularis pontis oralis. Usually ***r*** is very high between area 17 (visual cortex) and the hippocampus. Such high values of ***r*** may mean that theta waves arrive in such areas almost synchronously, coming from some other sites in the central nervous system. Nucleus reticularis pontis oralis is thought to contain the generator of theta rhythm (78,79) and is known to send direct efferents to the hippocampus and the cerebellar cortex, where we found theta waves that correlate closely with those in the hippocampus (Valle, Kubo, Iwamoto & Timo-Iaria, in preparation for publication).

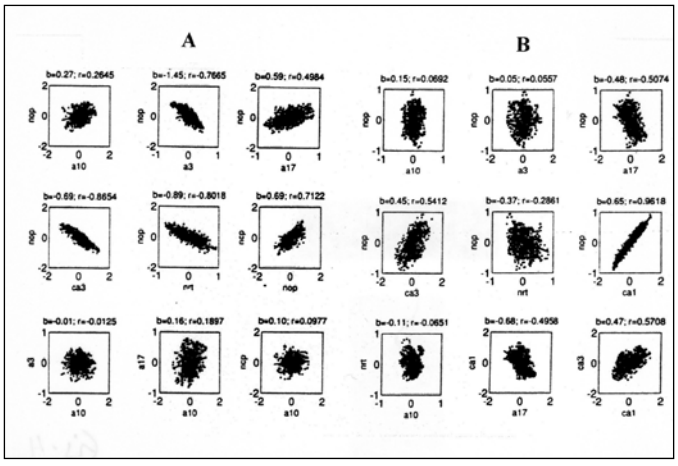

Figure 11. Scatter diagram displaying the correlation between concomitant theta waves voltage in several sites of the central nervous system during two periods (*A* and *B*) of desynchronized sleep of a rat. Each dot represents the peak voltage (expressed in mV), in a given instant, of concomitant theta waves in two sites and measured each 3.9 milliseconds. *n*=768 measurements, *r*: coefficient of correlation, *b*, slope of the regression line. The slope leaning to one side or the other is not relevant, inasmuch as it depends on the polarity of the potentials input in the amplification system. The sites of recording are indicated in the *x* and y axis. *NRPO*: nucleus reticularis pontis oralis. *NRT*: nucleus reticularis pontis caudalis. CA_3 and CA_1: hippocampal fields. A_{10}, A_3 and A_{17} correspond to cortical areas 10, 3 and 17, respectively. (*Simões, Valle & Timo-Iaria 1996*.)

Not only theta waves do occur in the cerebellar cortex during desynchronized sleep but also spindles and delta waves are found in this organ in synchronized sleep, just as in neocortical areas. In fact, all the phases of wakefulness and sleep, including desynchronized sleep, occur in the cerebellar cortex. Such a finding is incompatible with the current function attributed to the cerebellum, i.e., only correction of movements. Our hypothesis is that the cerebellum is involved in overall corrections of the components of all kinds of behavior, including sleep. Such hypothesis is grounded on the following steps. When any part of the brain programs a behavior it sends the program to the cerebellum. This organ receives information from the entire body, including the baroreceptors, as shown by Moruzzi (80). By comparing the program with the peripheral information, that tells it how the behavior is evolving, the cerebellum produces corrections, so that the execution can match the program. If this hypothesis is correct, it is no surprise that the phases of wakefulness and sleep are expressed in the cerebellar cortex by means of electrophysiological potentials.

From the spinal cord Marini (1997) recorded slow (delta) regularly oscillating waves during desynchronized sleep (81), which may be related to activation of spinal neurons during dreaming. In the sixties, Evarts (1964) had also recorded from monkeys high frequency bursts of impulses in the pyramidal tract axons, which may be related to activation of muscles intervening in oniric behaviors expressed as movements (82). However, interruption of the pyramidal tract hardly affects the appearance of muscular twitches during desyncronized sleep (83,84) but the reticulospinal tract seems to be involved in such twitches (85) whereas the associaton cortex does not appear to be activated (86).

