

## THE ROLE OF THE DOPAMINERGIC SYSTEM IN SLEEP-WAKE REGULATION

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### Abstract

**Aim:** The Role of the Dopaminergic System in Sleep-Wake Regulation.

**Material & Methods:** The study was conducted at the sleep laboratory of the department of psychobiology with the approval of the ethics Committee of the university as well as the radiation protection center. Fifty healthy male volunteers ranging from 20 to 30 years of age were randomly assigned to one of three experimental groups after giving written informed consent (10 non-sleep deprived, 10 total sleep deprived, and 10 REM sleep deprived). During the week preceding the study as well as during the study period, participants were asked to abstain from alcohol, chocolate, and caffeinated beverages and to maintain a standardized bedtime schedule in accordance with their regular habits. Blood samples were collected every morning from each volunteer during the experimental protocol to evaluate if the sleep deprivation promoted changes in cortisol, prolactin, and estradiol levels (hormones related to dopaminergic activity and stress).

**Results:** (ROIs) for [<sup>99m</sup>Tc] TRODAT-1 were used to estimate the concentration of DAT in the striatum (right and left at baseline) post SD, and post-sleep recovery. An elliptical ROI was placed on three consecutive slices in the occipital cortex, an area used for reference of non-specific DAT binding. The results show that after either 2 nights of total sleep deprivation or 4 nights of selective suppression of REM sleep, there were no statistical differences in prolactin, estradiol, and cortisol levels, as assessed by intra-group analysis.

**Conclusion:** We can't exclude that the hormones assessed did not lose their periodic pattern of excretion during sleep loss. Since the volunteers were injected with a radioactive isotope to perform the speCt, additional blood samples to evaluate hormone levels were not recommended after this intervention.

**Keywords:** Dopaminergic system, Neurotransmitter, EEG, REM & NREM Sleep, Sleep Regulation

### Introduction:

Sleep is a complex biological state characterized by behavioral, physiological, and electrophysiological parameters. All animals studied thus far show characteristic signs of sleep, including mammals, birds, fish, insects, roundworms, and jellyfish (Cirelli and Tononi, 2008; Eban-Rothschild and Bloch, 2008; Hendricks et al, 2000; Yokogawa et al, 2007). The transition between wakefulness and sleep involves profound changes in motor control, cognition, brain activity, and consciousness (McGinley et al, 2015). In mammals and birds, sleep and wake states are typically determined using electroencephalogram (EEG) and electromyogram (EMG) recordings, which measure global cortical and muscular activity, respectively. The awake state is heterogeneous, characterized by desynchronized EEG oscillations of low amplitude and mixed frequencies, and a variable amount of muscle activity. Active or motivated wakefulness is rich in theta (4–9 Hz) and gamma (40–300 Hz) EEG frequency ranges,

whereas quiet wakefulness is characterized by slower EEG frequencies, including alpha (7–15 Hz) and beta (8–30 Hz).

The EEG during REM sleep is dominated by theta and gamma oscillations, with a complete loss of muscle tone in axial posture muscles (REM muscle atonia). The sleep/wake states also differ in various physiological parameters, such as thermoregulation, cardiac activity, metabolism, and respiration (Brown et al, 2012; Saper et al, 2010). The characteristic EEG patterns are biomarkers of the different arousal states, and although most non-mammalian species lack the brain structures producing these EEG oscillations, they nonetheless show behavioral and physiological criteria for sleep (Allada and Siegel, 2008; Cirelli and Tononi, 2008; Zimmerman et al, 2008). Behaviorally, sleep is defined as a period of quiescence, with a species-specific body posture and/or sleeping site, and an elevated arousal threshold. Sleep is also defined by

its homeostatic regulation, which is manifested by an increase in sleep need following extended wakefulness.

