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Cholinergic mechanisms in cocaine-induced genital reflexes in paradoxical sleep-deprived male rats

M.L. Andersen*, L.A. Papale, S. Tufik

Department of Psychobiology, Universidade Federal de São Paulo, Escola Paulista de Medicina (UNIFESP/EPM), Rua Napoleão de Barros, 925, Villa Clementino, 4024-002, São Paulo, SP, Brazil

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Abstract

In view of the fact that paradoxical sleep deprivation (PSD) modifies cocaine-induced genital reflexes (penile erection [PE] and ejaculation [EJ]), the aim of this study was to address the interaction of cholinergic agents with the action of cocaine on the genital reflexes of PSD male rats. After a 4-day period of PSD, each group was administered with cholinergic drugs 1 h prior to cocaine and was placed in observation cages. The administration of nicotine (0.12, 0.25, 0.5 and 1 mg/kg sc) reduced the frequency and number of animals displaying PE and increased PE latency. Pretreatment with mecamylamine (1.25, 5, 10 and 20 mg/kg sc) also significantly reduced PE frequency for all doses used. The percentage of rats showing EJ was significantly reduced in the group pretreated with 1 mg/kg of nicotine compared with the saline group. The administration of pilocarpine (1.25, 2.5, 5 and 10 mg/kg sc) and atropine (1.25, 5, 10 and 20 mg/kg sc) led to a reduction in the frequency of PE displayed by the rats. These data show that agonist and antagonist cholinergic drugs inhibit genital reflexes in PSD male rats injected with cocaine. The data also suggest that the stimulating action of cocaine in potentiating the sexual effects in PSD rats does not override the effects of the cholinergic mechanisms of sexual behavior.
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Keywords: Paradoxical sleep deprivation; Genital reflexes; Cholinergic drugs; Cocaine; Rat

1. Introduction

Many drugs known to interfere with central neurotransmitter systems have different effects in rats submitted to paradoxical sleep deprivation (PSD) compared with controls (Tufik et al., 1978; Andersen et al., 2000, 2002, 2003a,b,c, 2004). For instance, PSD induces a number of changes in rat hormones (Spiegel et al., 1999; Andersen et al., 2002, 2003b,c), blood parameters (unpublished) and behavior, including reduced yawning induced by cholinergic agonists such as pilocarpine and physostigmine (Tufik et al., 1987). This has led to the hypothesis that PSD induces a down-regulation of muscarinic receptors in the brain, as later described by Nunes et al. (1994a). Of the different behaviors altered by PSD, however, its effect on sexual behavior remains controversial. Morden et al. (1968) reported an increased sexual behavior in PSD rats. Moreover, PSD for 3 to 4 days, using the platform procedure, was also associated with heightened sexual behavior in male rats in the presence of a receptive female (Verma et al., 1989). On the other hand, 4 days of PSD did not significantly alter the male sexual behavior (Hicks et al., 1991).

Andersen et al. (2000) reported that 96 h of PSD in adult rats induce genital reflexes after cocaine injection, 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, 2004) and young PSD rats (Andersen et al., 2003b). Indeed, the combination of PSD and cocaine elicited much more marked genital reflexes than when either is applied separately (Andersen and Tufik, 2002). The group in which PSD was followed by acute cocaine administration showed the largest percentage of animals presenting PE and EJ (100% and 60%, respectively), against 50% PE and 20% EJ in the PSD and saline injection group. Although not

* Corresponding author. Tel.: +55-11-5539-0155; fax: +55-11-5572-5092.
E-mail address: mandersen@sti.com.br (M.L. Andersen).

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statistically significant, the cocaine group induced 10% PE and 10% EJ.

