

Fluoxetine effects on paradoxical sleep deprivation

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RESUMO

O objetivo desse estudo foi avaliar os efeitos da privação de sono paradoxal (PSP) sobre o metabolismo, bem como a influência da serotonina nesta condição. Cinqüenta e dois ratos machos Wistar, pesando entre 210 e 320g, foram divididos em quatro grupos: Grupo PSP: 13 ratos submetidos à PSP; Grupo PSP/F: 13 ratos submetidos à PSP e à fluoxetina; Grupo CTL: 13 ratos controle (não PSP); Grupo CTL/F: 13 ratos controle (não PSP) com fluoxetina. Os animais do grupo PSP e PSP/F foram privados de sono paradoxal durante três dias, seguido da medida do consumo de oxigênio. Os grupos PSP/F e CTL/F receberam quatro tomadas de fluoxetina na dose de 0,75mg.Kg⁻¹ durante sete dias. Os dados foram apresentados como média mais ou menos desvio padrão. Os animais submetidos a PSP demonstraram aumento significativo do consumo de oxigênio em comparação aos dos grupos controle (38,67 ± 2,26*; 11,64 ± 1,84, respectivamente, * p<0,05). Contudo, não houve diferença significativa entre os grupos PSP/F e CTL ou entre CLT e CTL/F em relação ao consumo de oxigênio. Portanto, a privação de sono paradoxal aumenta o consumo de oxigênio, o que pode ser evitado pelo uso de fluoxetina.

Palavras-chave: sono REM, serotonina, consumo de oxigênio, metabolismo energético.

ABSTRACT

The objective of this study was to evaluate the effects of the paradoxical sleep deprivation (PSD) on the metabolism, as well as to verify the impact of serotonin on this deprivation. Fifty-two Wistar male rats, weighing between 210 and 320 g were divided into four groups: Group PSD: 13 rats subjected to the PSD; Group PSD/F: 13 rats exposed to the PSD with fluoxetine administration; Group CTL: 13 control rats non deprived of paradoxical sleep; Group CTL/F: 13 control rats with fluoxetine administration. Animals from PSD and PSD/F were deprived from paradoxical sleep for three days, followed by oxygen consumption measurement. Groups PSD/F and CTL/F received daily intraperitoneal injections of 0.75mg.Kg⁻¹ fluoxetine for seven days. Data are presented as average and standard deviation. Animals deprived of paradoxical sleep showed a significant increase in the oxygen consumption in comparison to control groups (38.67 ± 2.26 *; 11.64 ± 1.84, respectively, * p <0, 05). Nevertheless, no significant differences were observed between PSD/F and CTL groups, or between the CTL and CTL/F groups regarding the oxygen consumption. Therefore, the paradoxical sleep deprivation increases oxygen consumption that could be prevented by fluoxetine administration.

Key words: REM sleep deprivation, serotonin, oxygen consumption, metabolism.

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INTRODUCTION

The studies performed so far have shown that either total or paradoxical sleep deprivation is escorted by signs of fatigue, attention disturbances, and irritability with a reduction in the perceptiveness²⁴. In rats, deprivation of sleep for 7 to 9 days leads to behavior alterations followed by signs of intense physical exhaustion: hyperphagia, weight loss, increased plasma catecholamines indicating that sleep deprivation induces elevated energy consumption. As a consequence, the death of the animal occurs with signs of malnutrition despite the increase in the food intake^{6,13}.

The negative energetic balance is justified by the elevated metabolism. It was shown that sleep deprivation reduces anabolic hormones, including GH, prolactin⁵, thyroid hormones^{7,18} and testosterone¹, whereas other studies have shown increased production of catabolic hormones, such as adrenocorticotrophic hormone and corticosterone^{4, 25}. However, in sleep deprivation studies, oxygen consumption has rarely been evaluated.

It is known that serotonin plays an important role in central nervous system control without having any depressing effect³. It modulates several behavioral functions including the circadian rhythm, appetite, mood, sexual and autonomous functioning. Thus the evaluation of serotonin effects on the metabolism during rapid eye movement (REM) sleep deprivation would be important as well.

Therefore, the purpose of this study was to evaluate the effects of deprivation of paradoxical sleep and the influence of serotonin on the metabolism.

MATERIAL AND METHODS

Fifty two Wistar male rats, weighing between 210 and 320 g were used. Three days prior to the experimental procedures, the animals were dislocated to the Physiology laboratory of the State University of Health Sciences of Alagoas (UNCISAL) and maintained in conditions of natural light with

food and water *ad libitum*. The animals were separated into individual cages and placed in the same room. Experimental procedures were approved by Institucional Ethical Committee.

