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Effects of morphine or naloxone on cocaine-induced genital reflexes in paradoxical sleep-deprived rats

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Abstract

The involvement of opioidergic neurotransmission in the modulation of genital reflexes induced by paradoxical sleep deprivation (PSD) and cocaine in rats was the aim of the present study. Morphine (0, 1, 5 and 10 mg/kg) and naloxone (0, 0.3, 3 and 30 mg/kg) were administered prior to saline or cocaine to rats that had been deprived of sleep and the incidence of penile erections (PE) and ejaculations (EJ) was measured. PSD alone induced PE in 50% and EJ in 20% of the rats, but these behaviors were not influenced by morphine or naloxone. Cocaine potentiated the incidence of genital reflexes in PSD rats to 90% (PE) and 70% (EJ). Morphine and not naloxone significantly reduced the percentage of rats displaying this response at the highest doses. Morphine also significantly reduced PE and EJ frequencies at 10 mg/kg. Furthermore, this inhibitory effect of morphine on genital reflexes was prevented by the prior injection of naloxone. Although a number of factors are involved in such a complex phenomenon as PE and EJ, our data show that activation of the opioidergic systems by the agonist morphine reduces genital reflexes-induced by cocaine in PSD males while the antagonist, naloxone, did not have any significant effect. The findings suggest that the stimulating effects of cocaine in potentiating genital reflexes in PSD rats can be unidirectionally modified by opioidergic systems.

Keywords: Sleep deprivation; Genital reflexes; Morphine; Naloxone; Opioid; Cocaine; Sexual behavior; Rat

1. Introduction

Endogenous opioid peptides are commonly assumed as having an inhibitory role in male sexual behavior (Argiolas, 1999). This hypothesis derives mainly from studies showing that copulatory behavior in male rodents is depressed with the systemic administration of morphine or other opioids when given either acutely or chronically (McIntosh et al., 1980; Pfaus and Gorzalka, 1987; Serra et al., 1988). Clinically, sexual dysfunction has been observed in men chronically using opiates (Cushman, 1972; Crowley and Simpson, 1978). The involvement of opioid peptides in sexual behavior has also been supported by studies showing that opioid receptor antagonists (e.g. naloxone) induce penile erection [PE] (Bertolini et al., 1978) and facilitate sexual behavior (Gessa et al., 1979) in male rats and enhance sexual response to masturbation in men (Sather et al., 2001). On the other hand, naloxone has been also suggested to have inhibitory effects on sexual behavior, in that it increased the postejaculatory interval and decreased the number of mounts and ejaculations (EJ) in castrated males (Lieblich et al., 1985; Miller and Baum, 1986; Bitran and Hull, 1987), indicating that there is less agreement concerning the effects of the antagonist on sexual behavior.

An interaction of opiate and dopaminergic systems has been described in the regulation of PE/seminal emission (Bitran and Hull, 1987). Specifically, naloxone potentiated PE/seminal emission induced by apomorphine (Berendsen

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and Gower, 1986). Otherwise, morphine blocked dopaminergic drugs-induced PE/seminal emission (Ferrari and Baggio, 1982; Berendsen and Gower, 1986). Moreover, the dopaminergic antagonist haloperidol prevented the stimulating effects of naloxone on PE/stretching–yawning syndrome (SYS) (Ferrari and Baggio, 1982), suggesting that the effects of naloxone, in the absence of haloperidol, could be related to the potentiation of dopaminergic function (Bitran and Hull, 1987).

Sleep deprivation induces several behavioral (Tufik et al., 1978; Andersen and Tufik, 2002; Andersen et al., 2003a,b; Frussa-Filho et al., 2004), hormonal (Spiegel et al., 1999; Andersen et al., 2003b) and neurochemical (Farooqui et al., 1996; D'Almeida et al., 1998; Martins et al., 2004; Pedrazzoli et al., 2004; Silva et al., 2004) alterations. In this respect, sleep deprivation has been associated to modifications in opioid receptors (Fadda et al., 1991, 1993).