There is emerging evidence that the cholinergic system and, particularly, the nicotinic receptors play an important role in cocaine addiction. Indeed, cocaine inhibits nicotinic receptors and monoamine transporters and interferes with nervous system functioning (Hess et al., 2000). Mecamylamine, a nicotinic receptor channel blocker, has been shown to reduce cocaine craving in rats (Levin et al., 2000) and in human cocaine addicts (Reid et al., 1999). Conversely, the prototypic nicotinic agonist, nicotine, increased cue-elicited cocaine craving in human addicts (Reid et al., 1998). Acetylcholine is released from cholinergic interneurons within the nucleus accumbens, a key neural substrate implicated in cocaine reinforcement and addiction (Koob et al., 1998) and in the regulation of male sexual behavior. Acetylcholine acts in synchrony but oppositely to dopamine (DA) on the nucleus accumbens neural circuit. It is therefore important to investigate the effects of cocaine in PSD rats underlying cholinergic interaction.

Although the role of the cholinergic systems in regulating male sexual behavior has been studied, the sexual responses of PSD rats when challenged with cholinergic drugs have not been assessed. Alterations in the effects of such drugs could occur, at least theoretically, as a consequence of an altered dopaminergic influence (Tufik et al., 1978) on the cholinergic system or as a result of the direct influence of PSD on the dynamics of central cholinergic receptors. Cholinergic agents, regardless of being agonists or antagonists, suppress sexual behavior in male rats (Bignami, 1966; Soulairac and Soulairac, 1975). Such a fact raises the question: Would cocaine override the inhibition brought about by cholinergic drugs in PSD rats? Thus, the present study examined the mechanisms by which PSD modifies the action of cocaine and the possible participation of cholinergic receptors in this effect. To do such, we evaluated, for the first time, the influence of the roles of muscarinic and nicotinic agents in the genital reflexes of PSD male rats.

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 12:12-h light–dark cycle (lights on at 0700 h) and were 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.

Four doses of each drug were administered, permitting the derivation of dose response for the percentage and frequency of genital reflexes and latency to onset of these behaviors. The nicotinic drugs were nicotine (0.12, 0.25, 0.5 and 1 mg/kg; Soulairac and Soulairac, 1975) and

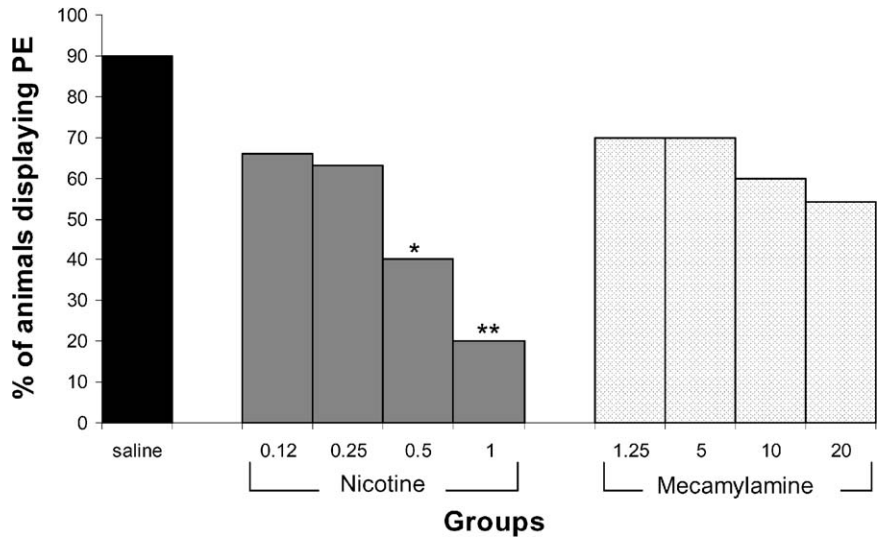

Fig. 1. Effect of nicotinic drugs (mg/kg sc) on the percentages of rats displaying penile erection (PE) in PSD-cocaine (7 mg/kg ip) males. PSD- and cocaine-induced facilitation of PE was effectively antagonized by pretreatment with nicotine (0.5 and 1 mg/kg). Mecamylamine had no significant effect on these genital reflexes. $*P<.05$, $**P<.01$, relative to saline (Fisher Exact Test).