## Methods

The study was conducted at the sleep laboratory of the department of psychobiology with the approval of the ethics Committee of the university as well as the radiation protection center. Fifty healthy male volunteers ranging from 20 to 30 years of age were randomly assigned to one of three experimental groups after giving written informed consent (10 non-sleep deprived, 10 total sleep deprived, and 10 REM sleep deprived). The REM sleep deprived persons were selected by the examination of their regular routine sleep cycle. The regular sleep cycle is observed by the EEG graph and in this graph,  $\alpha$ ,  $\delta$  and  $\gamma$  waves observed and the person who showed normal EEG pattern, were selected for the REM sleep. The 10-year age range and the gender of volunteers were chosen based on neuro imaging studies that have documented an age-related decline in striata dat and the influence of sexual hormones in DAT density (Lammers et al., 1999; Van et al., 1995). Volunteers were carefully screened by obtaining a detailed medical history and performing physical and neurological examinations, routine blood tests, and urine toxicology for psychotropic drugs to ensure they fulfilled inclusion and exclusion criteria. Exclusion criteria included the following: shift work, sleep disorders, extreme morningness eveningness, a history of neurological or psychiatric diseases and medical conditions, smoking, and alcohol or substance abuse. Volunteers underwent urine analysis to detect amphetamine, methamphetamine, cocaine, tetra- hydrocannabinol, barbiturates, opiates, and benzodiazepines. All participants had normal results on validated questionnaires, including the pittsburgh sleep Quality index, the epworth sleepiness scale, and the beck depression inventory. Normal sleep-wake rhythms and average sleep durations (between 7 and 9 h of sleep per night, with a morning wake time between 06:00 and 09:00) were verified by a sleep diary and actigraphy for a period of one week before participation in the study. Volunteers underwent a polysomnography recording to verify that they had no kind of sleep disturbances. This screening night also helped the volunteers to adapt to the recording equipment and the study environment.

## Experimental Protocol

During the week preceding the study as well as during the study period, participants were asked to abstain from alcohol, chocolate, and caffeinated beverages and to maintain a standardized bedtime schedule in accordance with their regular habits. Subjects received 4 meals per day (08:30, 12:00, 16:00, and 20:00) plus a late evening snack at 00:00 for the total sleep deprivation group during the sleep

deprivation period. The volunteers wore a wrist activity monitor to verify the compliance of the subjects with scheduled bedtimes for one week before and during the entire experimental protocol. All subjects underwent an adaptation night followed by a baseline polysomnography at the sleep institute. In the laboratory, subjects were instructed to be in bed between 23:00 and 08:00, and sleep was recorded each night. The control subjects had regular nights of sleep monitored by polysomnography during the entire experimental protocol, and naps were not permitted.

## Image Analysis

The speCt images were reconstructed using an algorithm of filtered back projection and a Butterworth filter of 0.4 cut-off with pixels of the 10th order. Photon attenuation correction was performed using Chang's first-order correction method with an attenuation coefficient of 0.11/cm (Chang LT, 1978). The speCt studies were evaluated through visual inspection and quantitative evaluation of the regions of interest (roi). The rois were different shapes (some more round and some more oblong, ranging from 7 to 14 pixels) to accommodate the different structures, including the str and occipital region. Average str images based on 3 consecutive slice rois were used to estimate the concentration of dat in the striatal body (right and left). An elliptical roi was placed on three consecutive slices in the occipital cortex, an area used for reference of non-specific DAT binding (Figure 2). To quantitatively evaluate dat, the binding potential (bp) was calculated, where  $bp = [STR-OCC]/OCC$  (Chou et al., 2004).

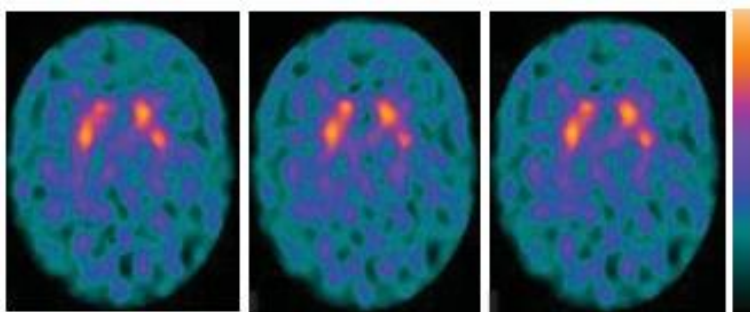
## Blood Sampling

Blood samples were collected every morning from each volunteer during the experimental protocol to evaluate if the sleep deprivation promoted changes in cortisol, prolactin, and estradiol levels (hormones related to dopaminergic activity and stress). Blood samples were centrifuged immediately at 4°C, and serum and plasma were stored at -80°C until the end of the study. Serum cortisol (coefficient of variation (CV): 5.3%; immulite 2000, dpC Corporation, usa), plasma prolactin and estradiol concentrations were measured by the chemiluminescence method (prolactin CV: 5.7% and estradiol CV: 6.7%; Ad- via Centaur, bayer Corporation, usa).