By recording potentials from large ensembles of rat hippocampal neurons related to the body position in space (*place cells*) during behavioral tasks, Wilson & McNaughton (87) found that neurons that fired together when the animals occupied particular locations in the environment (hence the name *place cells*) also exhibited an increased tendency to fire together during subsequent sleep, in comparison to sleep episodes *preceding* the behavioral tasks. On the other hand, cells that were silent during the behavioral task did not show the increase in frequency. The authors concluded that the correlation they found was probably involved in memory consolidation but such coincidence may indicate that during dreaming memorized information is being revoked to integrate a given dreaming pattern. Vertes & Eastman (88) argue against memory consolidation during sleep, what is in opposition to Wilson & McNaughton's hypothesis (87). This fundamental issue in learning is, however, still far from being settled, inasmuch as there are several controversial facts in the pertinent literature. It may be more appropriate to explain the latter authors' results by reasoning that dreams are originated in memorized information and are, accordingly, closely related to events occurring before sleep.

Genesis of dreaming

Generation of sleep is reasonably well known but not that of dreaming. In 1963 we found that cholinergic stimulation of a descending pathway (within Nauta's *limbic-mesencephalic system*) causes sleep (33). The caudalmost portion of this system (then labeled *descending hypnogenic cholinergic*) comprises the ventral and dorsal Gudden's nuclei, whose stimulation with carbachol triggered sleep in nearly 20 seconds. The previous station of these nuclei is the interpeduncular nucleus, whose stimulation with carbachol caused sleep within nearly 30 seconds. In both instances sleep evolved according to the phases of synchronized and then of desynchronized sleep, during which eye movements always

occurred. In more recent years several approaches confirmed these findings (89).

After transection of the brain stem at the pontomesencephalic transition, rostrally to locus coeruleus, desynchronized sleep still occurs below the transection (10,90-93). Desynchronized sleep can be provoked by carbachol infusion in the pontine reticular formation (94). Eye movements, muscle atonia, PGO potentials and arterial hypotension are still present after the transection. Above the transection, synchronized and desynchronized sleep keep occurring but without eye movements. In this preparation body temperature is not regulated anymore and the animal has to be artificially warmed at nearly 37ºC. Interestingly enough, if body temperature in cats subjected to pontomesencephalic transection is lowered, the amount of desynchronized sleep increases. At 36ºC, for instance, desynchronized sleep spans to about 10% of time, at 23ºC it occupies nearly 80% of the time, what has not been explained so far.

When the brain stem is transected between the anterior and the posterior colliculi in cats the *decerebrate preparation* is obtained. The main purpose of experimental decerebration is to study the mechanisms of the *fundamental posture*, that is, the standing posture. It has, however, been utilized with a great success in sleep studies. In decerebrate cats eye movements do occur and are integrated below the midbrain (67,95). Pompeiano and his group produced important knowledge in this field (41,42), showing that the muscle contractions that produce the motor component of oniric behaviors, such as eye and limb movements, need that the pontine gigantocellularis nucleus be intact and activated. Reticulospinal and reticulobulbar tracts are involved in conveying to the motoneurons the impulses that cause oniric movements. Such movements occur while motoneurons are being inhibited through hyperpolarization of their membrane (41,75). The gamma-alpha loop has been shown to play no role in producing the movements that characterize dreaming. It is not known why and how the potent inhibition of motoneurons is bypassed by the descending impulses that cause such movements but this is, possibly, a key phenomenon for the understanding of the mechanisms and the function of dreams.

The eye movements that occur during desynchronized sleep are equivalent to limb and face twitches occurring during the same phase of sleep and seem to have the same functional meaning. There is experimental evidence that eye movements are generated near the nucleus of the abducent nerve but Pompeiano (1967) does not agree with this view (10,41). Electrical potentials recorded from the medial vestibular nuclei precede eye movements by 20 to 30 milliseconds, which points to these nuclei as the last synaptic stations in the pathway that produces eye movements during desynchronized sleep. Such electrophysiological studies demonstrate that the abovementioned sites in the central nervous system are involved in the oniric movements but they do not prove that such structures generate them. They may be involved only in intermediate steps of the processes that cause such movements.

Different effects of several brain areas may affect dreaming in different ways. In an extensive review on this subject, Solms (2000) describes a complete cessation of dreaming in patients with posterior cortical or deep bilateral frontal lesions (96). The posterior areas affected in this syndrome are the visual areas V3, V3a and V4 (97). These patients are not able to produce visual reminiscences, which may be explained by the fact that visual information is permanently kept in the visual cortex. Since evoking visual reminiscences during wakefulness and the building up of dreams with visual information are dependent on the visual cortex, both facts can be correlated.