The animals were distributed in four groups: Group PSD (n=13): deprivation of paradoxical sleep; Group PSD/F (n=13): deprivation of paradoxical sleep with fluoxetine administration; Group CTL (n=13): control (animals non deprived from paradoxical sleep); Group CTL/F (n=13): control (animals non deprived from paradoxical sleep) with fluoxetine administration.

The deprivation method adopted was the single platform technique. Each rat was placed inside a metal chamber with water (24.0 cm long x 24.0 cm wide x 36.0 cm high) onto a platform of 10.0 cm diameter (to provide more space and mobility and minimize confinement as a restraint-like stressor) immersed in water up to 1.0 cm above the platform upper surface^{12,19}. This platform method is selective for abolishing REM sleep as when rats on 10 cm platforms lapse into REM sleep, they lose muscle tone, make facial contact with or fall into the surrounding water, abruptly awaken¹³. CTL and CTL/F rats were placed inside the metal chamber but, instead of water, the chamber was filled with sawdust bedding.

Rats from groups PSD/F and CTL/F were treated with daily intraperitoneal injections of 0.75mg.Kg-1 fluoxetine for seven days (8:00 am) starting 4 days prior to oxygen consumption measurement procedures. Rats from groups PSD and CTL received normal saline injections as the same way as PSD/F and CTL/F rats.

The oxygen consumption was measured (4:00-6:00 pm) in an open flow system containing 5 sealed chambers with 5L of water in each one, ventilated by air pressure at 2,0 L/min flow rate. A sample of 0.5 mL/min was removed to a sensor of oxygen (Sensor Medics Corp, Anaheim, CA, USA) and an electromagnetic valve allowed the measurement of one cage at a time. The concentration of oxygen was recorded for 6 minutes following the three days of experimental procedures.

The amount of oxygen consumed by the animals was compared among the groups by one-way ANOVA and whenever there was a difference among the groups, a Tukey post test was carried out. The T paired test was applied to compare weight variation in each group. Results were considered significant for $p \leq 0.05$.

All experiments were carried out in accordance with institutional guidelines for animal care.

RESULTS

Table 1 shows the analysis of oxygen consumption measurements (mL). We observed a significant increase in the oxygen expending from the PSD group compared to the CTL group ($38.67 \pm 2.26^*$ and 11.64 ± 1.84 , respectively). In contrast, no significant difference was observed in the consumption of oxygen among the groups PSD/F, CTL/F and CTL (18.04 ± 4.76 , 18.91 ± 4.12 , and 11.64 ± 1.84 , respectively).

Table 1. Consumption of oxygen in milliter ($X \pm SD$), in the control groups (CTL), deprived of paradoxical sleep (PSP), control group with fluoxetine (CTL/F) and deprived of paradoxical sleep with fluoxetine (PSP/F) after three days of deprivation. Paired with groups without deprivation of paradoxical sleep.

Groups	CTL	CTL/F	PSP	PSP/F
O_2 Consumption ($X \pm SD$)	11.64 ± 1.84	18.91 ± 4.12	$38.67 \pm 2.26^*$	18.04 ± 4.76

$p \leq 0,001$

Table 2 illustrates the evaluation of animals' weight (g) before and after the three days of experimental procedures. A significant increase in weight of CTL group was observed before (230.77 ± 8.81) and after ($236.76 \pm 7.61^*$) the experimental trial. In addition to, a statistically significant reduction in weight of the animals from the PSD/F group was detected before and after sleep deprivation (308.78 ± 18.74 and $276.70 \pm 17.19^*$, respectively). Nevertheless, no significant difference in weight was noticed in the groups CTL/F and PSD.

Table 2. Weight of the animals in control groups (CTL), deprived of paradoxical sleep (PSP), control group with fluoxetine (CTL/F) and deprived of paradoxical sleep with fluoxetine (PSP/F) before and after the three days of the experimental procedure ($X \pm SD$).

Groups	CTL	CTL/F	PSP	PSP/F
Before	230.77 ± 8.81	287.71 ± 20.84	247.88 ± 34.15	308.78 ± 18.74
After	$236.76 \pm 7.61^*$	297.80 ± 18.70	249.74 ± 32.39	$276.70 \pm 17.19^*$

$p \leq 0,001$

DISCUSSION

Our results revealed an increased consumption of oxygen in the animals subjected to REM sleep deprivation and this effect was reverted when fluoxetine was administered².