Behaviorally, we have demonstrated that paradoxical sleep deprivation (PSD) induces PE and EJ in male rats (Andersen and Tufik, 2002, 2003a). It has been shown that this PSD effect is dramatically potentiated by dopaminergic drugs such as cocaine (Andersen et al., 2003a, 2004a), methamphetamine (Andersen et al., 2003b) as well as other dopaminergic agonists (Andersen et al., 2003c). In an attempt to further elucidate the mechanisms behind these genital events induced by cocaine in PSD male rats, the neurochemical basis of such behaviors has been the focus of our investigations. The systemic administration of cholinergic (Andersen et al., 2004b), GABAergic (Andersen and Tufik, 2004) and dopaminergic (unpublished) drugs alter the expression of genital reflexes and suggests that cocaine-induced PE and EJ in PSD rats is related to a complex chain of events ruled by various systems.

Since the role of endogenous opioidergic transmission in the modulation of sexual behavior has been described, this study addressed the question whether the opioidergic systems also participate in the cocaine-induced genital reflexes in PSD male rats. To this purpose, male rats were submitted to the PSD method for 96 h (Andersen et al., 2003a) and were treated with opioid (morphine or naloxone) drugs prior to cocaine or saline injection.

2. Methods

2.1. Subjects

Naïve male Wistar strain rats aged 90 days were bred and raised in the animal facility of the Department of Psychobiology, Universidade Federal de São Paulo. The animals were housed in a colony maintained at 22 °C with 12:12-h light–dark cycle (lights on at 0700 h) and allowed free access to food and water inside standard polypropylene cages. Rats used in this study were maintained and treated in accordance with the guidelines established by the Ethical and Practical Principles of the Use of Laboratory Animals (Andersen et al., 2004c). The experimental protocol was approved by the Ethical Committee of UNIFESP (CEP N. 482/01).

2.2. Drugs and experimental design

All drugs were obtained from Sigma (St. Louis, MO, USA). Cocaine was mixed with sterile saline immediately before testing. The solution was injected i.p. in a volume of 1 mL/kg.

In a first experiment, three doses of morphine–HCl (1, 5, 10 mg/kg) and naloxone–HCl (0.3, 3, 30 mg/kg) were administered allowing for the derivation of dose–response in percentage and frequency of genital reflexes in PSD rats (N=9–10 animals/per dose). In the second experiment, the same doses of morphine and naloxone were tested in PSD-cocaine-treated animals. In a third experiment, naloxone (3 and 30 mg kg) and morphine (10 mg/kg) were co-treated prior to cocaine injection in PSD rats. Both drugs were dissolved in sterile saline. Drugs were prepared fresh before each test session, and were administered intraperitoneally (i.p.). Morphine was administered 15 min (Melis et al., 1997) and naloxone 30 min (Rodriguez-Manzo and Fernandez-Guasti, 1995) prior to cocaine or saline injection. The doses, the route and latencies of administrations were selected based on the above studies. The PSD group designed as control group was pretreated with sterile saline.

2.3. Paradoxical sleep deprivation (PSD)

The animals were submitted to PSD over a period of 96 h using the modified multiple platform method. This period of PSD was chosen since it has been shown that the most genital reflexes are produced during this span of time (Andersen et al., 2003a). The rats are placed inside a tilled water tank (123×44×44 cm), containing 14 circular platforms, 6.5 cm in diameter, in water up to within 1 cm of their upper surface. The rats could thus move around inside the tank by jumping from one platform to another. When they reached the paradoxical phase of sleep, muscle atonia set in and they fell into the water and woke. Throughout the study, the experimental room was maintained under controlled temperature (23±1 °C) and a 12-h light–dark cycle (lights on 0700–1900 h). Food and water were provided ad libitum by placing chow pellets and water bottles on a grid located on top of the tank. Tank water was changed everyday throughout the PSD period.

2.4. Genital reflexes evaluation

The animals were observed in experimental wire mesh cages (15×31×26 cm) containing neither water nor food.

The behavioral observations were carried out between 0900 and 1100 h in a temperature-controlled room, where the animals were monitored by trained observers unaware to which group they belonged with inter-rater reliability established in previous studies. PE was counted only when the rat stood on its hindlimbs, bent its body forward, bent its head down to reach the genital area, held and licked its penis in full erection and displayed hip movements. The erect penis was always visible. EJ was scored by the number of ejaculatory plugs. The frequency of spontaneous PE and EJ (total number of genital reflex divided by the number of rats) was assessed for 45 min. Each animal was tested only once. Observations of the genital reflexes of each animal took place immediately after acute i.p. saline or cocaine injection (7 mg/kg) which was applied immediately after each animal was removed from the tank and after the administration of the opioid drugs according to its respective latency and route of administration. Since we had found, in previous studies, that there were no genital reflexes in home-cage non-sleep-deprived control animals, we used only PSD male rats pretreated with saline for this study, thus avoiding the use of a large number of animals.