The meaning of dreams

This is for sure the most enigmatic issue about dreaming. There are many hypotheses to account for the existence of dreams but it is still a matter of debate why and what for we dream. In fact, we ignore almost completely why we dream. Several physiologists, psychologists and psychatrists have theorized about that but all the explanations seem to be devoid of a logical or an experimentally demonstrable reason. Some presently available explanations seem science fiction, rather than true science.

To discuss this issue we will concentrate only on a few hypotheses. As mentioned above, Plato, preceding by twenty four centuries one of the dogmas of psychoanalysis, believed that "forbidden" dreams, such as incestuous or criminal dreams, were only a way of doing incestual sex or killing someone without punishment. As such, this explanation may be interpreted as a way of doing something that we should never be allowed to do without paying for it. Dreams are still taken by a majority of the human kind as premonitory, ascribing them the function of telling us that something important will happen.

A theory that has many followers is the one that connects dreams, in particular, desynchronized sleep in general, with memory consolidation. Despite several demonstrations that this hypothesis is correct, a few argue against such a view. Vertes & Eastman (2000), for instance, believe that the stressful conditions in experiments intended to demonstrate a role of desynchronized sleep and dreaming in consolidation of memory spoil the results (88). These authors argue that despite the marked suppression of desynchronized sleep provoked by tricyclic antidepressants neither selective serotonine reuptake inhibitors and mono-amino-oxydase nor learning and memory are disrupted.

Recently a more acceptable evidence in favor of the consolidation hypothesis arises from the study of a gene involved in neuronal activation This gene protein, zif-268 (98), binds to a specific DNA molecule present in the promoters of a variety of genes expressed in the nervous system (99) and its up-regulation is thought to initiate a program of gene regulation leading to neuronal plasticity (100). For instance, zif-268 has been shown to induce the expression of a synapse-specific protein, synapsin II (101), and has been linked to the induction of hippocampal long-term potentiation (102,103) and other plasticity phenomena. In addition, zif-268 is up-regulated in several novelty or learning behavioral paradigms, including two-way active avoidance (104), brightness discrimination (105), and enriched environment exposure (106). Fos-like immunoreactivity was also found in association with cholinergically induced REM sleep (107,108). In 1999, Ribeiro et al., assaying zif-268 expression in control rats and in rats subjected to a rich environment training, found that in control animals this gene protein generally decreased, mainly in the cerebral cortex,

from wakefulness to synchronized sleep and from synchronized to desynchronized sleep (109). However, in the animals subjected to a rich-environment zif-268 increased significantly from synchronized to desynchronized sleep but decreased from wakefulness to synchronized sleep. Such activation of zif-268, which is likely to be correlated with the effect of learning on desynchronized sleep, was larger in the frontal and hippocampal cortices, where memorization is well known to occur.

Foulkes (1982) considered that dreams are so easily forgotten because the brain in desynchronized sleep is in a "reflective state". We suppose, instead, inasmuch as dreams are forgotten if we are not aroused while dreaming or within ten to fifteen minutes immediately after the dream has ceased, that it may well be that dreams are forgotten because the reticular activating system is highly deactivated during desynchronized sleep and thus the memory of the dreams cannot be consolidated (110).

Electrophysiologically, it has been shown that the same type of hippocampal cells that are activated during training in a radial maze are also endogenously reactivated during sleep, which accounts for memory consolidation and for a close correlation between dreams and events preceding sleep (87).

The hypothesis has been recently put forward by Revonsuo (2000) that the function of dreaming is to simulate threatening events, and to rehearse threat perception and threat avoidance (111). This seems to be a highly improbably conception, among other reasons because, as dreaming is concerned, threatening events are as dangerous to the organism as bad news we hear and as crossing a street or watching a movie-film full of violence are as well. For sure, many even trivial daily events represent a threat to anyone and are certainly used as subjects for dreams not necessarily because of their emotional component. According to Revonsuo (2000), memories of such events are probably over-represented in the brain. Since memorized information is the basic material to build up dreams, it is understandable that many (but not all) dreams are threatening and emotionally highly charged (111).

