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Inhibitory effect of GABAergic drugs in cocaine-induced genital reflexes in paradoxical sleep-deprived male rats

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Abstract

The aim of this study was to seek whether GABAergic drugs were involved in the action of cocaine on spontaneous genital reflexes (penile erection—PE, and ejaculation—EJ) of paradoxical sleep-deprived (PSD) male rats. After a 4-day period of PSD, each group was administered with GABAergic drugs 1 h prior to cocaine and placed in observation cages. The administration of gamma-aminobutyric acid (GABA)-A agonist (muscimol, 2 and 3 mg/kg sc) reduced the number of animals displaying PE, whereas all doses tested of muscimol and bicuculline significantly reduced the frequency of PE. Pretreatment with the lower doses of GABA-B antagonist, phaclofen (1 and 2 mg/kg sc), also significantly reduced the percentage of rats showing PE; however, after the higher dose injection, the proportion of animals with PE was similar to those seen after vehicle pretreatment. Both GABA-B agonist and antagonist significantly reduced the PE frequency for all doses used compared with the vehicle group. There were no significant differences between control and GABA-A drugs in EJ behavior, whereas phaclofen 2 mg/kg pretreatment increased the ejaculatory latency. These data show that GABAergic compounds inhibited PE in male PSD rats suggesting that this inhibition points to a differential role of GABA receptor subtypes.

Keywords: Paradoxical sleep deprivation; Genital reflexes; GABA; Cocaine; Rat

1. Introduction

Several neurotransmitters have been proposed to influence the control of sexual behavior in the male rat. The amino acid gamma-aminobutyric acid (GABA) is believed to serve as a major inhibitory neurotransmitter in the mammalian brain (Curtis and Johnston, 1974) and binds to the functionally and pharmacologically distinct GABA-A and -B receptors. GABA-A receptors are activated by GABA and by some agonists, including muscimol, and are blocked by bicuculline and picrotoxin; GABA-B receptors are activated by baclofen, a potent agonist while phaclofen is a specific antagonist.

Several studies have documented a wide range of doses and have associated systemic and central administration of GABAergic drugs in different brain areas with sexual behavior. In addition to GABA's well-documented role in striatonigral function (Scheel-Kruger, 1986), high concentrations of GABA have been reported in the medial preoptic area (MPOA) of male rat brains (Elekes et al., 1986; Andersson, 2001) that happens to be a site of essential importance for the expression of male sexual behavior. In male rats, the injection of GABA-A agonists into MPOA decreases (Fernández-Guasti et al., 1986a), whereas the injection of GABA-A antagonists into this region increases copulatory behavior (Fernández-Guasti et al., 1985). Another potential site of action of GABAergic drugs in the brain in altering erection response is thought to be the paraventricular nucleus (PVN) of the hypothalamus. The activation of GABA-A receptors in the PVN reduced apomorphine-, *N*-methyl-D-aspartic acid, and oxytocin-induced erection in male rats (Rosaria Melis et al., 2000). Intracerebral injection of bicuculline stimulated the rat male sexual activity whereas injection into the nucleus caudatus putamen of compounds that affect the GABAergic transmission did not cause any alteration in the mating pattern (Fernández-Guasti et al., 1986a).

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Agmo and Paredes (1985) reported that systemic administration of GABA-B rather than GABA-A receptor drugs was involved in the control of sexual behavior. Later, antagonizing GABAergic neurotransmission by bicuculline resulted in a marked shortening of the post-ejaculatory intervals and a reduction of the ejaculatory latency (Fernández-Guasti et al., 1986a). GABA-B agonist baclofen resulted in a dose-related decrease in the proportion of male rats displaying erections in a supine penile reflex test while bicuculline did not affect penile reflex (for a review, see Bitran and Hull, 1987). These findings suggest that GABAergic neurotransmission could be involved in the process underlying male sexual behavior (Agmo and Paredes, 1985; Andersson, 2001) as an inhibitory modulator in the autonomic and somatic reflex pathways involved in erection (de Groat and Booth, 1993).