2.5. Statistical analysis

For the statistical analysis of the numbers of animals displaying PE and EJ, the Fisher Exact Probability test (two-tailed) was used to assess differences between groups. Frequency data was analysed by one-way ANOVA test followed by Tukey test for comparison between the treatment and vehicle groups. Values are expressed as mean± S.E.M. The level of significance was set at $p<0.05$.

3. Results

3.1. Experiment 1—Effects of opioid drug pretreatment in PSD male rats

After saline injections, PE was observed in 50% of the PSD rats, 20% of which ejaculated as depicted in Fig. 1. Morphine and naloxone had no effect on genital reflexes-induced by sleep deprivation ($p>0.05$) neither on the percentage of animals displaying them nor on their frequencies (Table 1).

3.2. Experiment 2—Effects of opioid drug pretreatment in PSD+cocaine male rats

Cocaine administration in PSD rats elicited PE in 90% and EJ in 70% of the animals as shown in Fig. 2. The administration of morphine prior to cocaine (15 min) significantly reduced the number of animals displaying PE at 5 mg/kg ($p<0.01$) and at 10 mg/kg for both PE ($p<0.001$) and EJ ($p<0.02$) compared to vehicle-pretreated rats by Fisher Exact test (Fig. 2A). No statistically significant alterations were found in the percentage of animals displaying PE and EJ after the injection of naloxone at any dosage level.

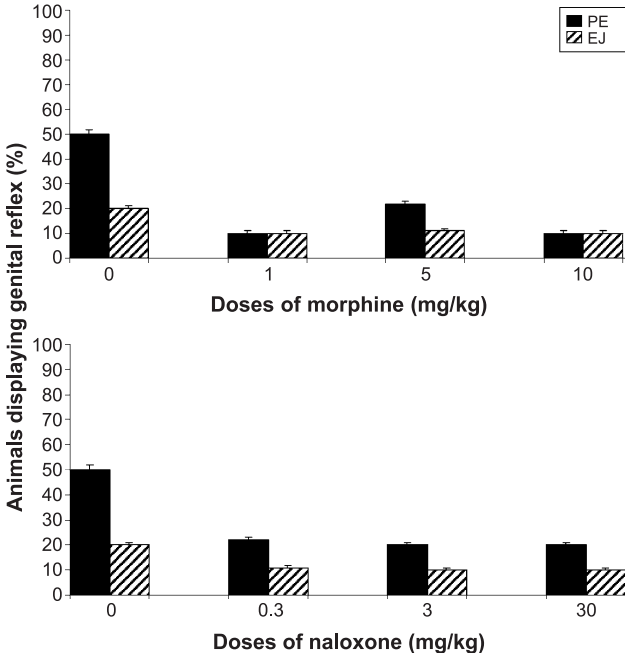

Fig. 1. Effect of morphine and naloxone on penile erection (PE) and ejaculation (EJ) in paradoxical sleep-deprived (PSD)-saline rats. Both compounds were injected i.p. 15 min (morphine) and 30 min (naloxone) prior to saline administration. $N=10$ (morphine 5 mg/kg, $N=9$).

ANOVA followed by Tukey test for PE [$F_{(3,36)}=26.00$; $p<0.00000$] and for EJ [$F_{(3,36)}=4.65$; $p<0.007$] revealed that the genital reflex frequencies observed in the morphine-treated groups were significantly lower than in vehicle-pretreated rats (PE; $p<0.0001$ and EJ, $p<0.02$). No dose–response relationships were found for the antagonist naloxone.

