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DMI-induced sexual effects in male rats: Analysis of DMI's acute and chronic actions on copulatory behavior and on the genital motor pattern of ejaculation

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ABSTRACT

Desipramine (DMI) is a tricyclic antidepressant that alters male sexual function. This work was designed to study the effects of acute and chronic DMI treatments on male rat copulatory behavior, discriminating between spinal and behavioral DMI actions on the ejaculatory response. To this aim, sexually experienced male Wistar rats received DMI (7.5 or 15 mg/kg, i.p.) for 14 days and were tested for sexual behavior on Days 1, 7 and 14 of treatment. Besides, the genital motor pattern of ejaculation (GMPE) was recorded in anaesthetized, spinal male rats after acute (1–10 μg, i.v.) or 14-day chronic DMI (15 mg/kg, i.p.) treatment. Results showed that acute and chronic DMI treatments reduced the ejaculatory threshold by decreasing intromission number and ejaculation latency of male rats, in successive copulatory series. The intensity of the effects depended on the dose and treatment duration. DMI acute treatment activated GMPE expression only at the lower doses and these responses exhibited modified parameters. Chronic DMI reduced the number of discharges and increased the frequency of discharge of spontaneous GMPE responses, affected mechanically evoked ones and increased the number of GMPEs expressed after repeated genital stimulation as compared to control rats. DMI treatments affected copulatory behavior both at brain and spinal levels and its effects on ejaculation, assumed to be similar in behavioral and spinal models, differed.

1. Introduction

Tricyclic antidepressants (TCA) are among the several classes of antidepressant drugs used to treat major depressive disorder (Harrison et al., 1986; Baldwin, 2004). Sexual dysfunction is one of the serious side-effects of antidepressant treatments (Taylor et al., 2005). Thus, it has been reported that TCA affect male sexual behavior by producing erectile dysfunction, alterations in orgasm and ejaculation and in some cases even anorgasmia and anejaculation (Ferguson, 2001; Montgomery et al., 2002). Desipramine (DMI) is a clinically useful TCA frequently used in animal models of depression (Deupree et al., 2007). Clinical studies show that chronic DMI administration inhibits male sexual behavior, particularly affecting the ejaculatory response (Ferguson, 2001). Both, DMI and its metabolite desmethyldesipramine are known to inhibit the norepinephrine transporter (NET) and to down-regulate β-adrenergic receptors (Argenti and D'Mello, 1994); however the mechanism by which DMI alters male sexual behavior is not entirely understood.

In the experimental analysis of male sexual behavior, a distinction has been made between two physiological mechanisms involved in its expression: the motivational component and the copulatory–ejaculatory component (Beach, 1956). Sexual motivation is controlled exclusively by the brain (Paredes and Agmo, 2004) while the ejaculatory response is regulated by the central nervous system (CNS) both at brain and spinal levels (Motofei and Rowland, 2005; Carro-Juárez and Rodríguez-Manzo, 2008). In the brain, the medial preoptic area and paraventricular nucleus exert an excitatory influence on ejaculation, while the nucleus paragigantocelullaris exerts an inhibitory one (Coolen et al., 2004). At the spinal level behavioral, clinical and experimental data revealed the existence of a pattern generator involved in the control of ejaculation that is located within the lumbosacral spinal cord (Coolen et al., 2004; Carro-Juárez and Rodríguez-Manzo, 2008). It has been proposed that the central pattern generator for ejaculation (CPGE) coordinates the neural information to produce the two phases of ejaculation, seminal emission and expulsion (Carro-Juárez and Rodríguez-Manzo, 2008).

The present work was designed to study the effects of acute and chronic DMI treatments on male copulatory behavior and specifically on the ejaculatory response. In this last case, we decided to distinguish spinal DMI actions from behavioral ones by evaluating their effects on the ejaculatory motor pattern of anaesthetized, spinal male rats.

2. Materials and methods

2.1. Animals

Sexually experienced male adult Wistar rats weighing 250–350 g were used in this study. Female Wistar rats were used as sexual stimuli. The animals were maintained under an inverted light dark

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cycle (12 h light:12 h dark, lights on at 22:00 h) and had free access to rat chow and tap water. The Local Committee of Ethics on Animal Experimentation approved all experimental procedures used in this research, which followed the regulations established in the Mexican official norm for the use and care of laboratory animals NOM-062-ZOO-1999.

2.2. Sexual behavior tests

Sexual behavior observations were conducted in a room under dim red light and during the dark phase of the cycle. The animals were tested in polycarbonate cylindrical arenas (43×32.5×18 cm), 2 h after the beginning of the dark phase of the cycle. A 5-min adaptation period was allowed to the males before introducing a receptive female. Females' receptivity was induced by the sequential s.c. injection of estradiol benzoate (4 μg/0.1 ml, Sigma Chemical Co., MO) followed 44 h later by progesterone (2.0 mg/0.1 ml; Sigma Chemical Co., MO). Male rats were rendered sexually experienced by testing them with receptive females in three different sexual behavior sessions. Those males that ejaculated in less than 15 min in at least two tests were considered sexually experienced and selected for the study. Sexual behavior was recorded for 60 min to be able to record several ejaculatory series. The following parameters were assessed: a) intromission latency (IL, time elapsing from the introduction of the female to the arena until the appearance of the first intromission); b) ejaculation latency (EL, time between the first intromission and ejaculation); c) postejaculatory interval (PEI, time from ejaculation to the first intromission of the next copulatory series); d) number of mounts preceding each ejaculation (*M*); e) number of intromissions preceding each ejaculation (*I*); and f) number of ejaculations exhibited during the observation period (E).