It is interesting that the representation of animals in dreams of infants is quite conspicuous. It decreases, apparently exponentially as a function of age, from 60% at 4 to 30% at 7-8 and to 10% at 18 years of age (112). This may be related to the presence of pet animals in most families in the Western countries and consequently this "subject" probably becomes the main thought of children. Later, school and work dominate the mental field and the main features in dreams also change accordingly, supporting this hypothesis.

Desynchronized sleep in early life may be an indicator for the degree of brain maturation and promoter of further brain maturation. Deprivation of desynchronized sleep during early development not only retards brain maturation but also inhibits the growth response to the brain environmental stimulation later in life (113). In rats subjected to early desynchronized sleep deprivation, ejaculation was deeply reduced in adulthood (114,115), what is a profound impairment of a very important instinctive behavior. The authors suggest that such a disturbance of reproduction occurs because desynchronized sleep (and consequently dreaming) was prevented to occur normally in infancy but the functional meaning of this interesting phenomenon.

Another hypothesis to account for desynchronized sleep function is that this phase of sleep is programmed to occur when central temperature is low and that it has a thermoregulatory function. Therefore, desynchronized sleep should be ascribed a homeothermic function (116). Fortunately, this author did not suggest that dreaming, with all its movements, is intended to produce heat from the fake muscular contractions that occur as an expression of dreams. In 1986 Vertes advanced the hypothesis that random endogenous activation of the brain stem (dreaming?) during desynchronized sleep prevents sustained brain inactivity, which might occur during sleep.

A related point of view was put forward by Krueger & Obal (1993), who proposed that, on the basis of use-dependent synaptic stabilization, the neuronal assembly not activated during wakefulness will be activated during sleep, to prevent it from atrophy (117).

Another fancy hypothesis is the one that proposes that we dream to forget, in order to delete "unwanted" information by reverse learning or unlearning (118). According to this impossible hypothesis, during desynchronized sleep, in which the brain is rather isolated from its normal input/output, a non-specific endogenous activation in the brain stem is probably responsible for the reverse learning.

Jouvet (12,119), one of the most important researchers on sleep, suggests that dreaming is "a guardian and programmer of the hereditary part of our personality" and as such it plays a role in our general behavior. Thanks to the extraordinary possibilities of functional connections that take place in the brain when the "basic circuitries of our personality are programmed", dreams do contribute to shape new solutions for new problems. Jouvet believes that dreaming activity plays a key role during the earliest years of life and thus may be involved in continuously programming some of the most subtle reactions of our consciousness during wakefulness.

Whereas Freud was convinced that dream forgetting was an active function of repression, Hobson, Pace-Schott & Stickgold (2000) attribute the failure to recall a dream to a state-dependent amnesia caused by aminergic demodulation of the sleeping brain (120). The waking level of aminergic modulation falls to 50% during synchronized sleep and to nearly zero in desynchronized sleep (121,122). It would appear that the intense activation of desynchronized sleep must overcome this demodulation and persist into subsequent waking, in order for very vivid dreams to be remembered.

It has been proposed (120,123,124) that presleep mentation is infrequently incorporated in top dreams and that "naturalistic" day time events rarely enter dream content, but several authors correlated dream content to the previous day events, starting with Aristotle 2,400 years ago and with Calkins in 1893. Hobson, Pace-Schott & Stickgold (2000) do not take into consideration that a single object or a brief key fact or image occurring in the day preceding a given dream may be enough to trigger an entire dreamed "story" related to it (120). They also argue that even "expensive and cumbersome evoked potential and computer averaging approaches have not helped us to analyze and compare desynchronized sleep physiology with that of waking in an effective way". This statement is incorrect, inasmuch as electro-oscillograms during both

states in humans are not so similar as to confound an observer and in rats we have found that theta waves that occur in both attentive wakefulness and in desynchronized sleep are largely different. During wakefulness theta waves consistently exhibit a lesser voltage and are less regular than during desynchronized sleep, what makes it easy to tell wakefulness from desynchronized sleep from the shear inspection of the electro-oscillograms (21,30,31,125).