3.3. Experiment 3—Effects of co-treatment of opioid drugs in PSD+cocaine male rats

The inhibitory effect of morphine observed after the 10 mg/kg dose (Fig. 2A) was prevented by prior administration of naloxone in PSD-cocaine rats as depicted in Fig. 3A. Both doses of naloxone induced a significant increase in the number of rats displaying PE and EJ (naloxone 3 mg/kg, p's<0.05 and naloxone 30 mg/kg, p's<0.01) compared to morphine-treated rats and returned to the values seen in saline PSD-cocaine rats. As previously demonstrated, the PSD-cocaine group pretreated with saline also differed from the morphine group (PE, $p<0.001$ and EJ, $p<0.01$).

ANOVA test revealed that PSD-cocaine rats co-administrated with naloxone and morphine, and PSD-cocaine displayed higher PE [$F_{(3,36)}=5.82$; $p<0.002$] than in the morphine group (PSD-cocaine, $p<0.01$; naloxone 3 mg/kg, $p<0.06$ and naloxone 30 mg/kg, $p<0.01$). No differences

Table 1
Effect of pretreatment with morphine and naloxone on frequency of penile erection (PE) and ejaculation (EJ) in paradoxical sleep-deprived (PSD) rats challenged with saline or cocaine injection (7 mg/kg, i.p.)

Groups	Treatment (mg/kg)	ER Frequency	EJ Frequency
Saline	0	0.5±0.2	0.2±0.1
	Morphine		
	1	0.1±0.1	0.1±0.1
	5	0.2±0.1	0.1±0.1
	10	0.1±0.1	0.1±0.1
	Naloxone		
	0.3	0.3±0.2	0.1±0.1
	3	0.2±0.1	0.1±0.1
	30	0.3±0.2	0.1±0.1
Cocaine	0	3.0±0.4	0.9±0.2
	Morphine		
	1	0.8±0.2[a]	0.4±0.2
	5	0.3±0.2[a]	0.2±0.1[a]
	10	0.1±0.1[a]	0.1±0.1[a]
	Naloxone		
	0.3	2.1±0.4	1.1±0.2
	3	2.7±0.6	1.1±0.2
	30	2.8±0.6	0.9±0.2

Data presented as means±S.E.M.
See text for p values.
[a] Different from vehicle-injected group (ANOVA followed by Tukey test).

were observed between the naloxone groups and PSD-cocaine rats. Ejaculation frequencies were significantly more numerous in the PSD-cocaine ($p<0.03$) and naloxone-30 mg/kg ($p<0.01$) groups than in the morphine group

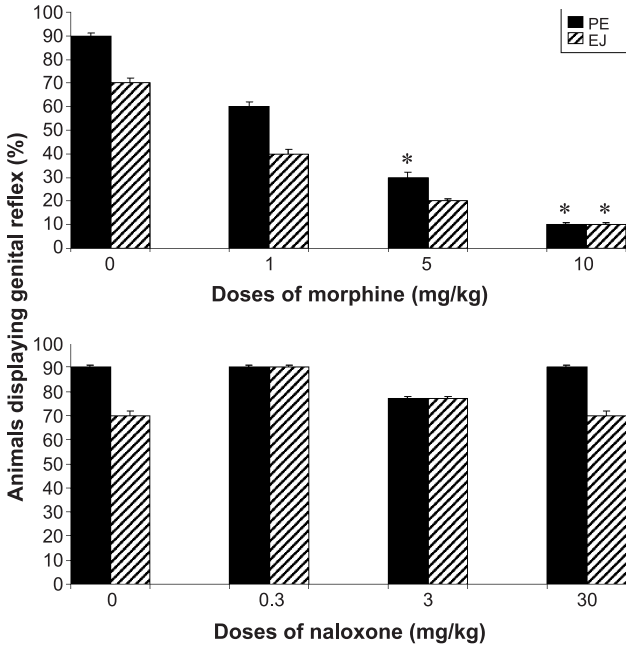

Fig. 2. Effect of morphine and naloxone on penile erection (PE) and ejaculation (EJ) in paradoxical sleep-deprived (PSD)-cocaine rats. Both compounds were injected i.p. 15 min (morphine) and 30 min (naloxone) prior to cocaine administration (7 mg/kg, i.p.). *Values are significantly different from vehicle-injected group. N=10 (naloxone 3 mg/kg, N=9) by Fisher Exact test.