2.3. Motor activity test

In order to discard non-specific effects of the treatments that might interfere with the execution of sexual behavior, the animals' spontaneous activity was recorded. Thus, male rats were placed into an acrylic box (33×44×20 cm) with the floor divided into 12 squares (11×11 cm for each quadrant). The number of crossings from one quadrant to another during a 5-min period was recorded.

2.4. Surgical procedures

Sexually experienced male rats were laparatomized, under urethane anesthesia (0.7 g/kg, i.p.), by a single midline incision to expose the pelvic muscles. The adequacy of the anaesthesia was assessed by the absence of a withdrawal reflex after noxious paw pinch. Animals were implanted with a PE-50 catheter (0.965 mm OD) inserted into the pelvic urethra via a bladder incision that was firmly tied to the bladder neck. The catheter was connected to a Harvard syringe pump (model 2000) to mechanically stimulate the urethra. Two platinum wires (Grass) were inserted into the bulbospongiosus muscles to record electromyographic activity. The wires were connected to a polygraph (Grass M7) for conventional recording. The femoral vein was dissected and cannulated for drug administration. At the end of the surgical procedures, animals were spinalized by transection at T6 level.

2.5. Activation of the GMPE

Immediately after spinal cord transection, spontaneously expressed GMPEs appeared and could be recorded in the genital muscles. Sensory-elicited GMPEs were obtained by mechanical distension of the urethra. This distension was achieved by injecting saline solution (200 μl/min) with a syringe pump, during 10 s, through the catheter inserted into the pelvic urethra, simultaneous to the occlusion of the urethral meatus. When the GMPE was repeatedly evoked, the urethral stimulation was applied at 3-min intervals.

2.6. Recording of the GMPE

In order to assess the ability of the spinal cord to produce the ejaculatory response, after spinalization the GMPE was activated by urethral stimulation and the recorded responses served as control data. Then, to assess the effects of acute pharmacological treatments, the selected dose of the drug was i.v. injected and the response obtained under its influence registered. Three additional urethral stimulation episodes were applied after drug injection and the resulting genital responses, if present, recorded. Only the first of these last ejaculatory motor patterns was considered (all 3 responses were almost identical). When no response was obtained within a 10 minute period, the experiment was ended. The GMPE parameters evaluated were the number of discharges and the frequency of discharge (number of discharges/motor train duration).

For the establishment of the effects of chronic treatment with DMI on the expression of the GMPE the following protocol was followed in animals chronically treated with vehicle or the antidepressant (15 mg/kg DMI for 14 days): after spinal cord transection, the spontaneous ejaculatory activity, if present, was registered. In the absence of a spontaneous response the recording was maintained for 10 min. Thereafter, the GMPE was mechanically evoked by urethral stimulation and registered. After this, the GMPE was repeatedly evoked by urethral stimulation, at 3-min intervals, until the inhibition of the response (Carro-Juárez and Rodríguez-Manzo, 2000, 2003, 2008). In this case, the number of GMPEs that could be evoked in control and DMI-treated rats before response inhibition was registered.

2.7. Drug treatments

DMI was purchased from Sigma-Aldrich and dissolved in physiological solution (0.9% NaCl). Animals in the sexual behavior groups were i.p. injected either with vehicle (control group) or DMI (7.5 or 15 mg/kg) in a volume of 1 ml/kg. Treatments were administered 15 min prior to the sexual behavior tests. DMI doses were selected on the basis of their reported antidepressant-like effect in rats after chronic injection (Connor et al., 2000; Zhao et al., 2008).

For the evaluation of the acute effects of DMI on the GMPE, increasing doses of DMI (1, 3 or 10 μg/0.4 ml/rat, i.v.) were tested in independent groups of animals to build a dose–response curve. The effect of chronic DMI treatment on the GMPE was assessed in male rats treated daily with DMI (15 mg/kg, i.p.) for 14 days and tested 2 days after the end of treatment.

2.8. Experimental design

2.8.1. Effect of acute and chronic DMI treatments on copulatory behavior

Twenty-six sexually experienced animals were randomly divided into three experimental groups. Two groups ($n=8$, each), were subjected to chronic daily treatment with 7.5 or 15.0 mg/kg DMI, for 14 days. In these groups sexual behavior was recorded on Days 1, 7 and 14 of treatment. A third group ($n=10$) receiving saline solution for 14 days and tested at the same intervals was used as control.

2.8.2. Effect of acute and chronic DMI treatments on the GMPE

Thirty sexually experienced male rats were divided into five experimental groups. Groups 1 to 3 were used to analyze the acute effect of different doses of DMI (1, 3 or 10 μg/rat; $n=4$, each) on GMPE expression. In these animals, the GMPE was evoked by mechanical stimulation of the urethra prior to pharmacological treatment and those responses served as control data.

Group 4 was constituted by animals ($n=8$) chronically treated with 15 mg/kg DMI (i.p.) daily for 14 days. A two-day drug holiday was allowed to animals in this group to be able to distinguish the effects of the chronic DMI treatment on the functioning of the spinal generator for ejaculation, in the absence of circulating DMI. Thus, on Day 16, males were subjected to the surgery and, in the absence of drug treatment; the

GMPE was recorded both immediately after spinal cord transection (spontaneous response) and after urethral stimulation (mechanically evoked response). This last stimulation was repeatedly applied and the respective GMPEs recorded until response inhibition. The control group (Group 5, $n=8$) consisted of animals chronically injected with saline solution that were stimulated and recorded in the same way.