Paradoxical sleep deprivation (PSD) causes substantially different effects in neurotransmitter systems. For instance, GABA levels, albeit high, do not seem to be significantly altered during PSD; however, they are increased during the rebound period (Bettendorff et al., 1996). Behaviorally, PSD induces genital reflexes after saline (Andersen and Tufik, 2002) or cocaine injection (Andersen et al., 2003a), as reflected by the number of animals displaying penile erection (PE) and ejaculation (EJ) and backed by the fact that none of the control rats displayed these behaviors. Furthermore, PE was also described in old (Andersen et al., 2002; 2004a), young (Andersen et al., 2003b), and castrated PSD rats (Andersen et al., 2004b). Indeed, the combination of PSD and cocaine induced a dramatic intensification of genital reflexes than either applied separately (Andersen and Tufik, 2002).

The present study addressed the question whether GABAergic neurotransmission, besides being involved in processes generated during sexual activity, also exerts an influence 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 GABAergic drugs prior to cocaine injection.

2. Methods

2.1. Subjects

Male Wistar strain rats 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 a 12:12-h light–dark cycle (lights on at 0700 h) and allowed free access to food and water inside standard polypropylene cages. All procedures used in the present study complied with Guide for the Care and Use of Laboratory Animals and the experimental protocol was approved by the Ethical Committee of UNIFESP (CEP N. 482/02).

2.2. Drugs

All drugs were obtained from Sigma (St. Louis, MO, USA). Cocaine was mixed with sterile saline immediately before testing. The solution was injected intraperitoneally in a volume of 1 ml/kg. Three doses of each GABAergic drug were administered permitting the derivation of dose–response for the percentage and frequency of genital reflexes ($n = 10$/per dose, except for baclofen 5 mg/kg and bicuculline 0.5 mg/kg whose $n = 9$). The GABA-A drugs were muscimol (1, 2, and 3 mg/kg; Fernández-Guasti et al., 1986a) and bicuculline (0.5, 1, and 2 mg/kg; Agmo and Paredes, 1985; Fernández-Guasti et al., 1986a). The GABA-B drugs were baclofen (1.25, 2.5, and 5 mg/kg; Agmo and Paredes, 1985; Shafizadeh et al., 1997) and phaclofen (1, 2, and 4 mg/kg; Zarrindast and Farahvast, 1994). Only the doses of the drugs and not route or latencies of administrations were selected based on the above studies. The data produced by pilot tests indicated that the chosen protocol design did not result in motor impairment and led to genital reflexes.

All GABAergic drugs were dissolved in drops of Tween and sterile saline and administered subcutaneously 60 min prior to cocaine injection. The PSD group designed as control group was pretreated with sterile saline and drops of Tween. No animal received more than one experimental treatment.

2.3. Paradoxical sleep deprivation

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 12:12-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 interrater reliability estab-

lished in previous studies. PE was counted only when the rat displayed and bent down to lick its penis in full erection. EJ was scored by the number of ejaculatory plugs. The number of spontaneous PE and EJ and latency (time elapsed between the injection to the first genital reflex) were assessed for 60 min. Each animal was tested only once. Observations of the genital reflexes of each animal took place immediately after acute intraperitoneal cocaine injection (7 mg/kg), which was applied immediately after each animal was removed from the tank and 1 h after the subcutaneous administration of the GABAergic drugs. 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 injected with cocaine and pretreated with saline for this study, thus avoiding the use of a large number of animals.

2.5. Statistical analysis

For 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 and latency data were analyzed by one-way ANOVA test, followed by Duncan test for comparison between the treatment and saline groups. Values are expressed as mean ± S.E.M. The level of significance was set at $P < .05$.

3. Results

3.1. Effects of GABA—a drug pretreatment

All PSD and cocaine-administrated rats pretreated with saline presented PE (Fig. 1A) and 70% presented EJ. The injection of muscimol (2 and 3 mg/kg) reduced the number of animals displaying this behavior compared to saline pretreated rats ($P < .03$ and .01, respectively, Fisher's Exact Test). No statistically significant alterations were found in the percentage of animals displaying PE after the injection of bicuculline. In the group given 1 and 2 mg/kg dose of bicuculline, six animals showed PE but there was only a tendency for significant differences ($P < .08$).