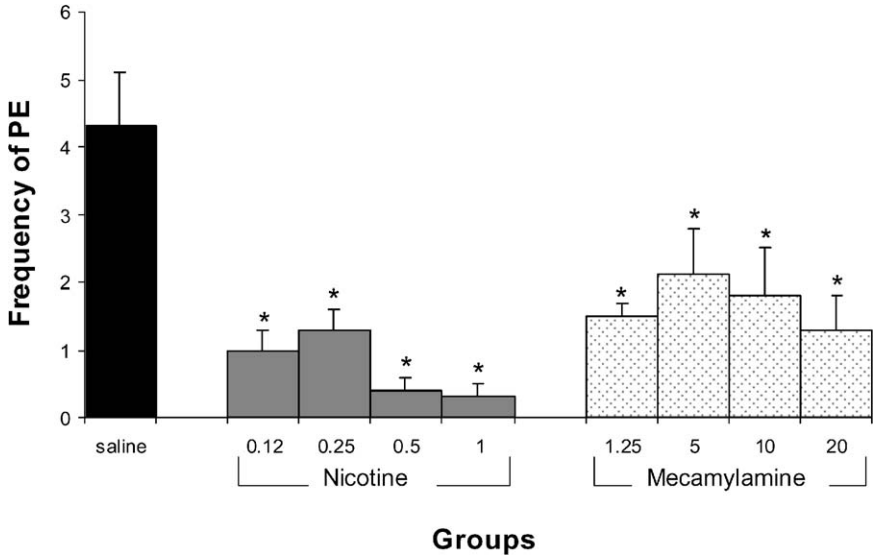

Fig. 2. Inhibitory effect of nicotinic pretreatment (mg/kg sc) with nicotine and of mecamylamine on the frequency of penile erection (PE) events in PSD-cocaine (7 mg/kg ip) male rats. Data are expressed as mean ± S.E.M. $N = 10$. * $P < .01$, relative to saline (ANOVA followed by Duncan test).

mecamylamine (1.25, 5, 10 and 20 mg/kg; Levin et al., 2000). We tested the effects of the muscarinic drugs pilocarpine (1.25, 2.5, 5 and 10 mg/kg; Ahlenius and Larsson, 1985) and atropine (1.25, 5, 10 and 20 mg/kg; Soulairac and Soulairac, 1975). All drugs were dissolved in drops of Tween and sterile saline and were administered subcutaneously 60 min prior to cocaine injection. The control group was pretreated with sterile saline and drops of Tween.

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 because 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, on water up to within 1 cm of the 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 wake. 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 behavioral observations were carried out between 0900 and 1100 h in a temperature-controlled room where the animals, unaware of which group they belonged to, were monitored by trained observers with interrater reliability established 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 was 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 cholinergic drugs.

2.5. Statistical analysis

For the statistical analysis of the number of animals displaying PE, 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

Table 1
Effects of nicotinic drugs (mg/kg sc) on percentage of PSD-cocaine (7 mg/kg ip) rats ejaculating

Groups	Ejaculation (%)
Saline	70
Nicotine 0.12	30
Nicotine 0.25	30
Nicotine 0.5	20
Nicotine 1.0	10 *
Mecalamine 1.25	60
Mecalamine 5	40
Mecalamine 10	30
Mecalamine 20	30

* $P < .01$, relative to saline (Fisher's Exact Test).

