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The effect of testosterone and DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) on male sexual behavior of rats

Maristela J. Padoin, Aldo B. Lucion *

Department of Physiology, Institute of Biological Sciences, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

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Abstract

The effects of a 5-HT_2 receptor agonist, DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; 0.5 mg/kg), on the behavior of male rats at different ages when given alone or with different levels of testosterone, in the presence of sexually receptive and non-receptive females are presented. DOI increased mounting and/or mount plus thrusting behavior in adult males with receptive females. In pre-pubertal males, DOI increased the frequency of pursuit and genital sniffing in the presence of receptive females, but not of non-receptive ones, when no mounts or thrustings were recorded. In castrated rats treated with testosterone and tested with receptive females, DOI increased the frequency of thrusting behavior, but in castrated rats without testosterone treatment, DOI produced no change. DOI did not induce mounting in pre-pubertal or castrated rats without testosterone substitution therapy. These results suggest that DOI influences male sexual behavior through a neural system that is modulated by testosterone.

Keywords: Sexual behavior; 5-HT (5-hydroxytryptamine, serotonin); 5-HT_2 receptor agonist; DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane); Testosterone; Castration; Puberty

1. Introduction

Serotonin (5-HT) modulates a number of behaviors, including sexual behavior. The various receptor subtypes seem to have different roles in sexual behavior. It has been repeatedly demonstrated that the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), facilitates sexual behavior of male rats (see Ahlenius and Larsson, 1989; and Gorzalka et al., 1990 for a review). On the other hand, studies on 5-HT_2 receptor agonists and sexual behavior of males have produced a more complex picture. Previous studies showed that the 5-HT_2 receptor antagonists, ketanserin and pirenperone, inhibited male sexual behavior, suggesting a facilitatory role of 5-HT_2 receptors (Mendelson and Gorzalka, 1985). However, DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane), a 5-HT_2 receptor agonist, inhibited copulatory behavior in male rats and this inhibitory effect was reversed by serotonin receptor antagonists like pirenperone, ritanserin and ketanserin (Foreman et al., 1989; Watson and Gorzalka, 1991). The suppressive effect of DOI on sexual activity was also reversed by amperozide, a selective 5-HT_2 receptor antagonist. However, amperozide and DOI had similar effects in that both decreased the number of intromissions prior to ejaculation and prolonged the post-ejaculatory interval. Amperozide specifically restored the ejaculatory capacity in animals pretreated with DOI (Klint et al., 1992).

Serotonergic neurons have also been considered mediators of the effects of hormones on behavior since they participate in the central regulation of secretion in the anterior pituitary. However, the relationship between serotonin and hormones is complex, its study producing results that sometimes are contradictory. In adult male rats, serotonin, injected in the 3rd ventricle of the brain, has an inhibitory effect on the secretion of

* Corresponding author. Fisiologia, Biociencias, UFRGS, Sarmento Leite 500, Porto Alegre, RS 90050-170, Brazil. Tel. +55 51 2281633 ext. 3453, fax +55 51 2267191, e-mail ALUCION@VORTEX.UFRGS.BR.

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gonadotrophins in the pituitary (Justo et al., 1989). On the other hand, castration increases the biosynthesis of serotonin in the limbic system and diencephalon of the rat; this increase can be reversed by testosterone in the diencephalon, but not in the limbic system (Long et al., 1983). The presence of neurons sensitive to androgens and to serotonin in the limbic system suggests a potential interaction between them in the regulation of behaviors.

The purpose of this study was to analyze the effects of a 5-HT_2 receptor agonist (DOI) on sexual behavior of male rats at different ages, castrated or not, and to attempt to establish a functional interaction between this 5-HT receptor agonist and testosterone.

2. Materials and methods

2.1. Animals

Male Wistar rats from the stock of the Federal University of Rio Grande do Sul were used in the following experiments.

Experiment 1 – Effect of the injection of DOI on sexual behavior of young adult male rats. A total of 30 male rats, 60 days old, weighing 240–280 g, were divided into 2 groups of 15 animals each: group 1 = injection of saline; group 2 = injection of DOI.

Experiment 2 – Effect of the injection of DOI on sexual behavior of pre-pubertal male rats. A total of 60 male rats, 33 to 35 days old, weighing 80–100 g, were divided into 4 groups of 15 animals each: group 1 = males were injected with saline and were tested with a non-receptive female; group 2 = males were injected with DOI and were tested with a non-receptive female; group 3 = males were injected with saline and were tested with a receptive female; group 4 = males were injected with DOI and were tested with a receptive female.