As indicated in Fig. 1B, a statistically significant decrease in the frequency of PE in relation to control rats ($P < .0001$) was also found in all muscimol and bicuculline doses as indicated by ANOVA followed by Duncan's test [$F(6,62) = 15.81$; $P < .000000$]. The frequency of PE was lower in the rats injected with 3 mg/kg of muscimol compared to the ones injected with 1 mg/kg ($P < .01$). Pilot experiments were undertaken in which the animals were administered with 4 mg/kg of muscimol. However, these data were not included since the majority of the animals showed marked behavioral alterations (tremor, freezing) and prostration, leading us to use a lower dose (3 mg/kg) that did not induce behavioral alterations.

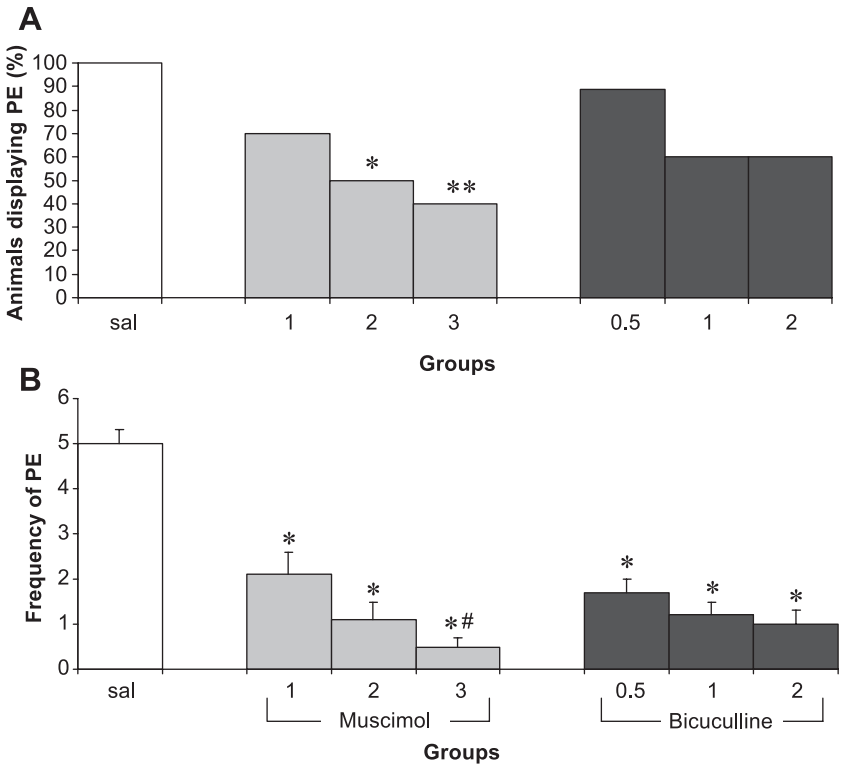

Fig. 1. Effect of GABA-A drugs (muscimol and bicuculline, mg/kg sc) on the penile erection (PE) in paradoxical sleep-deprived (PSD) cocaine (7 mg/kg ip) rats. Panel A: Percentage of animals displaying PE. * $P < .03$, * * $P < .01$, relative to saline (Fisher's Exact Test). Panel B: Frequency of PE events. * $P < .0001$, relative to saline, $^{\#}P < .01$, relative to muscimol—1 mg/kg (ANOVA followed by Duncan's test). Data are expressed as mean ± S.E.M. for $n = 10$ (bicuculline 0.5 mg/kg, $n = 9$).

No significant alterations were seen in the parameters of EJ (proportion of animals ejaculating, frequency, and latency; Table 1).