In rats bilateral lesion of the midbrain reticular formation is followed by a long lasting state of synchronized sleep, with predominance of phase III (Timo-Iaria, Assumpção & Bernardi, unpublished observations). If the animal is kept alive by forced feeding and is kept warm, in six days frontal desynchronization and theta waves in the other cortical areas reappear and then not only wakefulness is fully recovered but also desynchronized sleep, including oniric activity. Such a recovery means that other mechanisms are put into action that are able to generate not only wakefulness but desynchronized sleep as well. When only one side of the reticular formation is also destroyed, the same pattern of recovery does occur; if the other side of the reticular formation is also destroyed after two or three weeks, recovery of wakefulness and desynchronized sleep is even faster than when both sides are lesioned at the same time. This is an additional fact to point to the activation of other mechanisms capable of producing wakefulness and desynchronized sleep, including dreaming.

According to Hobson, Pace-Schotter & Stickgold (2000), since image studies show activation of "limbic" and "paralimbic" structures of the forebrain during desynchronized sleep, as compared to wakefulness (120,126-128), emotion may be a primary shaper of dream plots, rather than playing a secondary role plot instigation. However, we all know that many dreams are not emotional at all.

Braun et al. (1997) found during desynchronized sleep a consistent activation of the pons, midbrain, anterior hypothalamus, caudate and medial prefrontal, caudal orbital, anterior cingulate, parahippocampal and inferior temporal cortices (126). These findings do not necessarily mean that such areas are involved in generating dreaming. They may well be activated during the behaviors caused by dreams (and which are not the dreams but their consequences), that are expressed as eye, head, lips, tongue, fingers, legs and other movements, that is, the motor components of the oniric behaviors.

Selective deactivation of the dorsolateral prefrontal cortex has been found in desynchronized sleep. Maquet et al. (1996) and Braun et al. (1997), in their PET studies, found a significant deactivation, in desynchronized sleep, of a large portion of the dorsolateral prefrontal cortex, what was found also by Madsen et al. (1991) and Lovblad et al. (1999) (126,127,129,130). No wonder that dream recall is impaired in brain-damaged patients (97). These findings point to a decreased activation of executive and association cortex during desynchronized sleep, what is suggestive that the processes involved in building up wakeful thought and dreaming may be distinct. Despite such discrepancies, however, during synchronized sleep PRT studies reveal a decrease in global cerebral energy metabolism relative to both waking and desynchronized sleep. Metabolism during desynchronized sleep tends, in fact, to be equal to or even larger than that of waking (131,132). In addition, blood flow velocity in the middle cerebral artery decreases during synchronized sleep whereas in desynchronized sleep it is similar to that occurring in waking (133).

Winson (1990) believes that dreams "reflect an individual strategy for survival. The subjects of dreams are broad-ranging and complex, incorporating self-image, fears, insecurities, strengths, grandiose ideas, sexual orientation, desire, jealousy and love". According to this author, in children at the age of two, when the hippocampus, which is still in the process of development at birth, becomes functional, REM sleep takes on its interpretive memory function (134).

According to Mancia (1995), the brain "produces dreams" as "a symbolic process of elaborating, interpreting and reorganizing in narrative sequences all the material accumulated in the memory during waking hours". This author "thus proposes a psychoanalytical model of dreaming, in which dreams constitute a way of representing the individual's inner world with internal objects related with one another and with the self" (135).

Considering dreams as hallucinations, Hernández-Peón (1966) theorized that they are possible because the system responsible for wakefulness is inactivated during sleep, releasing memory tracings which are brought to consciousness. Although related to the information fluxogram displayed in figure 2 of the present review, Hernandez-Péon's process involves the function of participating in "adaptive waking behavior", which does not seem to have a real meaning (136).

The meaning of dreams is therefore still an unsolved problem. Many hypotheses have been advanced but so far they do not explain why and what for we do dream. Despite the fact that many studies have found that mental activity during wakefulness differs from that during dreaming, the mechanisms involved in both may differ as to the degree of control over the release and combination of memorized information in wakefulness and in desynchronized sleep.

Inasmuch as dreaming seems to occur in most birds and mammals, it is unlikely that it has no function in the animal organism. Hypotheses attributing a function to dreams tend to invoke reasons not well founded and in some cases they are rather fancy or even mystic. Much experimental work is needed before a convincing function can be ascribed to the fascinating physiological phenomenon that is dreaming.

