Sleep deprivation increases seizures induced by cocaine in rats

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ABSTRACT
Objective: Medical emergency units have increasingly to deal with seizures associated with cocaine abuse, which are seen as a determinant of cocaine-related lethality in humans. Cocaine is an excitatory drug that inhibits catecholamine reuptake and induces sleep deprivation. In view of the fact that stress plays an important role in modulating drug action, the aim of this study was to compare whether paradoxic sleep deprivation and other modalities of chronic stress influence the proportion of cocaine-induced seizures in male rats. Methods: The incidence of seizures was measured for 60 minutes after acute administration of cocaine to rats (40, 45, 50 and 70 mg/kg) that have been submitted to different modalities of stress (paradoxical sleep deprivation, footshock, swimming and immobilization), applied repeatedly for 4 days, and then compared with non-stressed rats. Results: Cocaine induced seizures in 10% of control rats, and among stressful events this effect was potentiated only by paradoxical sleep deprivation, since 90% of the rats had seizures. Conclusion: Our data show that selective sleep loss triggers a marked increase in the number of cocaine-induced seizures, suggesting that the absence of sleep per se has a relevant effect in modulating such events.

Keywords: Sleep deprivation; Cocaine/adverse effects; Seizures; Stress, psychological; Dopamine; Disease models, animal; Rats

RESUMO
Objetivo: As crises convulsivas resultantes do uso de cocaína têm se tornado um fato recorrente nas unidades de emergência médica, e isso é reconhecido como um fator potencial de mortalidade associada à cocaína em humanos. A cocaína é uma droga excitatória que inibe a recaptatação de catecolaminas e induz a privação de sono. Tendo em vista que o estresse potencialmente aumenta o uso de drogas e que a privação de sono modifica a transmissão dopaminérgica, o objetivo deste estudo foi avaliar os efeitos da privação de sono paradoxal e outras modalidades de estresse crônico sobre a ocorrência de crises convulsivas induzidas pela cocaína em ratos. Métodos: Ratos machos adultos foram submetidos a diferentes modalidades de estresse (privação de sono paradoxal, choque nas patas, natação e imobilização) durante 4 dias e, após esse período, receberam injeção de cocaína nas seguintes doses (40, 45, 50 e 70 mg/kg). Logo após a aplicação, a ocorrência de crises convulsivas foi determinada durante 60 minutos em todos os grupos submetidos ao estresse e comparada com o grupo de animais não-estressados. Resultados: A cocaína induziu crises convulsivas em 10% dos animais do grupo controle. O grupo submetido à privação de sono paradoxal apresentou maior incidência do fenômeno ictal (90% dos animais), enquanto nos grupos com outras modalidades de estresse não houve diferença significativa. Conclusão: Os dados mostram que a privação de sono aumenta significativamente a incidência de crises convulsivas induzidas pela cocaína. Sugere-se que a ausência de sono per se pode modular a ocorrência desses fenômenos.

Descritores: Privação do sono; Cocaína/efeitos adversos; Convulsões; Estresse psicológico; Dopamina; Modelos animais de doenças; Ratos

INTRODUCTION
Cocaine dependence is a great matter of public health. The 1990’s saw a dramatic increase in the number of emergency incidents and deaths resulting from stimulant overdosing(1,2). Loss of conscience and occurrence of seizures was detected in 18% of cocaine users in a cross-sectional survey conducted in São Paulo, Brazil(3). These data illustrate some of the harmful effects of this drug abuse in the central nervous system(4). Status epilepticus, a life threatening clinical emergency, has been frequently associated with cocaine use(5-7). This finding suggests that failure of the normal homeostatic seizures-suppressing system occurs and is possibly resistant to therapeutic measures(7,8).

Cocaine enhances the release of noradrenalin and inhibits the reuptake of dopamine, noradrenalin and serotonin in the central nervous system. These effects result in abnormal behaviors such as anxiety, euphoria, psychosis, delirium and seizures(4,9,10). In addition to the detrimental effects of cocaine on neurobiological system, several studies have reported a positive association between drug abuse and stressful events(11,12).

Current theories of addiction postulate that stress plays an important role in increasing drug abuse. In-
Sleep deprivation and cocaine-induced seizures

deed, an augmentation of the toxic effects and fatalities of cocaine have been observed following stressful conditions\(^{(13)}\). Concurrently, stressful situations are capable of inducing marked alterations leading to poor sleep quality in humans\(^{(16)}\) and in rats\(^{(15)}\). For instance, an extensive study showed that, in rats, each kind of stressor condition (restraint, electrical footshock, swimming and cold) promotes a distinct sleep response, inducing major alterations in sleep homeostasis\(^{(15)}\).

Sleep loss, which is increasingly frequent due to the hectic life styles of the modern world\(^{(16)}\), involves some degree of stress. It has also been shown that sleep loss leads to catecholamines, hormonal and behavioral alterations in rats\(^{(17-23)}\). Previous studies show that neurobiological alterations caused by sleep loss affect the threshold for seizures in humans\(^{(24)}\) and animal models\(^{(25)}\). For instance, Shouse showed lower thresholds and higher cortical excitability in cats after nearly total sleep deprivation during 24 hours\(^{(26)}\). In agreement with these findings, Cohen and Dement\(^{(27)}\) demonstrated that rats with 6-hour paradoxical sleep deprivation have lower threshold for seizures induced by electroconvulsive shocks. At present, few studies correlating the effects of drugs abuse on threshold of seizures in sleep-deprived animals are available.