2.9. Statistical analysis

Sexual behavior specific parameters and motor activity data were compared with their respective control group on each testing day and were statistically analyzed by means of the Kruskal–Wallis ANOVA followed by Dunn's test. Repeated measure analysis of ejaculatory capacity (number of ejaculations) during the treatment was conducted by means of a Friedman RM ANOVA followed by Dunn's test. Fisher *F* test was used to assess differences in the percentage of animals displaying the GMPE. The specific parameters from these GMPEs (number and frequency of discharge), as well as the total number of GMPEs expressed, were compared with their respective control group by means of the Mann–Whitney *U* test. For all tests a $p<0.05$ was taken as statistically significant. The Sigma Stat program version 3.1 was used for the analyses.

3. Results

3.1. Acute and chronic DMI effects on male rat sexual behavior

During the 60-min sexual behavior test, 90% of the control sexually experienced animals were able to execute 4 successive ejaculatory series. Repeated testing (on Days 1, 7 and 14) did not significantly modify the number of ejaculations exhibited by control animals. Rats treated acutely (Day 1) with either dose of DMI showed no significant differences in the ejaculation number when compared to the control group. However, the comparison between the two groups treated with DMI (low vs. high dose) showed a significant increase of the ejaculation number in the group treated with the high DMI dose (Fig. 1, first panel).

After chronic DMI treatment, a statistically significant reduction in the ejaculation number was found in the group treated with the high dose of DMI (15 mg/kg) on Day 7 as compared to its own performance on Day 1 (Fig. 1, first panel). This difference disappeared after 14 days of treatment. Chronic treatment with the low dose of DMI did not significantly change the ejaculation number in any testing day.

Fig. 1 also shows the different sexual behavior parameters of the first copulatory series of animals receiving both DMI doses (7.5 or 15 mg/kg) on Day 1 (acute effects), Day 7 and Day 14 of treatment (chronic effects). As it can be seen, with the low dose of DMI, the only parameter that was modified is the number of intromissions, which was statistically significantly reduced both after acute injection and after 14 days of treatment. Statistically significant reductions in this parameter were also observed in the 2nd copulatory series (see Table 1) after the acute treatment and in the 2nd and 3rd copulatory series after a 14-day chronic treatment. The high dose of DMI reduced both the intromission number and the ejaculation latency. The intromission number was reduced in the 1st and 2nd copulatory series, both acutely and after a 7-day chronic treatment, while after 14 days of treatment the reduction was observed in all 4 copulatory series (see Fig. 1 and Table 1). As to the ejaculation latency, it was significantly reduced in the first 3 copulatory series after acute treatment; after 7 days of treatment, with this high dose the reduction was statistically significant only in the 1st and 3rd copulatory series (Fig. 1 and Table 1), and after 14 days a statistically significant reduction in this parameter was only obtained in the 4th copulatory series (Table 1). None of the treatments had significant effects on motor activity (data not shown).