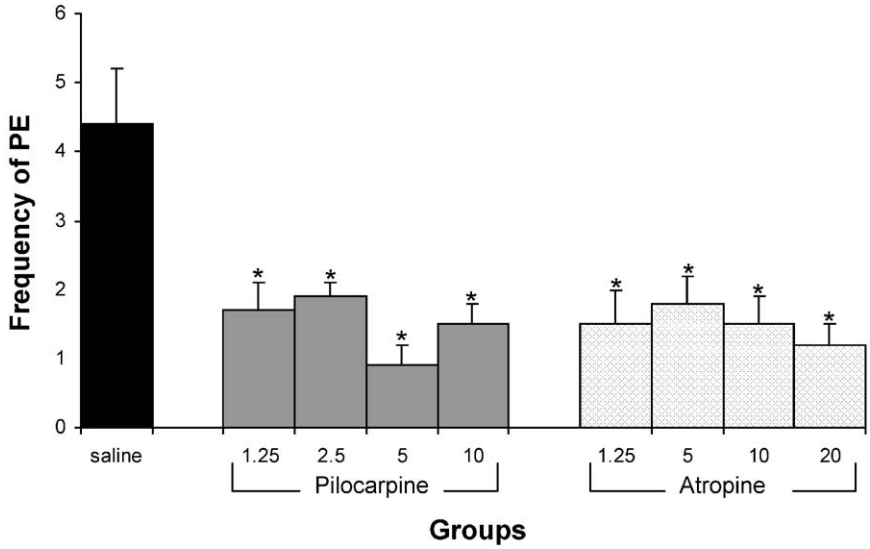

Fig. 3. Systemic pretreatment with muscarinic drugs (in mg/kg sc) 60 min prior to testing reduced the frequency of PE, displayed by the 7 mg/kg ip cocaine-injected PSD rats. Data are expressed as mean ± S.E.M. * $P < .001$, relative to saline (ANOVA followed by Duncan).

expressed as mean ± S.E.M. The level of significance was set at $P < .05$.

3. Results

3.1. Effects of nicotinic pretreatment

The effects of administering nicotinic drugs on genital reflexes induced by PSD and cocaine are shown in Fig. 1. PSD and acute cocaine administration, pretreated with saline, elicited 100% of the animals displaying PE and 70% displaying EJ. The administration of nicotine produced a dose-dependent inhibition of male rat PE, with a reduction in the frequency and number of animals displaying this behavior, and an increase in PE latency. With doses of 0.5 and 1 mg/kg, the percentages of animals displaying PE decreased significantly (40% and 20%, respectively) compared with the PSD rats pretreated with saline ($P < .05$ and $P < .01$, respectively, by Fisher test).

Systemic pretreatment with nicotine and mecamylamine, 60 min prior to cocaine administration, significantly reduced PE frequency in all doses used, as revealed by one-way ANOVA [$F(8,82) = 4.68$; $P < .0001$], depicted in Fig. 2. No statistically significant mecamylamine effects were found for any of the other parameters studied.

An examination of PE latency showed that the time lag increased with nicotine pretreatment ($P < .05$). The onset for erection in the saline PSD rats was 7.3 min, while the latency for the nicotine groups rose to 24 min (0.12 mg/kg), 26 min (0.25 mg/kg), 22.6 min (0.5 mg/kg) and 21.5 min (1 mg/kg).

The Fisher Exact Test showed that the percentage of rats showing EJ was significantly reduced in the group pre-treated with 1 mg/kg of nicotine compared with the saline group ($P < .01$), as illustrated in Table 1. In the group given 0.5 mg/kg of nicotine, two animals ejaculated, but there was only a tendency to significant differences as indicated by statistical test ($P < .06$). Mecamylamine had no statistically significant effects on EJ in the 1.25–20 mg/kg dose range.

3.2. Effects of muscarinic pretreatment

The administration of pilocarpine, 1.25, 2.5, 5 and 10 mg/kg, and atropine, 1.25, 5, 10 and 20 mg/kg (60 min), reduced PE frequency. ANOVA test revealed significant differences between the groups [$F(8,82) = 4.07$; $P < .001$]. The PE frequencies observed after pretreatment with muscarinic drugs differ from those of saline group ($P < .001$), as shown in Fig. 3. There were no significant differences in the percentage of animals displaying PE and EJ or in the latency to PE.