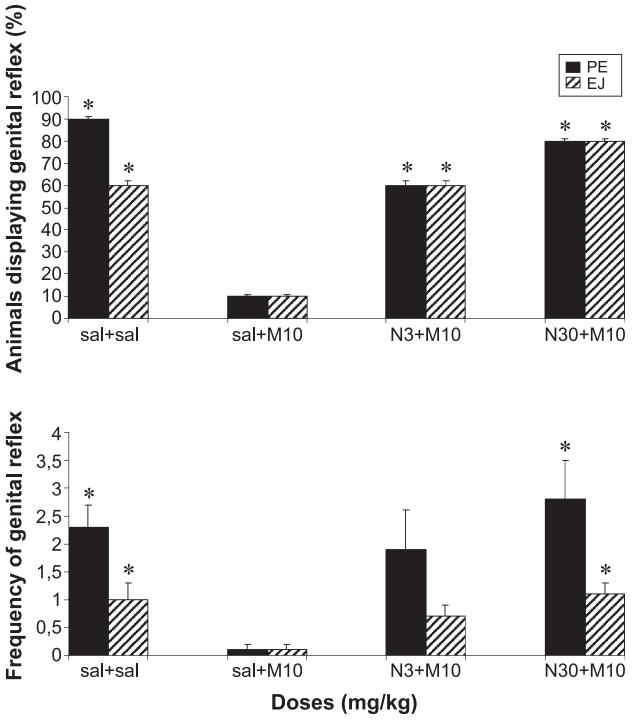

Fig. 3. Effect of co-treatment of naloxone (N) and morphine (M) on penile erection (PE) and ejaculation (EJ) in paradoxical sleep-deprived (PSD)-cocaine rats. *Values are significantly different from morphine 10 mg/kg-injected group (n=10/group).

[$F_{(3,36)}$=4.07; $p<0.01$] as indicated by ANOVA followed by Tukey test and illustrated in Fig. 3B.

4. Discussion

The present results show that morphine given systemically to PSD rats and treated with cocaine reduced the percentage of animals displaying PE at 5 mg/kg and PE/EJ at 10 mg/kg. Moreover, it also reduced the frequency of PE and EJ at the highest doses. Morphine injected in PSD vehicle-treated males did not produce statistically significant effects neither in the number of animals nor in the frequency of genital reflex episodes. In contrast, naloxone did not differ in any parameter of genital reflexes evaluated in PSD rats treated with vehicle or cocaine. This seems to suggest that only activation of the opioid system by the agonist morphine reduces genital reflexes induced by cocaine in sleep-deprived males whereas the antagonist, naloxone, did not cause any alteration.

In respect to the inhibitory nature of the opioid system modulation, while heroin addicts have a suppressed sex drive (Mintz et al., 1974; Cicero et al., 1975), most preclinical studies have focused on the effects of morphine on sexual behavior. Morphine has been reported to decrease the proportion of intact males copulating (Hetta, 1977; Mumford and Kumar, 1979; McIntosh et al., 1980). Morphine injected into the paraventricular nucleus (PVN) also

prevents copulation and noncontact PE (Melis et al., 1999). These PE episodes occur in a male rat in the presence of an inaccessible estrous female rat (see Argiolas, 1999 and references therein). These inhibitory effects of morphine are prevented by naloxone (Argiolas, 1999) and probably mediated by the prevention of nitric oxide synthase activation that occurs in the PVN during genital events (Melis et al., 1998, 1999).

Concerning the magnitude of the effects, the method of PSD seems to exert a favorable effect over male sexual reflexes. Together with our previous data in this series of experiments (Andersen et al., 2003a,b,c, 2004a,b), the present observations support the notion that this sexual behavioral effect is a consequence of the changes in the levels of several brain monoamines and subsequent physiological alterations induced by deprivation of sleep. Both morphine and naloxone act preferentially at the μ-receptor and although this opioid receptor was not significantly altered after PSD in 33 areas examined by autoradiographic study (unpublished data), it was reported that impotent male rats have higher levels of opioid peptide mRNAs in the PVN than in those of sexually potent males (Arletti et al., 1997).

Studies with naloxone have yielded conflicting results. While a higher number of males copulated (Gessa et al., 1979; McIntosh et al., 1980), it has also been reported that there is reduction in the number of intromissions necessary to achieve EJ as well as in EJ latency (McIntosh et al., 1980; Pellegrini-Quarantotti et al., 1979). Conversely, others studies have found that naloxone increases the postejaculatory interval (Sachs et al., 1981) and decreased the number of mounts and EJ in recent castrated animals (Lieblich et al., 1985) indicating that enhancement of sexual performance by systemically administered opioid receptor antagonists occurs, but only under certain experimental test conditions (Van Furth et al., 1995).