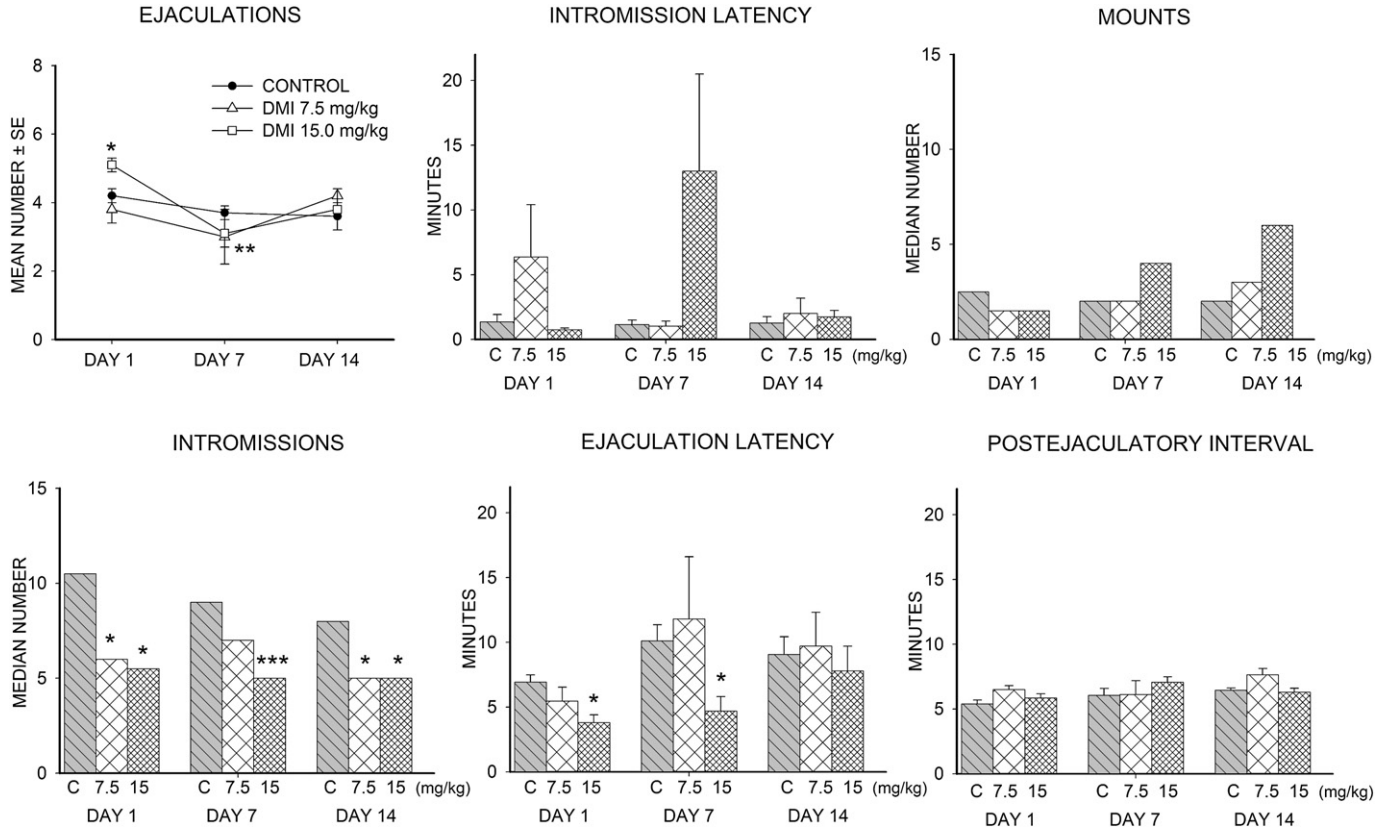

Fig.1. Specific sexual behavior parameters. Average number of ejaculations exhibited by control and DMI-treated rats in a 60 min test and sexual behavior parameters of the first copulatory series exhibited by these animals. Male rats were tested after acute (DAY 1) or chronic DMI (7.5 or 15 mg/kg i.p., $n=8$, each) treatment for 7 (DAY 7) or 14 days (DAY 14). The number of ejaculations and all latencies are expressed as mean ± SEM, the number of mounts and intromissions as medians. Friedman RM ANOVA followed by the Dunn's test, $^{**}p<0.01$ for DAY 7 vs. DAY 1. Mann–Whitney *U* test,$^{*}p<0.05$ for DMI 15 mg/kg vs. DMI 7.5 mg/kg. Kruskal–Wallis ANOVA followed by Dunn's test, $^{*}p<0.05$, $^{***}p<0.001$ vs. control.

Table 1
Specific sexual behavior parameters from the 2nd copulatory series onwards of control rats ($n=10$) and animals treated daily with 7.5 mg/kg ($n=8$, DMI 7.5) or 15 mg/kg DMI ($n=8$, DMI 15) for 14 days.

Copulatory series	Mounts			Intromissions			Ejaculation latency			Postejaculatory interval		
	Control	DMI 7.5	DMI 15	Control	DMI 7.5	DMI 15	Control	DMI 7.5	DMI 15	Control	DMI 7.5	DMI 15
Day 1												
2nd	1	2	1	6.5	4***	2.5***	5.7±0.9	3.0±0.6	3.4±1.4*	7.0±0.6	7.4±0.7	7.3±0.4
3rd	0.5	0	1.5	5	4	3	4.4±0.7	2.5±0.5	2.2±0.3	8.3±0.8	10.6±0.8	8.8±0.7
4th	0.5	0	2	5.5	4	3	5.3±1.0	2.2±0.5	3.7±1.2	8.0±0.7	12.9±2.2	10.6±1.6
Day 7												
2nd	1	1	1	6	5	3*	5.5±1.6	4.8±1.6	2.4±0.5	7.8±0.5	7.4±0.8	7.8±0.4
3rd	1.5	1	1	5	7	2	5.8±1.1	3.0±1.0	1.8±0.3*	8.2±0.7	9.2±0.9	10.8±1.5
4th	1	0.5	1.5	5	3.5	5	8.1±3.5	1.9±0.4*	3.2±0.9	12.3±2.1	10.5±0.4	18.4±3.2
Day 14												
2nd	0	0.5	0	5	2**	2**	6.1±1.3	3.1±0.4	5.3±1.1	9.0±1.1	8.0±0.3	8.5±1.1
3rd	1	2	1	6	3***	3***	5.6±1.7	3.0±0.5	2.5±1.0	9.2±1.0	9.3±0.6	9.8±1.3
4th	1	3	0	5	3	1**	4.3±1.1	2.9±0.5	1.2±0.4*	8.2±0.7	12.8±1.5	10.0±2.7

Sexual behavior was recorded on Days 1 (acute), 7 and 14 (chronic) of treatment.
Mount and intromission numbers are expressed as medians, ejaculation latency and postejaculatory interval as mean±SEM. Kruskal–Wallis ANOVA followed by Dunn's test ***$p<0.001$; **$p<0.01$; *$p<0.05$ vs. control.

3.2. Acute effects of DMI on the GMPE

In all animals, spontaneously expressed GMPEs appeared immediately after spinal cord transection. Each ejaculatory motor pattern consisted of a first motor train followed by an after-discharge activity component. Individual muscular contractions were always accompanied by the potent expulsion of the urethral contents and clusters of penile erectile responses, including flaring and cups. Urethral stimulation produced the same type of response which was used as control. The specific parameters of the GMPEs that were analyzed were the number and frequency of discharge.

DMI acute treatment induced the expression of the GMPE at the low and intermediate doses, but not at the highest dose (Fig. 2). Thus, with the low dose of DMI (1 µg) a GMPE was expressed in all animals, after the intermediate dose (3 µg) only 25% of them exhibited a GMPE and after the high dose (10 µg) none of the rats responded with a GMPE (Fig. 2a). However, 75% of the males treated with the intermediate and high doses of DMI were able to respond with a GMPE to a subsequent mechanical stimulation of the urethra (Fig. 2a, right side).

In the GMPEs expressed in response to DMI injection, the number of discharges, but not the frequency of discharge was altered as compared with mechanically evoked ones. After the lowest DMI dose, the number of discharges was statistically significantly reduced when compared to the mechanically evoked responses obtained after the pharmacological treatment (2ME) (Fig. 2b). In the case of the higher DMI doses, the reduction was significant as compared to the first mechanically evoked responses, prior to drug treatment (Fig. 2c and d). Comparison between mechanically evoked GMPEs, i.e. before (1ME) and after (2ME) pharmacological treatment, showed no change in their parameters (Fig. 2b, c and d).