3.2. Effects of GABA-B drug pretreatment

No statistically significant differences were seen in any of the baclofen doses (1.25, 2.5, and 5 mg/kg) administered when the percentage of animals with PE were analyzed by the Fisher test. The dose of 1 mg/kg of phaclofen reduced the percentage of animals displaying PE to 40% and 2 mg/kg to 50% compared to saline-pretreated rats ($P<.01$ and .03, respectively) (Fig. 2A). Baclofen and phaclofen significantly reduced the frequency of PE events in all doses used as revealed by ANOVA [$F(6,62)=15.72$; $P<.000000$] followed by Duncan's test. Rats injected with phaclofen (4 mg/kg) differ between 1 and 2 mg/kg pretreated groups ($P<.02$) as shown in Fig. 2B.

Table 1 shows the effects of different doses of GABAergic drugs on EJ parameters. The only effect found was with phaclofen, which led to an increase in ejaculatory latency at 2 mg/kg compared to control rats ($P<.01$). Moreover, this dose also differs between 1 and 4 mg/kg ($P<.05$).

4. Discussion

Present data show that pretreatment by systemic administration of GABAergic drugs altered cocaine-induced genital reflexes in PSD male rats. In particular, GABA-A agonist (muscimol) reduced the percentage of animals displaying PE whereas both agonist and antagonist (bicuculline) drugs reduced the frequency of PE. In contrast, treatment with GABA-B agonist (baclofen) did not alter the proportion of animals displaying PE while the lower doses of the antagonist (phaclofen) reduced that proportion. However, both drugs also significantly reduced the frequency of PE. Thus, common to all drugs, whether it was GABA-A or -B (agonist or antagonist), was their capacity to strongly reduce the frequency of PE. Overall, these data suggest that GABAergic mechanisms are involved in the PE induced by sleep deprivation and cocaine.

In respect to the inhibitory nature of the GABAergic receptor subtypes modulation, most studies have focused on the GABA-B receptor. It appears that the stimulation of GABA-B receptors by baclofen inhibits precopulatory behaviors (Paredes and Agmo, 1989). The role of the GABA-A receptor is less clear. Infusion of GABA-A agonist muscimol into MPOA produced a marked inhibition of most aspects of sexual behavior (Fernández-Guasti et al., 1986a). In contrast, the infusion of GABA-A antagonists in the same area facilitated sexual behavior in castrated testosterone-treated rats (Fernández-Guasti et al., 1986b), suggesting that subtypes A and B receptor activation reduces sexual-related activities. Pretreatment with GABA-A antagonist (bicuculline) and with GABA-B agonist (baclofen) does not alter the proportion of animals displaying PE and may result from the particularities of protocol design of sleep deprivation associated to cocaine.

It is particularly notable that several studies have focused on the ejaculatory response induced by GABAergic drugs. The concentration of GABA in the cerebrospinal fluid of male rats significantly increases after EJ (Qureshi and Södersten, 1986). This is a moment when sexual activity is inhibited and may suggest that GABA is related to the postejaculatory behavioral inhibition (Paredes et al., 1997). In addition, intra-MPOA injection of bicuculline dramatically reduced the postejaculatory refractory period (Fernández-Guasti et al., 1986a). In contrast, the present data show that EJ response was not significantly altered after GABAergic drug treatment, especially with GABA-A drugs. Phaclofen at 2 mg/kg increased the latency of EJ and was the only significant result obtained in EJ observation. Possibly the number of animals utilized in the experiments ($n=10$/group) may be the reason for the absence of significant effects induced by the drugs. At any rate, one must take into account the fact that this result, although in general is not significant, is similar to the overall reduction observed in PE.

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., 2002; 2003a,b; 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

Table 1
Effects of GABA-A (muscimol and bicuculline) and GABA-B (baclofen and phaclofen) drugs on EJ parameters in PSD cocaine male rats

Treatment (mg/kg)	Proportion (%)	Frequency (number/rat)	Latency[a] (min)
Saline	70	0.9 ± 0.2	6.3 ± 0.9
Muscimol			
1	60	0.6 ± 0.1	5.3 ± 0.6
2	50	0.5 ± 0.2	10.2 ± 1.0
3	40	0.4 ± 0.2	11.3 ± 2.0
Bicuculline			
0.5	33	0.3 ± 0.2	12.3 ± 1.3
1	30	0.4 ± 0.2	7.0 ± 0.8
2	50	0.5 ± 0.2	11.4 ± 2.5
Baclofen			
1.25	30	0.3 ± 0.2	6.5 ± 0.2
2.5	30	0.3 ± 0.2	9.3 ± 1.3
5	33	0.3 ± 0.2	10.7 ± 1.1
Phaclofen			
1	40	0.4 ± 0.2	8.8 ± 0.8
2	40	0.4 ± 0.2	14.3 ± 2.3 *,†
4	70	0.8 ± 0.2	8.4 ± 0.5

Data presented as means ± S.E.M.