Experiment 3 – Effect of the injection of DOI on sexual behavior of castrated adult male rats. A total of 60 adult male rats, castrated at 60 days and tested at 150 days of age, weighing 360–430 g, were divided into 4 groups of 15 animals each: group 1 = no testosterone substitution therapy and saline injection; group 2 = no testosterone substitution therapy and DOI injection; group 3 = testosterone substitution therapy and saline injection; group 4 = testosterone substitution therapy and DOI injection.

Experiment 4 – Effect of the injection of DOI on sexual behavior of adult male rats. A total of 16 adult male rats, 180 days old, weighing 320–420 g, were divided into 2 groups: group 1 = injection of saline ($n = 7$); group 2 = injection of DOI ($n = 9$).

The animals were kept in groups of 2–5 males per cage (41 × 34 × 17 cm) according to body weight until the experiment began. They were kept in an environment with a light/dark cycle of 12/12 h (lights on at 4 a.m.) and temperature about 23°C. The animals had free access to water and food throughout the experiment. Except for experiment 2 (pre-pubertal males), the rats had 2 previous contacts (10 min each) with a receptive female, when they were selected on the basis of sexual performance. Only those with a frequency of mounting above 6 in the second 10-min session were used.

The females used to observe the sexual behavior of the males (experiments 1, 3 and 4) were also Wistar rats 60 to 90 days old, weighing an average of 270 g. In experiment 2, due to the size of the males, the females were 45 to 48 days old, weighing around 80 g. The females were oophorectomized, and brought to sexual receptiveness by sequential intramuscular injections of estradiol benzoate (5 μg/rat) 48 h and progesterone (5 μg/rat) 4 h before behavioral recording began. All females were first tested with a sexually active male and only proven lordotic ones were used.

2.2. Treatments

DOI was always administered in a dose of 0.5 mg/kg body weight diluted in 0.9% saline (0.25 mg/ml). DOI was kindly donated by Dr. B. Olivier, Duphar, Weesp, Netherlands. In experiments 1, 2 and 3, the drug and vehicle (saline) were injected intraperitoneally (i.p.), and, in experiment 4, subcutaneously (s.c.). Saline or DOI was always injected 30 min before behavioral recording.

In experiment 3, at the age of 60 days, the males were castrated under general anaesthesia. Substitution therapy consisted of intramuscular injections of testosterone (Durateston 250, Organon), in a dose of 5 mg/rat, every 3 weeks, during a 90-day period, when a new recording, previous to saline or DOI injection, was performed to check on the effectiveness of the substitution therapy.

Plasma testosterone levels were determined in duplicate by radioimmunoassay. The animals were killed by decapitation at 3 p.m. Blood was collected from the trunk. Samples were centrifuged for 10 min at 3000 rpm; the plasma was separated and stored frozen until estimation of testosterone (Kit DSL, intra-assay coefficient = 9.7%, inter-assay coefficient = 12.9% and sensitivity = 0.05 ng/ml).

2.3. Behavioral testing

Sexual behavior was recorded in cages, size 70 × 70 × 35 cm, with steel walls, except for the front wall that was glass, which allowed complete viewing of the animals. Behavior recording was performed 1–2 h after the beginning of the dark phase, with light from a

40-Watt lamp. Initially, the male was placed in the observation cage 10 min before the female for adaptation to the environment. After this period, the behaviors of the male were videotaped during a 10-min session. Frequency (number of times each behavior occurred during a recording session) was the parameter analyzed.

The following behaviors were recorded (Clemens, 1974; Olivier and Mos, 1988; Hillegaart, 1991): pursuit – the male runs after the female; sniffing – the male sniffs the female's body; genital sniffing – the male approaches the female and sniffs her anogenital area; mount – the male places his forepaws on the female without pelvic movements; thrusting – the male places his forepaws on the female, performing repeated deep pelvic thrusting (mount plus thrusting).

2.4. Statistical analysis

In experiments 1 and 4, frequency of the behaviors was compared between the two groups (saline × DOI) using Student's *t*-test. The plasma testosterone level was compared between the two groups (60 × 180 days) using Student's *t*-test. In experiments 2 and 3, an analysis of variance (ANOVA) among groups was performed. When appropriate, the different means were compared using the Newman-Keuls test. A $P < 0.05$ was considered statistically significant in all experiments.