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events

23rd ANNUAL MEETING OF THE ASSOCIATED PROFESSIONAL SLEEP SOCIETIES - APSS

June 6 - 11, 2009
Seattle / Washington / USA
www.sleepmeeting.org

PNEUMOSUL 2009 – CONGRESSO DE PNEUMOLOGIA DA REGIÃO SUL

II Congresso de Atendimento Multidisciplinar em Doenças Respiratórias
April 18 - 21, 2009
Hotel Mabú – Foz do Iguaçu / PR / Brasil
www.eventos2009.net/2009/02/24/pneumosul-2009-congresso-de-pneumologia-da-regiao-sul

22º CICLO DE AVANÇOS EM CLÍNICA PSIQUIÁTRICA

May 8 - 9, 2009
Centro de eventos AMRIGS – Porto Alegre / RS / Brasil
www.aprs.org.br

5º CONGRESSO GAÚCHO DE NEUROLOGIA E NEUROCIRURGIA

3º Simpósio Gaúcho de Reabilitação Neurofuncional
2º Simpósio Latino-americano de Monitorização Neurofisiológica Intra-operatória
Encontro do DC de Moléstias Neuromusculares da ABN
May 14 - 16, 2009
Hotel Plaza São Rafael – Porto Alegre / RS / Brasil
www.ccmeventos.com.br/neuro2009

VII CONGRESSO PAULISTA DE MEDICINA DO SONO

May 15 -16, 2009
Associação Paulista de Medicina – São Paulo / SP / Brasil
E-mail: eventos@apm.org.br

XII SIMPÓSIO - DOENÇAS DE INVERNO RINITE, RINOSSINUSITE E ASMA

X Workshop de Otorrinolaringologia Pediátrica
XI Fórum Interativo de Otites
May 15 - 16, 2009
Hotel Sheraton – Porto Alegre / RS / Brasil
www.doencasdeinverno.com.br

5º CONGRESSO BRASILEIRO DE CÉREBRO, COMPORTAMENTO E EMOÇÕES

Simpósio de Atualização em Doenças do SN
Jornada Nacional de Neuropsiquiatria Geriátrica
Sono, Epilepsia e Comportamento
June 11 - 13, 2009
ExpoGramado / Gramado / RS / Brasil
www.cbcce.com.br

3rd WORLD CONGRESS ON SLEEP MEDICINE OF WORLD ASSOCIATION OF SLEEP MEDICINE – WASM

November 7 - 11, 2009
WTC Sheraton – São Paulo / SP / Brazil
www.wasm2009.com.br

VII CONGRESSO PAULISTA DE NEUROLOGIA

June 25 - 27, 2009
Hotel Sofitel Jequitimar Guarujá / SP / Brasil
www.apm.org.br/neurologia

19TH INTERNATIONAL SYMPOSIUM SHIFTWORK AND WORKING TIME. HEALTH AND WELL- BEING IN THE 24-H SOCIETY

August 2 - 6, 2009
Venice / Italy
http://osha.europa.eu/en/campaigns/hw2008/19th_international_symposium_shiftwork_working_time_01.26012009

XXIV REUNIÃO ANUAL FESBE – FEDERAÇÃO DE SOCIEDADES DE BIOLOGIA EXPERIMENTAL

August 19 - 22, 2009
Centro de Convenções do Hotel Monte Real / Águas de Lindóia / SP / Brasil
www.fesbe.org.br/fesbe2009

8º CONGRESSO IBERO AMERICANO DE OSTEOLOGIA Y METABOLISMO MINERAL

3º BRADOO – Congresso Brasileiro de Densitometria, Osteoporose e Metabolismo Ósseo
October 1 - 3, 2009
Hotel Bourbon – Foz do Iguaçu / PR / Brazil
www.ccmeventos.com.br/osteo2009

6º ENCONTRO NACIONAL DE DISTÚRBIOS DO MOVIMENTO

October 5 - 7, 2009
Costão do Santinho – Florianópolis / SC / Brasil
www.ccmeventos.com.br/dm2009

X LATIN AMERICAN SYMPOSIUM ON CHRONOBIOLOGY

October 21 - 24, 2009
Eurosol Tibau (Pipa) Resort – Natal / RN / Brazil
www.cb.ufrn.br/labcrono/lasc

36th WORLD HOSPITAL CONGRESS – IHF RIO 2009

November 10 - 12, 2009
Windor Barra Hotel & Congressos
Rio de Janeiro / RJ / Brazil
www.ihfrio2009.com/portugues/index.html

Sleep science

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