4. Discussion

Cholinergic drugs were associated with pronounced and significant inhibition of genital reflexes on most of the parameters studied, as seen particularly in the reduction in the frequency or number of animals displaying PE and the delayed onset of this behavior. Pretreatment with all doses of nicotine and mecamylamine induced a significant decrease in the frequency of PE. The two highest doses of nicotine (0.5 and 1 mg/kg) significantly reduced the percentages of animals displaying PE (40% and 20%, respectively) compared with the PSD rats pretreated with saline (90%). In addition, a 1 mg/kg nicotine dose significantly reduced the percentage of animals ejaculating

(Table 1). The fact that the actions observed usually depended on drug dosage suggests that the latter may play an inhibitory modulating role in relation to cocaine-induced genital reflexes in PSD rats.

In animals pretreated with the muscarinic drugs, both pilocarpine and atropine, at the doses used, also significantly reduced the frequency of PE induced by cocaine associated to PSD, but had no effect on other variables. Among the PSD rats injected with saline prior to cocaine administration, 90% showed PE at the frequency of 4.3 ± 0.8 events, thus corroborating previous studies (Andersen et al., 2000, 2002, 2003a,b,c, 2004).

We found that the nicotinic antagonist mecamylamine reduced PE in approximately the same degree in all four doses used. Indeed, mecamylamine, a nicotinic antagonist, markedly attenuated responses for the self-administration of cocaine in rats (Levin et al., 2000). For instance, after mecamylamine treatment, cocaine self-administration was 34–50% below the levels observed after the administration of saline and was not dose-related over the range of doses tested. Even when using the higher doses mentioned above, we did not observe nonspecific sedative effects. The finding that higher doses of mecamylamine did not reduce cocaine self-administration also argues against mecamylamine-induced sedation (Levin et al., 2000). The decreased effect of PSD and cocaine in inducing erection responses due to mecamylamine supports the view that the latter may decrease the reinforcing value of cocaine.

The interaction of nicotine receptors with DA systems has been found to play an important role in addiction. Nicotine can directly inhibit DA re-uptake, which is blocked by mecamylamine (Izenwasser and Cox, 1992). We know that cocaine acts on the binding of dopamine transporter and prevents DA re-uptake into the presynaptic terminal, which, in turn, has been associated with sexual drive and the pleasurable effect of cocaine (Buffum et al., 1988). However, in addition to this route, cocaine may have some of its effects mediated via the antagonism of nicotinic receptors (Damaj et al., 1999). Indirectly, nicotinic systems may also have interactions with cocaine. Nicotinic receptor stimulation promotes the release of neurotransmitters such as norepinephrine, serotonin, acetylcholine, glutamate and DA. There is recent evidence that mecamylamine-sensitive DA release may be due to direct actions on DA terminals via nicotinic receptor subtypes (see Levin et al., 2000). Since monoamines (particularly DA) have been implicated in rewarding brain effects of substances of abuse, mecamylamine is being investigated for its potential in treating various types of substance dependence. Antagonism of nicotinic receptors involved in the release of neurotransmitters may have interactions with cocaine effects, which may serve to decrease its reinforcing value (Levin et al., 2000).

It is reasonable to speculate that cholinergic sexual inhibitory effects are mediated through their actions on neurotransmitters. Indeed, PSD causes substantially different effects in DA, serotonin and GABA receptors. For instance, PSD leads to the supersensitivity of brain DA receptors, as suggested initially by behavioral observations (Tufik et al., 1978), and later confirmed by direct autoradiographic analyses that showed a significant up-regulation of D2 receptors (Nunes et al., 1994b). In turn, stimulation of D2 receptors is known to have facilitatory effects on sexual behavior and to play a major role in the control of male copulatory behavior (Melis and Argiolas, 1995).