[a] For rats displaying ejaculation.

* $P<.01$ different from saline, ANOVA followed by Duncan's multiple range test.

† $P<.05$ different from phaclofen 1 and 4 mg/kg, ANOVA followed by Duncan's multiple range test.

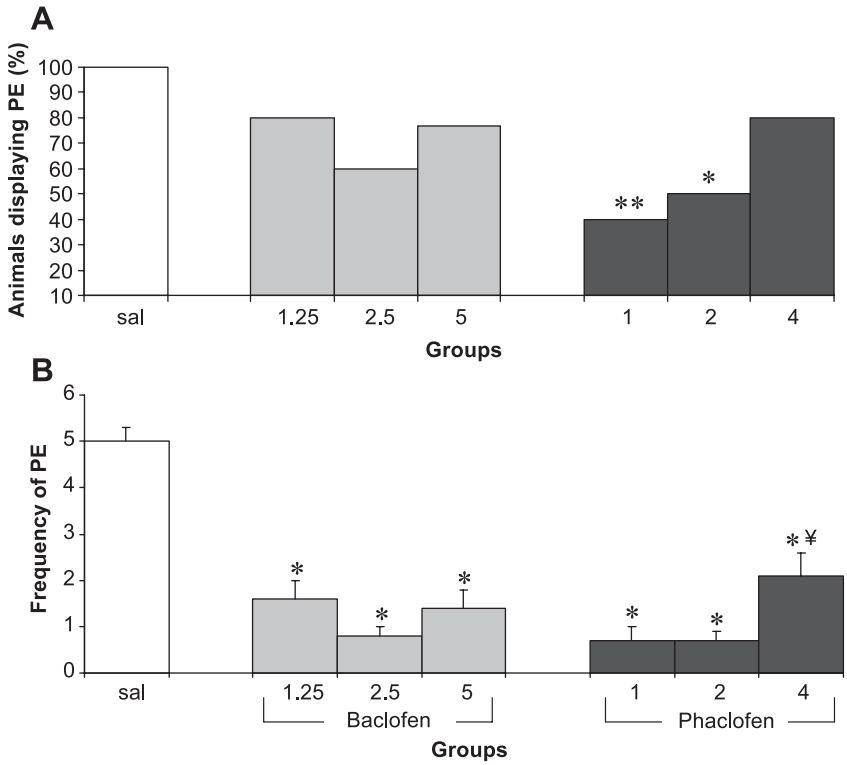

Fig. 2. Effect of GABA-B drugs (baclofen and phaclofen, mg/kg sc) on the penile erection (PE) in paradoxical sleep-deprived (PSD) cocaine (7 mg/kg ip) rats. Panel A: Percentage of animals displaying PE. * $P<.03$, ** $P<.01$, relative to saline (Fisher's Exact Test). Panel B: Frequency of PE events. * $P<.0001$, relative to saline, $^{¥}P<.02$, relative to phaclofen—1 and 2 mg/kg (ANOVA followed by Duncan's test). Data are expressed as mean ± S.E.M. for $n=10$ (baclofen 5 mg/kg, $n=9$).

and subsequent physiological alterations induced by deprivation of sleep.

In 1978, Tufik et al. described supersensitivity of dopaminergic receptors, with consequent behavioral changes in response to dopaminergic drugs following PSD. This was later confirmed by direct autoradiographic analyses by Nunes et al. (1994) showing that 4 days of PSD under conditions similar to those in the present study lead to a significant up-regulation of D_2 receptors. In turn, stimulation of D_2 receptors is known to have facilitatory effects on sexual behavior and to play a major role in the control of male copulatory behavior whereas dopaminergic antagonists seem to reduce sexual motivation (Melis and Argiolas, 1995).