3.3. Chronic effect of DMI on the GMPE

The GMPEs of animals that were chronically treated with the high dose of DMI (15 mg/kg, i.p., daily for 14 days) were recorded two days after the last DMI injection. In this group one animal failed to exhibit the GMPE, both after spinal cord transection and in response to mechanical stimulation (Fig. 3, panel a). The rest of the subjects ($n=7$) were all able to respond both spontaneously and to urethral stimulation. The GMPEs of these animals also consisted of a first motor train followed by an after-discharge activity that was accompanied by expulsion of urethral contents and by clusters of erectile responses including flaring and cup penile movements. Analysis of the spontaneous GMPE responses (S) exhibited by chronically DMI-treated animals, after spinal cord transection, showed a statistically significant reduction in the number of discharges and an increase in the frequency of discharge when compared to the spontaneous GMPEs exhibited by control animals that received vehicle for 14 days (see Fig. 3, panels b and c). In the mechanically evoked GMPEs (ME) of the treated animals a statistically significant increase in the frequency of discharge (panel c), without changes in the number of discharges (panel b) was observed when compared to mechanically evoked responses of control animals.

In response to repeated urethral stimulation, animals chronically injected with vehicle exhibited a mean number of 7 successive GMPEs, before inhibition of the ejaculatory response. The rats that were treated chronically with DMI, by contrast, expressed a statistically significantly increased number of GMPEs before inhibition of the response (Fig. 3d).

Fig. 4 depicts sample tracings of the GMPEs obtained after acute (panel a) and chronic (panel b) DMI treatments.

4. Discussion

The main findings of the present series of experiments can be summarized as follows: changes in copulatory behavior after acute and chronic DMI administration were reductions in the number of *I* and the EL in successive copulatory series. The intensity of the effects depended both on the dose used and the treatment duration. The most pronounced reduction in the *I* number was observed after 14 days of treatment at both DMI dose levels, while the effects on EL were seen only with the high DMI dose and disappeared after the 14 day treatment. This TCA did not affect the ejaculatory capacity of male rats when compared to control animals. However, acutely, DMI increased the *E* number in the group treated with the high dose as compared to the one receiving the low dose. Finally, in the group chronically treated with the high dose of DMI, a transient decrease in ejaculatory capacity was seen at Day 7.

In relation to the motor pattern of ejaculation, acute treatment with DMI induced GMPE expression only at the lower doses. The highest DMI dose failed to induce the expression of the GMPE. In addition, these GMPEs had a decreased number of discharges, evidencing a disruptive effect of acute DMI on the response. Mechanical urethral stimulation subsequent to DMI acute treatments partially surmounted DMI's disruptive effects.