## STATISTICAL ANALYSIS

Average values are given as means  $\pm$  SDs. Statistical significance was assessed by a repeated-measures analysis of variance (ANOVA). If the day-by-group interaction showed a significant effect ( $p < 0.05$ ) a post hoc tukey test was used for comparison.

## Results



**Figure 1: Averaged brain images based on three consecutive slice regions of interest**

(ROIs) for [ $^{99m}\text{Tc}$ ] TRODAT-1 were used to estimate the concentration of DAT in the striatum (right and left at baseline) post SD, and post-sleep recovery. An elliptical ROI was placed on three consecutive slices in the occipital cortex, an area used for reference of non-specific DAT binding.

#### Effects of Sleep Deprivation on Hormonal Profile

The results show that after either 2 nights of total sleep deprivation or 4 nights of selective suppression of *rem* sleep, there were no statistical differences in prolactin, estradiol, and cortisol levels, as assessed by intra-group analysis. Correlation analyses were performed between sleep parameters and hormonal profiles to further investigate the

effect of total and selective sleep deprivation in each recovery day. After total sleep deprivation, estradiol levels were positively correlated with sleep latency and the percentage of SWS in r1, while prolactin levels were positively correlated with REM sleep latency in r2. Cortisol levels were negatively correlated with the percentage of REM sleep in R2. Additionally, we observed a tendency towards a positive correlation between cortisol and the percentage of SWS in r2. During sleep recovery in the *rem* sleep deprived group, estradiol levels were positively correlated with *rem* sleep latency in R3. In addition, prolactin levels were positively correlated with SWS latency in R1 and negatively correlated with *rem* sleep latency in R2.

**Table 1: Hormonal concentrations in control and sleep deprived groups at baseline during the nights of sleep deprivation and during nights of sleep recovery**

	<b>B</b>	<b>n1</b>	<b>n2</b>	<b>n3</b>	<b>n4</b>	<b>r1</b>	<b>r2</b>	<b>r3</b>
<b>Prolactin (ng/ml)</b>								
Control	12 ± 2.12	13.6 ± 3.44	13 ± 1.23	14.7 ± 4.55	13.9 ± 6.44	14.6 ± 5.76	12 ± 5.22	16.3 ± 6.22
REM Sleep Deprivation	13 ± 2.14	10.1 ± 2.45	10.8 ± 2.11	10.1 ± 2.22	9.6 ± 5.66	14.5 ± 6.77	13.2 ± 2.44	11.7 ± 1.11
Total Sleep Deprivation	8.8 ± 4.22	7.7 ± 5.66	8.2 ± 5.44			12 ± 7.54	11.3 ± 4.33	11.8 ± 1.45
<b>Estradiol (pg/ml)</b>								
Control	22.9 ± 6.44	23.5 ± 5.55	18.4 ± 4.33	24.5 ± 7.66	26.3 ± 3.44	20.9 ± 7.55	30.4 ± 2.33	25.1 ± 3.22
REM Sleep Deprivation	22.3 ± 7.55	21 ± 6.55	23.3 ± 2.45	19.7 ± 8.66	24.8 ± 5.42	20.8 ± 9.11	20.2 ± 4.22	19.9 ± 1.44
Total Sleep Deprivation	26.8 ± 3.44	23.1 ± 6.44	22.7 ± 7.55			27.9 ± 8.35	29.2 ± 1.34	29.7 ± 2.21
<b>Cortisol (µg/dl)</b>								
Control	16.2 ± 2.22	17.7 ± 4.22	16.2 ± 2.11	17.2 ± 3.22	15.5 ± 6.22	15.2 ± 5.33	14.9 ± 4.33	14.1 ± 4.22
REM Sleep Deprivation	18.7 ± 4.22	14.8 ± 3.12	14.9 ± 3.11	14.7 ± 4.12	14.1 ± 4.76	15.7 ± 4.67	15.3 ± 3.44	15.2 ± 9.12
Total Sleep Deprivation	16.9 ± 2.11	16.8 ± 4.22	16.1 ± 3.22			18 ± 7.22	17.8 ± 6.44	17.8 ± 2.12