Under our experimental conditions, genital reflexes remain unaltered after naloxone treatment. Furthermore, both naloxone and morphine had no significant effect on untreated sleep-deprived rats. The absence of effects on genital reflexes by the antagonist naloxone may be attributed to the possibility that it would only modify sexual behavior when such is inhibited or disrupted. Within this context, a third experiment was performed and showed that the inhibitory effect of morphine on genital reflexes was prevented by the prior injection of naloxone that by itself did not induce any effect. Thus, it was demonstrated that morphine induced its inhibitory effect by acting specifically on opioid receptors.

An interaction between opiate and dopaminergic systems has been documented. Morphine injected into the ventral tegmental area (VTA) facilitates sexual behavior and increases dopamine (DA) transmission in the nucleus accumbens in male rats (Mitchell and Stewart, 1990). On the other hand, morphine inhibits PE when injected into the PVN of the hypothalamus (Melis et al., 1999; Argiolas, 1999). Endogenous peptides may exert a dual effect on sexual behavior. Firstly, they facilitate the anticipatory phase of sexual behavior by improving sexual arousal and motivation acting in the VTA to increase the activity of the mesolimbic dopaminergic system. Secondly, they inhibit the consummatory phase of sexual behavior by impairing sexual performance acting in the medial preoptic area (Van Furth et al., 1995; for review Argiolas, 1999). Accordingly, naloxone, an opioid antagonist, injected into the VTA, decreased the number of anticipatory level changes that occur in the bilevel testing chamber before copulatory activity (Van Furth and van Ree, 1996). An increase/decrease in the number of level changes is considered to reflect increased/decreased sexual motivation (Everitt, 1990). The data presented in this study should be mostly related to the inhibitory effect of morphine in the PVN of the hypothalamus and not to the facilitative effect of the drug in the nucleus accumbens. Accordingly, we have verified that intra-accumbens injection of cocaine in PSD male rats does not significantly increase genital reflexes such as PE and EJ (unpublished data). According to Argiolas (1999), the rewarding effect of opiates might also be responsible, at least in part, for the inhibitory effect of these compounds on sexual behavior: These drugs would maximally stimulate reward centers, making sexual behavior less rewarding, and hence less motivated.

Erection requires optimal levels of different neurotransmitters. Cocaine, known to possess aphrodisiac properties, has been consistently related to potentiating genital reflexes in male PSD rats. Nevertheless, morphine did reverse the facilitative effects of cocaine on sexual behavior in PSD rats. This fact indicates that the inhibitory mechanisms of opioid agents override the excitatory mechanisms of cocaine associated to sexual behavior in PSD rats. Obviously our data do not exclude the possibility that other neurotransmitters may play an important role in modulating this behavior, directly or by interacting with the opioid systems. For example, since oxytocin induces PE by activating oxytocinergic neurons originating in the PVN and projecting to extrahypothalamic brain areas (Argiolas and Melis, 1995), it is possible that opioid peptides and opiates prevent this male sexual function by inhibiting central oxytocinergic transmission (see Argiolas, 1999).

Opioid peptides are one of the best known neuropeptides that influence sexual behavior. In the last years, much has become known about the neurochemical substrates involved in the regulation of male sexual behavior. Selective ligands of the three main opioid receptors (μ, δ and κ) identified so far have become available, and have been tested under different experimental procedures. This has led to a clearer picture of the possible sexual role of these peptides in both males and females. As we have already demonstrated, cocaine-induced genital reflexes are altered by cholinergic (Andersen et al., 2004b), GABAergic (Andersen and Tufik,

2004) and dopaminergic compounds (Andersen et al., 2003c). The present data show that this phenomenon can also be influenced by modifications in opioidergic transmission, thereby leading to the concept that genital reflexes may be an effect of interaction rather than be viewed as a consequence of a single component. Only further studies can elucidate the complex neurochemical and behavioral bases involved in genital reflexes-induced by cocaine in sleep-deprived male rats.

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