Taking together all this evidence that seizures are common in cocaine users, and that stress and sleep deprivation are potential risk factors for seizures, we proposed to evaluate the effects of sub-chronic stress modalities and paradoxical sleep deprivation on seizures induced by acute cocaine administration in rats.

**METHODS**

**Subjects**

Male Wistar rats (~300 g) were used and maintained at 22 ± 1°C, 50 ± 10% relative humidity on a 12/12 hours light/dark cycle (lights on at 7 a.m.). Food and water were available *ad libitum*. The research protocol was approved by the local Ethics Committee of UNIFESP (CEP N. 482/02) and was carried out in accordance with the Ethical and Practical Principles of the Use of Laboratory Animals\(^{(28)}\).

**Experimental procedure**

Rats were assigned randomly to one of the stressors applied repeatedly for 4 days as described below. Each group of stress modality was further distributed according to the dose (n = 10 for each dose). Pre-established cocaine doses were 40, 45, 50 and 70 mg/kg. Controls were treated with the same doses and were left undisturbed in their home cage.

**Stress modalities**

The delivery of each stressor occurred at a random time around 0900 and 1600h to avoid synchronization. The animals were submitted to one modality of chronic stress according to our previous studies\(^{(15,29,30)}\).

- **Paradoxical sleep deprivation (PSD):** the classical PSD method consists of placing 10 rats for 96 hours in a tiled water tank containing 14 circular platforms, 6.5 cm in diameter, standing in water up to 1 cm from their upper surface. In this procedure, animals are prevented from sleeping when the characteristic atonia that accompanies PSD causes the head or whole body to touch the surrounding water, which awakens the rat. We have recently noted a total suppression of paradoxical sleep in rats submitted to this method\(^{(31)}\).

- **Swimming:** the rats were introduced individually inside a 23 cm-high container filled with water at 22-24°C and swam twice a day for 1 hour each time. Afterwards, they were removed and allowed to dry and returned to their home cages. The swimming sessions were carried out at 900 and 1600h.

- **Immobilization:** the animals were maintained in plastic cylinders (21 cm in length x 6 cm in diameter) for 22 hours/day. In the intervening hours twice a day (1 hour periods), the rats were allowed to move freely in their cages to eat and drink at 0900 and 1600h.

- **Footshock:** the animals were placed individually in the compartments (14 x 25 x 28 cm) of an acrylic box, containing an electrified grid on the floor, through which the shocks were delivered. Shock intensity was 2 mA, lasting 0.25 seconds at intervals of approximately 15 seconds. Four to six shocks were delivered per minute with a variable inter-shock interval, which was changed every 5 minutes, in order to prevent anticipation by the animal. The shocks were applied twice a day for periods of 1 h at 0900 and 1600h.

**Cocaine treatment**

Cocaine HCl (Sigma Chemical CO., USA) was dissolved in physiological saline (0.9% NaCl solution). Drug treatment was allocated to animals according to group.

**Behavioral observations**

Immediately following i.p. injection, rats were placed in individual transparent behavioral observation chambers and recorded for 60 minutes, in which the behaviors of animals were analyzed. The seizures recorded were similar to Racine’s kindled stages III (bilateral forelimbs clonus) and IV (stage III and rearing). Behavioral observations were carried out between 0900 and 1100h.

**Statistical analysis**

Data were analyzed using the Fisher’s exact test (two-tailed). Significance level for all statistical tests was set at $p < 0.05$. 

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RESULTS
After acute cocaine injection, significant differences in seizure occurrences, according to the stress modalities, were observed. The incidence of seizures was significantly higher in PSD when compared to the control group (Fisher’s exact test, p < 0.001). In PSD group, 90% of the animals presented cocaine-induced seizures, against 10% in control group (Figure 1). In the other stress groups, seizure occurred in 50% of the immobilized groups, 30% of the footshock groups and 10% of the swimming groups (Figure 1) and these differences were no significant compared to controls. In addition, incidence of seizures was higher in the PSD group when compared to other stress groups (PSD versus immobilization, p < 0.03; versus footshock, p < 0.01; and versus swimming, p < 0.001).

As compared to control group, a significant increased incidence of seizures after acute cocaine injection at the doses of 50 and 75 mg/kg was observed in PSD group. However, no significant differences were observed at the doses of 40 and 45 mg/kg (Figure 2).