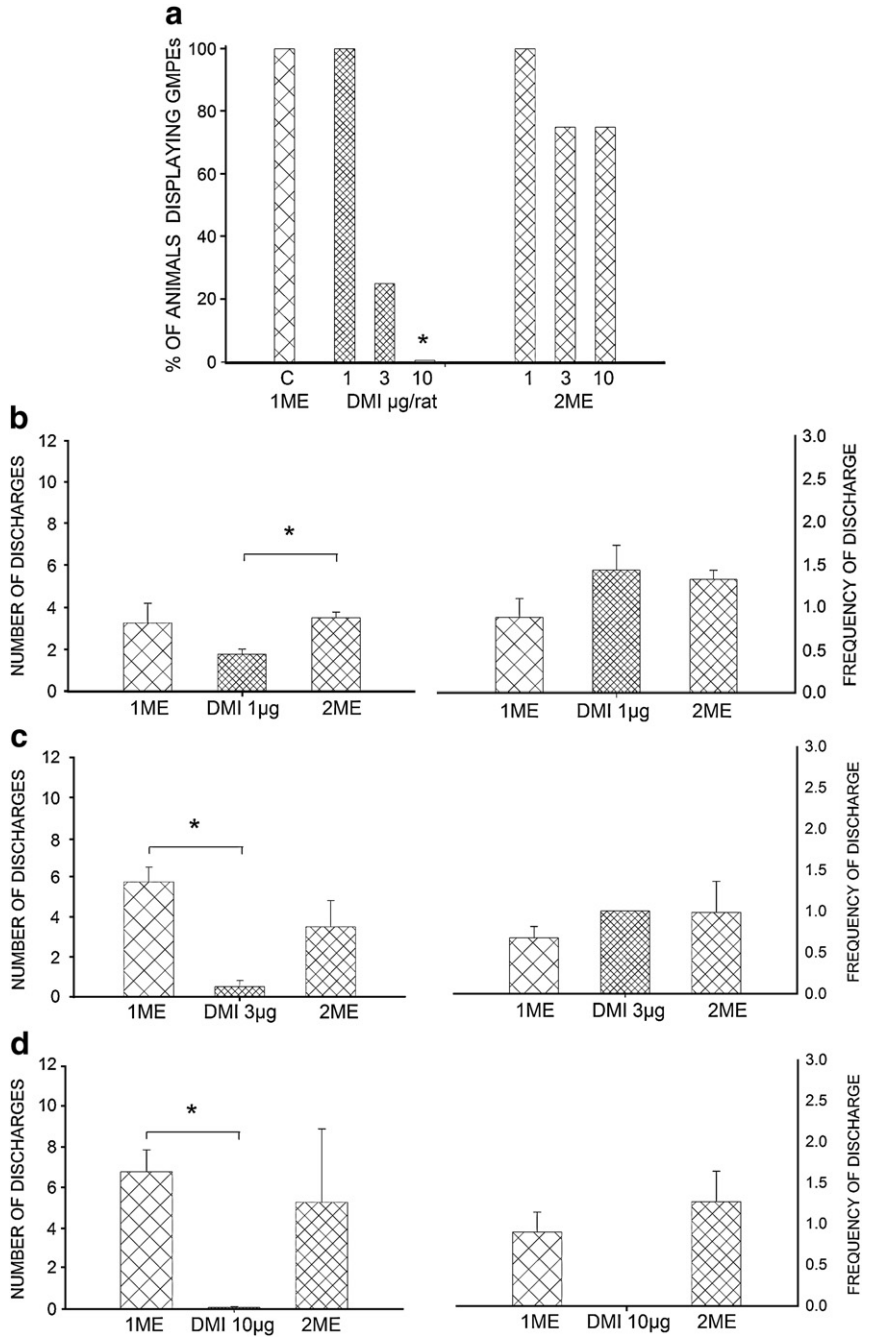

Fig.2. DMI acute effects on the genital motor pattern of ejaculation (GMPE). a: Percentage of animals ($n=4$, each group) showing a GMPE after acute i.v. injection with 1, 3 or 10 µg/rat DMI. Fisher *F* test, $^*p<0.05$ for the 10 µg-treated group vs. control. b–d: Mean ± SEM number and frequency of discharge in the GMPEs obtained by a first mechanical stimulation of the urethra (1ME), after 1, 3 or 10 µg/rat DMI and in response to a mechanically evoked GMPE after drug injection (2ME). Mann–Whitney *U* test, $^*p<0.05$.

After chronic DMI treatment the parameters of spontaneous GMPE responses, in the absence of circulating drug, were markedly diminished as compared to spontaneous GMPEs of the control group. Mechanically evoked GMPEs in DMI-chronically treated animals showed increased frequencies of discharge without changes in the number of discharges. Finally, the number of GMPEs that DMI-chronically treated animals were able to show after repeated mechanical stimulation was significantly increased in comparison to control males.

DMI acts at the CNS by blocking the NET, which results in increased noradrenergic (NA) availability at the synaptic cleft, thus augmenting the NA tone (Kitada et al., 1983). NA facilitates the expression of male rat copulatory behavior (Clark et al., 1985; Bitran and Hull, 1987) and plays an excitatory role in the control of ejaculation at the spinal level (Coolen et al., 2004; Carro-Juárez and Rodríguez-Manzo, 2006). Enhanced NA transmission induced by blockade of α_2-autoreceptors, has been found to augment copulatory behavior in sexually sluggish and castrated male rats (Smith et al., 1987; Tallentire et al., 1996) and to increase the percentage of sexually naïve animals that copulate to ejaculation (Benelli et al., 1993; Tallentire et al., 1996). In sexually experienced rats the increased NA tone decreases IL, EL and the PEI (reviewed in Meisel and Sachs (1994)).

NA exerts its effects by stimulating α and β-adrenoceptors. Stimulation of α_2-adrenoceptors impairs sexual behavior by increasing the IL and the PEI in sexually vigorous male rats, while stimulation of α_1-adrenoceptors has a facilitative effect evidenced by a decrease in the IL, EL and the PEI (reviewed in Meisel and Sachs (1994)). Non-selective β-