The data are means ± SD (n = 10 volunteers per group). No significant differences were detected. B, baseline night; N1, first night of sleep deprivation; N2, second night of sleep deprivation; N3, third night of sleep deprivation; N4, fourth night of sleep deprivation; R1, first night of sleep recovery; R2, second night of sleep recovery; R3, third night of sleep recovery.

## Discussion

The major finding reported here is that four nights of REM sleep deprivation and two nights of total sleep deprivation do not directly influence DAT availability in the STR. However, they do promote distinct and heterogeneous patterns of sleep recovery as well as significant cross-talk between DAT expression, the endocrine system and sleep parameters.

Adaptive hormonal responses, including changes in estradiol levels, have also been associated with sleep deprivation (Onzález-Santos *et al.*, 1989). Our data show that in the first night of recovery after total sleep deprivation, estradiol levels were positively correlated with the sWAs percentage. Moreover, in the REM sleep deprived group, estradiol levels were positively correlated with REM sleep latency in the third day of recovery. Interestingly, since this hormone is present in women in a much higher concentration than in men, these results corroborate recent data from our group showing that women spend significantly more time in SWS and have a higher REM latency than men (Silva *et al.*, 2008). Our results suggested that the levels of physiologic estradiol in women are related to their sleep pattern. In addition, a growing body of research has examined the modulation of brain dopaminergic systems by estrogen. Specifically, striatal DAT activity in rats has been correlated with estradiol concentrations (Morissette & Paolo, 1993). In support of this result, our examination of the total sleep deprived group revealed that higher estradiol levels were associated with a higher DAT density in the right STR after total sleep deprivation and recovery.

Clinical and experimental evidence indicates that increases in DA release occur after sleep deprivation periods (Davies *et al.*, 1984). In animal models, sleep deprivation is responsible for a number of alterations similar to the effects caused by psychostimulant drugs, especially those affecting dopaminergic pathways (Andersen *et al.*, 2005). Thus, experimental manipulations of the sleep-awake cycle have proven to be efficient in intensifying stimulant behaviors, such as aggressiveness, stereotypy, locomotion, erection, and ejaculation (Gessa *et al.*, 1995). Moreover, wake-promoting substances that act on the dopaminergic system, such as psychostimulant drugs, have acute and chronic effects on sleep architecture. A recent study suggests that both sleepiness and slow waves in the EEG observed after injection of psychostimulants might be explained by the close interaction between the DA activity and the retinoic acid receptors (Krezel *et al.*, 1998). Retinoic acid receptors are highly expressed in the brain, where they are involved in the regulation of neural functions such as the control of locomotion (Krezel *et al.*, 1998) and possibly in the neurobiology of parkinson disease, and addiction (Maret *et al.*, 2005), through their effect on DA neurotransmission (Krezel *et al.*, 1998). Delta oscillations, characteristic of the EEG of SWSs, estimate sleep depth and need and are thought to be closely linked to

the recovery function of sleep. Maret and colleagues demonstrate in the mouse that the gene encoding the retinoic acid receptor  $\beta$  determines the contribution of delta oscillations to the sleep EEG (Maret *et al.*, 2005). Thus, retinoic acid signaling, which is involved in the patterning of the brain and DA pathways, regulates cortical synchrony in the adult.

## Conclusion

Blood samples were collected once a day at 08:00. We can't exclude that the hormones assessed did not lose their periodic pattern of excretion during sleep loss. Since the volunteers were injected with a radioactive isotope to perform the SPECT, additional blood samples to evaluate hormone levels were not recommended after this intervention.

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