DISCUSSION
This study shows that PSD was the only stress modality that increased the frequency of cocaine induced seizures. Considering that the PSD method completely abolishes selectively paradoxical sleep (31), it is interesting to note that among all the other stress modalities that were applied, animals submitted to immobilization presented higher prevalence of seizures (50%) when compared to those submitted to methods that mildly affect sleep i.e. footshock (30%) and swimming (10%). This fact raises the hypothesis that it is the loss of sleep rather than the involved stress that leads to cocaine-induced seizures. In agreement with our hypothesis, Kumar and Raju(32), examining the effect of enhanced paradoxical sleep, showed that there was a significant decrease on cortical excitability, inasmuch as, the threshold for after discharge was significantly higher when compared to baseline condition in rats. Indeed, PSD per se decreased significantly the threshold for electroconvulsive shock in rats(27). De Paula and Hoshino postulated that PSD influences cortical excitability over long periods of time and could therefore modulate seizure activity(33).

Previous studies used different stress modalities in an attempt to isolate the stress produced by the PSD technique(15,19,29,34). Recently, Papale et al. performed a continuing electroencephalography (EEG) recording during the 4-day stress period as used in the present study(15). The findings indicated that each kind of stress promoted changes in a differential fashion. Unpredictable shocks and swimming led to a decreased sleep efficiency, slow wave sleep and paradoxical sleep in the light phase of the third and fourth days compared to baseline. Immobilization presented drastic alterations in all sleep parameters, since it reduced sleep efficiency, slow wave sleep and paradoxical sleep during the 4 days of the stress modality in relation to baseline. Moreover, a significant increase in corticosterone levels was found only in the PSD (299.8 ng/mL) and footshock (169.6 ng/mL) groups compared to home-cage rats(30). These changes may be thought to be the full steroidal response to stress of significant intensity. Thus, the data suggest that different stress modalities result in distinct steroid hormone responses.

Stressful manipulation is known to interfere with drug effects. One study reported that cocaine increases fatalities

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Figure 1: Incidence of seizures in stress groups or home-cage maintained rats after acute cocaine administration (50 mg/kg, IP). Stress groups: paradoxical sleep deprivation (PSD), footshock (FS), swimming (SW), immobilization (IM); and non-stressed home-maintained control rats (CTRL). Statistically significant differences were seen at the higher number of PSD animals, demonstrating seizures when compared to the respective control group (*p < 0.01).

Figure 2: Effects of different doses of cocaine (mg/kg, IP) on percentage of control (CTRL) and sleep deprived (PSD) rats with cocaine-induced seizures. Statistically significant differences were seen at the higher number of PSD animals, demonstrating seizures when compared to the respective control group (*p < 0.01).
threefold when injections of this drug were followed by 30 minutes of restraint stress\(^{(13)}\). Several mechanisms have been proposed to explain the effect of stress altering drug action. Stress is accompanied by the rapid modification of brain and body physiology that leads to the release of biogenic amines and adrenal steroids. Cocaine, as a stimulant, activates the same brain circuitry that is associated with the stress response. It stimulates the secretion of corticosterone and adrenocorticotropic hormone, probably through a corticotropin-releasing factor-related mechanism.

Stress leads to state-related changes in brain reward circuitry, resulting in an interaction with the neural action of drugs\(^{(13)}\). Most commonly abused drugs, such as cocaine, produce toxic effects and a variety of other physiological and behavioral effects through interaction with several distinct central nervous system receptor sites. It was suggested that convulsive and lethal effects of cocaine clearly depend on the serotoninergic (5-HT), muscarinic receptor sites, as well as dopaminergic site\(^{(35)}\). Animal studies have demonstrated that increases in both dopamine and glutamate transmission within the nucleus accumbens are important mechanisms involved with cocaine addiction\(^{(36)}\). Among the different glutamate receptors, the N-methyl-D-aspartate (NMDA) receptor plays an important role in the neural system, which becomes seriously compromised after an overdose of cocaine\(^{(36)}\). Alterations in the presynaptic release of glutamate and GABA within thalamocortical circuitry have been shown in several studies of seizures in animals\(^{(37)}\). Moreover, PSD increases the content of glutamate and glutamine in the cerebral cortex of rats and this is probably due to PSD effects rather than the stress associated with the experimental procedure\(^{(38)}\). Thus, the interaction of several neurotransmitter systems in the mediation of cocaine-induced seizures should also be considered.

The results of this study document substantial effects of PSD in susceptibility to seizures induced by acute cocaine administration. PSD was observed to be the factor that most induced seizures compared to other stress modalities. This suggests that the lack of sleep as such may play a major role rather than does the stressing conditions promoted to modulate cocaine-induced seizures. The nature and significance of the complex relationship between sleep loss, neuroendocrine and behavioral effects of cocaine are not clear and deserve further studies.

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REFERENCES

5. Shneker BF, Fountain NB. Epilepsy. Dis Mon. 2003;49(7):426-78. Review.
18. Frussa-Filho R, Gonçalves MT, Andersen ML, de Araújo NP, Chinen CC, Tufik S. Paradoxical sleep deprivation potentiates...


Susić V, Markovic O. Potentiation of metaphit-induced audiogenic seizures by REM sleep deprivation in rats. Physiol Behav. 1993;54(2):331-8.

Shouse MN. Sleep deprivation increases thalamocortical excitability in the somatomotor pathway, especially during seizure-prone sleep or awakening states in feline seizure models. Exp Neurol. 1988;99(3):664-77.