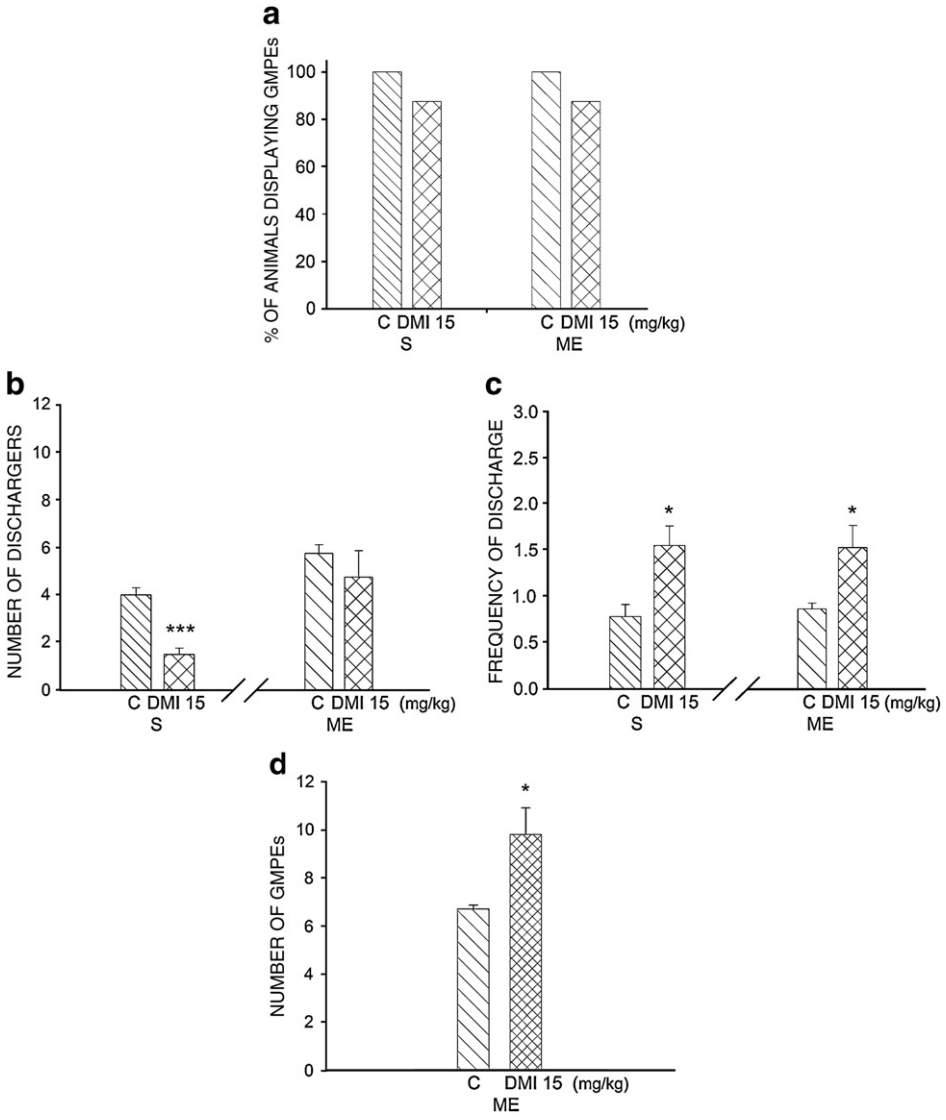

Fig. 3. Effect of chronic DMI treatment on the GMPE. a: Percentage of animals treated with vehicle (control, C; saline 1 ml/kg, i.p. daily for 14 days; $n=8$) or with DMI (15 mg/kg, i.p., daily for 14 days; $n=8$) exhibiting a GMPE either spontaneously (S) or after mechanical stimulation of the urethra (ME). b: Mean ± SEM number of discharges, Mann–Whitney *U* test, ***$p<0.001$ vs. control. c: Mean frequency of discharge in the spontaneously expressed (S) GMPEs obtained in DMI ($n=8$) and control ($n=8$) chronically treated rats, Mann–Whitney *U* test, *$p<0.05$ vs. control. d: Mean ± SEM number of successive mechanically evoked (ME) GMPEs recorded in control and in chronically DMI-treated rats before response inhibition, Mann–Whitney *U* test, *$p<0.05$ vs. control.

adrenoceptor agonists have been found to reduce *M* and *I* numbers preceding ejaculation and to increase the PEI in sexually vigorous male rats (Benelli et al., 1990).

The sexual behavior data obtained in the present work, showing a consistent reduction in *I* number as a result of both acute and chronic DMI treatments, suggest that the effects of this TCA on copulation could be mainly mediated by β-adrenoceptors. Besides, the transient reduction in the EL produced by the high dose of DMI is in line with the effects associated with an enhanced NA tone and could be ascribed to the stimulation of α_1-adrenoceptors. In any case, both effects are indicative of a reduced ejaculatory threshold. In line with this interpretation, there was an increase in the ejaculatory capacity of animals treated acutely with the high dose of DMI when compared to those treated with the low dose.

The fact that the actions of DMI on the EL were temporary, together with the transient disappearance of its facilitative effect on the *I* number (at Day 7) could be indicators of the occurrence of neuroplastic changes. It is known that sustained exposure to high levels of neurotransmitters produces adaptive changes in target neurons (Subhash et al., 2003). Thus, it could be assumed that adaptive changes are in progress after a 7-day treatment and become established after a longer exposure period, thus accounting for the diverse sexual behavior results. In line with this interpretation, it has been proposed that DMI exerts differential effects on NA brain function at distinct time points of treatment. Thus, acutely, DMI increases extra-cellular NA in the locus coeruleus (LC) by reuptake blockade. This increase is associated with α_2-adrenoceptor mediated decreases in firing of LC cells and subsequent decreased NA release into LC cortical terminal fields (Mateo et al., 1998). This decrease in firing persists during sub-chronic treatment (48 h) (Linnér et al., 1999). However, a gradual reversal of the reduction in LC firing accompanied by a greater accumulation of NA in cortical terminal fields, and a gradual increase in the release and extra-neuronal metabolism of NA in brain has been reported after chronic treatment with TCAs in animals (Schildkraut et al., 1971), and with DMI in humans (Mooney et al., 2008). Chronic treatment effects have been suggested to rely on a blockade of the NET (uptake1), increased accumulation of extra-cellular NA together with desensitization of α_2-adrenoceptors and increased conversion of NA to normetanephrine (NMN). The NMN increase would block the extra-neuronal uptake of NA (uptake2) thereby augmenting its levels at the synapse (Mooney et al., 2008). From these data it could be proposed that the

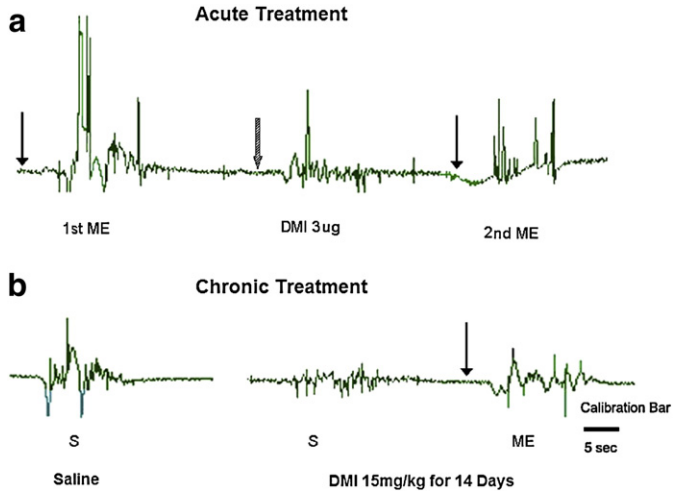

Fig. 4. Polygraphic sample tracings showing the effects of acute and chronic DMI treatments on the GMPE. a: Acute treatment, 1st ME: mechanically evoked response prior to drug injection; DMI 3 µg: response to acute DMI injection with 3 µg/rat DMI; 2nd ME: mechanically evoked response after drug injection. b: Chronic treatment (saline or DMI 15 mg/kg, i.p. along 14 days). S: spontaneously expressed GMPE, ME: mechanically evoked GMPE. Black arrows indicate application of mechanical stimulation, dashed arrow indicates drug injection moment. Calibration bar: 5 s.