The noradrenergic system is also altered by PSD. Another autoradiographic study shows generalized, but regionally heterogeneous, reductions in bindings to β receptors subtypes in the brain, as well as clear trends towards the down- and up-regulation of α_1 and α_2 sites, respectively (Hipolide et al., 1998a). Concerning the serotonin system, the concentration of serotonin and its metabolite 5-hydroxyindolacetic acid (HIAA) was reduced in the frontal and parietal cortexes of PSD rats (Farooqui et al., 1996), whereas no differences in serotonergic receptor sensitivity were found between the PSD and control groups (Hipólide et al., 1998b).

Tufik et al. (1987) hypothesized that the decrease of cholinergically induced yawning behavior after PSD could be due to a down-regulation of central muscarinic receptors. Later, Nunes et al. (1994a) provided direct support for this hypothesis by showing that PSD resulted in a generalized down-regulation of muscarinic receptors in the rat brain. In normal conditions, agonists act on receptors to induce facilitatory effects on sexual behavior. However, as noted above, PSD results in muscarinic receptor alteration. It may be that when the agonist is present, the lower level of sensitivity of the postsynaptic receptors decreases the drug-inducing effects. In this light, pilocarpine failed to induce tremor, a behavioral parameter that has been used to evaluate the activity of the central cholinergic system and the effects of cholinergic drugs after PSD (Santos and Carlini, 1988).

The blockade of muscarinic receptors appears to play an inhibitory role. Our data are in line with other studies that have shown that systemically administered scopolamine resulted in a variety of effects, including the inability to reach erection (Vliet and Meyer, 1982). In rats, blocking muscarinic receptors decreased the percentage of animals copulating, and fewer of those that did mount gained intromission or ejaculated (Hull et al., 1988). In this respect, PE frequencies were reduced to approximately the same extent for all doses tested. Given the experimental finding that both higher and lower levels of cholinergic activity reduce the percentage of animals displaying PE and its frequency, it is conceivable that any alteration in cholinergic systems may impair genital reflexes in cocaine-injected PSD male rats. Indeed, both cholinergic agonists and antagonists, injected into the ventricles or systemically, either delayed the initiation or decreased the number of animals copulating, respectively. Similarly, high doses of both agonists and antagonists indicate that distinct cholinergic mechanisms may contribute to the initiation of sexual behavior, in particular, copulation (for review, see Bitran and Hull, 1987).

An alternative interpretation could be based on the pharmacological properties of the agonists used (nicotine and pilocarpine). Nicotine interacts with nicotinic receptors but has agonist-like properties, only at low doses; at higher doses, it produces a depolarization block (Taylor, 2001). Thus, the high nicotine doses used might have acted as an antagonist. Pilocarpine is not a full agonist but a partial one (Clarldorp et al., 1985); therefore, at higher doses, this compound may also have acted functionally as an antagonist. Alternatively, higher doses of these agonists could have produced locomotor depression, and this nonspecific depression might have interfered with the sexual responses. These concerns notwithstanding, we demonstrated that considerably lower doses of nicotine and pilocarpine were also effective in inhibiting genital reflexes.

Once we take the above considerations together with previous data (Bignami, 1966; Leavitt, 1969), the possibility is raised that genital reflexes need an optimal level of cholinergic transmission. The present study suggests that this optimal level is required not only for the spontaneous expression of male genital reflexes but also for the potentiating effect of cocaine/sleep deprivation on such behavior.

Cocaine, known to possess aphrodisiac properties, has been related to potentiating genital reflexes in male PSD rats. Nevertheless, cocaine did not reverse the effects of cholinergic drugs (agonists and antagonists) over sexual behavior. This fact indicates that the inhibitory mechanisms of cholinergic drugs override those of cocaine associated to PSD.

Concerning the magnitude of the effects, our results are consistent with the notion that cholinergic receptors may participate in PSD- and cocaine-induced genital reflexes. Additional factors may be involved because cholinergic stimulation also alters other neurotransmitters such as the dopaminergic system. However, further research is needed to fully elucidate the mechanisms through which this influence is exerted.

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