Different studies demonstrated that paradoxical sleep is important for the stability of the cognitive processes. It has been shown that selective deprivation of paradoxical sleep leads to an enhancement in the nervous system excitability both in humans and animals. It is also known that a high level of energy is necessary to maintain the metabolism and/or activity of this hyper-excited tissue which in turn demands an increase of the nutrients and oxygen intake^{3,17}.

Nevertheless, as the cognitive processes are not so relevant in animals, paradoxical sleep deprivation might not be itself the fundamental factor responsible for the raise of metabolism as demonstrated by the results obtained in the animals from the PSD group. Furthermore, it is well established that the deprivation of REM sleep takes apart the entire architecture of sleep. When animals selectively deprived from paradoxical sleep fall asleep they go straight into REM sleep instead of following the distinctive periods of desynchronized sleep (NREM sleep)¹⁵. The phase of desynchronized sleep (that occurs at unrelated times) plays an important role in the reestablishment of the active and metabolic needs. During the wakefulness, the catabolic processes tend to outstrip the anabolic processes in intensity. In this phase of sleep, the reduction of musculature tone reduces the protein catabolism, allowing reestablishment of protein and ATP stocks⁸. Thus, it suggests that the increase in the metabolism exhibited by the animals deprived of paradoxical sleep could be a result of the disturbance in the structure of the desynchronized sleep^{6,3,17,8}

A study showed that the animals under paradoxical sleep deprivation but receiving fluoxetine, an inhibitor of serotonin recaptation at synaptic cleft, did not display an increment in the consu-

mption of oxygen. In fact, studies have demonstrated that serotonin exerts a regulatory effect on a variety of cognitive functions, acting mainly as an inhibitory neurotransmitter¹⁶. Based on this effect, we would suggest that the administration of fluoxetine prevented the increase of nervous system excitability in the animals deprived of paradoxical sleep. Nevertheless, the fluoxetine does not have any inhibitory effect on a nervous system with normal excitability²⁷. This information is in agreement with our results in the control animals treated with fluoxetine, in which none reduction in the oxygen consumption was observed.

It has been reported, in rats, that a reduction in the metabolic rate takes place predominantly in the paradoxical sleep. This drop of the metabolism is endorsed by the absence of cognitive activity during the REM sleep and the paralysis exhibited during this phase is responsible for the diminution in the body metabolism^{26,8, 21,9}. Interestingly the opposite effect was observed in humans, in whom the REM sleep produced an increase in the body metabolism as a result of the intense cognitive activity during this stage, enhancing the metabolism regardless the generalized muscular paralysis stage.^{22,20,8}

As shown in the table 2, no significant difference in weight was identified in the animals from the PSD group, the ones deprived of paradoxical sleep. In contrast the animals non deprived of paradoxical sleep and having a free supply of food showed a significant increase in weight. Clemens and Dement demonstrated that rats with withdrawal of paradoxical sleep presented a weight loss, despite of the increase in appetite¹⁷. Thus, according to this finding and to our results, we can assume that in the animals from the PSD group, the reduce in the animal growth could be a consequence of the increased metabolism, and even with *ad libitum* food it was still inadequate to supply the body requirements.

Table 2 also shows no significant increase in the animals' weight of the CTL/F group during the experimental procedure. This result suggests that fluoxetine might have interfered in the normal weight

increment. Many studies have demonstrated that fluoxetine acts in the hypothalamus at the hunger core causing attenuation of the neuronal activity. Numerous evidences illustrate that the increase in the post synaptic activity of different types of serotonergic receptors, under various experimental conditions leads to the reduction in the quantity of food intake and modifies the pattern of feeding^{23,14,10}. Therefore, it is reasonable the use of fluoxetine in humans as an appetite's moderator and/or the anorectic role of serotonin, especially in diets encompassing an imbalance of aminoacids¹¹. Accordingly, the results obtained from the animals of CTL/F group could be explained by the effect of fluoxetine in reducing the food intake. In the PSD/F group, the significant decline in weight is probably a consequence of the cumulative effects from the increased metabolism associated to the deprivation of paradoxical sleep in addition to the reduced food intake caused by fluoxetine.

Therefore, we demonstrated that deprivation of paradoxical sleep in rats enhances the metabolic requirements, revealed by the increase in the oxygen consumption. The hypothesis that serotonin is involved in the regulatory mechanisms of neuronal excitability is also reinforced. Additionally, the use of fluoxetine in the treatment of obesity as an appetite's moderator is very plausible.

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All experiments were carried out in accordance with institutional guidelines for animal care.

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