effects of DMI on copulatory behavior observed in the present work after acute and 14-day chronic DMI treatments could be explained by an increased NA tone (each one mediated by a different mechanism), while the effects seen after a 7-day treatment could be due to the changes in NA metabolism reported for the sub-chronic treatment. Needless to mention, specific experiments should be conducted to support this interpretation.

The NA system regulates male sexual functions through ascending pathways to the brain and descending pathways to the spinal cord (Rampin, 1999). Spinal neurons at the Onuf nucleus that control the striated muscles involved in sexual functions receive dense NA innervation (Giuliano and Rampin, 2004) that has been suggested to exert a modulatory role in favoring the expression of penile erection and ejaculation (Hansen and Ross, 1983; Kimura et al., 1980; Clark et al., 1985). In line with this suggestion the ejaculatory motor response is modulated by NA agents at the spinal level. Thus, it has been reported that selective stimulation of α_1-adrenoceptors or blockade of α_2-adrenoceptors both activate the central pattern generator for ejaculation (CPGE) and modulate the rhythmic expression of the GMPE. Conversely, activation of α_2- or blockade of α_1-adrenoceptors inhibits its expression (Carro-Juárez and Rodríguez-Manzo, 2006). In the present work, DMI acute treatments exerted a dual influence on GMPE expression. Thus, DMI activated the ejaculatory response at low doses, but these GMPEs exhibited a reduced number of discharges. On the other side, after higher doses of this TCA a dose-related reduction in the percentage of animals expressing GMPEs was observed. Although these results appear difficult to interpret, it could be speculated that, depending on the dose, DMI acts to a distinct extent at α_1- and α_2-adrenoceptors. The facilitative effects of the lowest DMI dose could be attributed to predominant α_1-adrenoceptor stimulation, while the inhibitory effects observed after the intermediate and high DMI dose levels could be ascribed to increasing α_2-adrenoceptor stimulation which activation exerts an inhibitory influence on GMPE expression (Carro-Juárez and Rodríguez-Manzo, 2006). Yet, the possible contribution of β-adrenoceptors cannot be discarded, since its participation on the ejaculatory response at a spinal level has not been established.

Interestingly, the doses of DMI that failed to activate the ejaculatory response did not cancel the response capacity of the spinal motor apparatus, since 75% of the treated animals responded with a GMPE to mechanical stimulation subsequent to drug treatment. The fact that the inhibitory actions of DMI could be surmounted by subsequent sensorial stimulation suggests that DMI acute effects could be exerted directly at the spinal neuronal circuits of the CPGE.

In the group chronically treated with DMI (for 14 days), only one out of 8 animals failed to express the ejaculatory response; however the intrinsic properties of both spontaneous and mechanically evoked GMPE responses were altered in these rats. It is important to recall that the recording of these responses occurred in the absence of circulating DMI, since 48 h elapsed between the last DMI injection and the day in which the experiment was conducted. Thus, the observed alterations were in all probability the result of neuroplastic changes induced by the chronic DMI treatment. Changes consisted in a decrease in the number of discharges and an increase in the frequency of discharge of spontaneous GMPEs, suggesting that chronic DMI treatment altered the functioning of the CPGE. This functioning could be partially restored by urethral stimulation, since the number of discharges in the mechanically evoked GMPEs showed control values, but the frequency of discharge remained increased. Hence, the spinal apparatus produced more rapid responses than those exhibited by control animals. This last effect is indicative of a facilitative action of chronic DMI treatment on the functioning of the CPGE. This fact is further confirmed by the remarkable increase in the number of successive GMPE responses that could be evoked, prior to its inhibition, in chronic DMI-treated rats as compared to control animals. Thus, the neuroplastic changes occurring at the CPGE promoted both inhibitory and facilitative actions. These apparently conflicting results could be explained by the fact that the facilitative effects observed are most probably due to the disruption of an inhibitory mechanism normally regulating the number of successive GMPEs that can be expressed. Thus, the facilitative effects would result from the inhibition of an inhibitory mechanism.

Together, results show that DMI treatment affects copulatory behavior both at brain and spinal levels. The effects in behaving rats were exerted on the ejaculatory threshold, reducing it. At the spinal level acute DMI treatment transiently inhibited CPGE functioning, an effect that does not appear to be related with the one observed in behaving animals acutely treated with this TCA. By contrast, chronic DMI treatment modified the functioning of the CPGE to produce on the one side, spontaneous motor trains with reduced number of discharges and increased frequency of discharge and on the other, more rapid and increased number of ejaculatory responses evoked by mechanical stimulation. This last effect might be related to the lowering of the ejaculatory threshold observed in behaving male rats.

Although in the literature drug effects on ejaculation are assumed to be similar in behavioral and spinal models; present data demonstrate that these results may differ. A statement in the same sense has been made earlier in relation to the control of erection (Sachs, 2000). Thus, in order to get a better picture of the effects of different treatments on sexual function these complementary approaches should be used.

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