It has been previously reported that GABAergic drugs decrease the apomorphine-induced PE dose-dependently (Zarrindast and Farahvast, 1994). Indeed, in agreement with these authors, our findings also show that GABA-A and -B receptor stimulation inhibit PE induced by cocaine. The authors suggested that a possible release of dopamine by picrotoxin and an increase in dopamine receptor stimulation might be a possible cause for the inhibitory effect of picrotoxin. Besides the indication of methodological adequacy of the present study, the reduction of PE found after GABA antagonists treatment may be related to the dopaminergic system activation promoted on both studies.

Taking these facts into account, one could speculate that this inhibitory effect on genital reflex is a consequence of the motor action caused by these drugs (Agmo et al., 1987). Pilot study data showed that baclofen at 10 mg/kg (data not shown) induces motor impairment and consequently reduces the occurrence of genital reflexes. At 5 mg/kg, no locomotor activity alterations were worth mentioning besides the 70% of the rats displaying PE. In turn, muscimol at 4 mg/kg (data not shown) produced serious motor effects and prostration, leading us to test 3 mg/kg that along with yet a smaller dose (2 mg/kg) no signs of behavioral alterations were observed. But both doses significantly reduced PE to 50% and 40% of the rats, respectively, lending some support that inhibition of genital reflex by GABAergic drugs probably occurs independently from their effects over locomotor activity (Agmo and Paredes, 1985). Thus, the inference of dopaminergic involvement in such genital reflexes is supported by the fact that the drugs in doses used in the present study cause no motor effect and by the absence of effects observed in studies with antagonists administered systemically in rats without such dopaminergic activation. In fact, there is extensive evidence showing that enhanced GABAergic activity inhibits the dopaminergic system (Fuxe et al., 1975; Lloyd et al., 1980), indicating that the similar effects of GABAergic agents and dopamine antagonists are the results of a common action (Paredes et al., 1997).

In our experimental conditions, there was loss of inhibitory action of phaclofen administered at 4 mg/kg compared to the lowest doses in the number of animals showing PE (Fig. 2A). The PE reducing mechanism by phaclofen treatment indicates that increasing inactivation of subtype B receptor seems to liberate one PE activation mechanism and the dopaminergic erecting mechanism could be considered as the control of these genital reflex events.

The inhibitory effects of GABAergic drugs on both apomorphine (Zarrindast and Farahvast, 1994) and on cocaine-induced PE give base to the possible interaction between dopaminergic and GABAergic systems, although there is no complete accordance between the reduction in the frequency of PE and the number of animals that do not present such genital reflex event. The complete role of the most important parameter that expresses GABAergic activity cannot be judged on the basis of the present data.

Thus, based on our data that both agonist and antagonist agents reduce the frequency of PE, it is conceivable that any alteration in GABAergic systems may impair genital reflexes in cocaine-injected PSD male rats. Moreover, the stimulating action of cocaine in potentiating the sexual effects in PSD rats does not override the inhibitory effects of GABAergic compounds on sexual behavior.

Our data showed that PE induced by cocaine in sleep-deprived rats is not a linear response. Erection requires optimal levels of GABAergic neurotransmission. Thus, lower level of activation of its mechanisms does not evoke the full response, neither its excessive activation. GABAergic mechanisms are involved in many processes, including anxiety. It is known that behavioral responses display an inverted U-shaped performance under progressive level of anxiety (Silva and Frussa-Filho, 2000). The dependence of a behavioral response emission on a particular level of steady state of activation is more adaptive than the linear response, and PE seems to be the case.

To summarize, the present study shows that GABAergic compounds inhibited genital reflexes in male PSD rats and offers evidence suggesting that this inhibition points to a differential role of GABA receptor subtypes. Although our data are in agreement with the current literature on the inhibitory effects of GABA, more studies are required for the complete understanding of the consequence of systemically administered GABAergic drugs on genital reflexes.

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