3. Results

In young adult male rats (60 days), tested with a sexually receptive female, DOI increased the frequency of mount and thrusting (Fig. 1).

In pre-pubertal male rats (35 days), DOI did not affect sniffing of the body of both sexually receptive (R) and non-receptive (NR) females (Fig. 2). However, DOI increased the frequency of genital sniffing and pursuit with a receptive (R) female, but not with a non-receptive (NR) one, as compared to saline injection under each set of conditions. Moreover, in the DOI-injected groups, the frequency of genital sniffing and pursuit was higher in the group of males that were in the presence of a receptive female than in those that were with a non-receptive female (Fig. 2).

In castrated adult male rats (Fig. 3), tested with a sexually receptive female, DOI increased the number of thrustings in animals that had received testosterone substitution therapy (T +), but had no effect in rats without the substitution therapy (T −). DOI had no effect on mount frequency when compared to saline injection in the substitution therapy (T +) group. After DOI injection in the animals that had received testosterone substitution therapy (T +) the frequency of mount and thrusting was higher than in animals without the substitution therapy (T −).

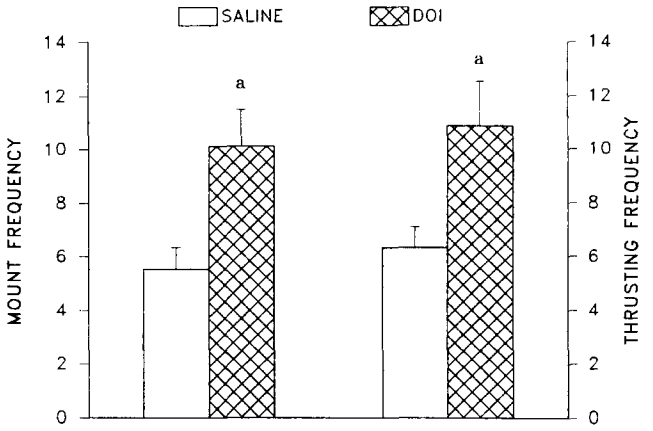

Fig. 1. Effect of i.p. injection of DOI (0.5 mg/kg) on mean frequency ± S.E.M. of mount (left side) and thrusting (right side of the figure) of young adult male rats (60 days old). Each group (saline and DOI) represents the average for 15 animals. [a] Significance versus the respective control group injected with saline ($P < 0.01$).

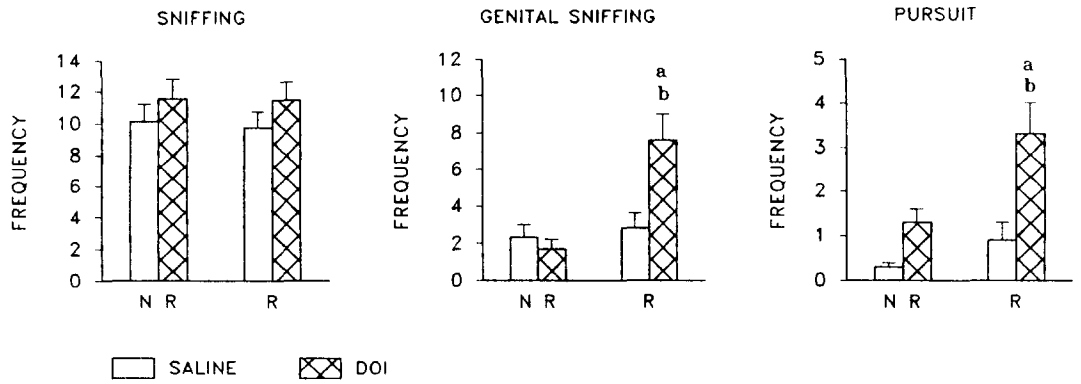

Fig. 2. Effect of i.p. injection of DOI (0.5 mg/kg) on mean frequency ± S.E.M. of sniffing, genital sniffing and pursuit by pre-pubertal male rats (35 days old) in the presence of a non-receptive female (NR) or in the presence of a sexually receptive female (R). Each group (saline and NR; saline and R; DOI and NR; DOI and R) represents the average for 15 animals. [a] Significance versus the respective control group injected with saline ($P < 0.05$). [b] Significance versus the injection of DOI and NR ($P < 0.05$).

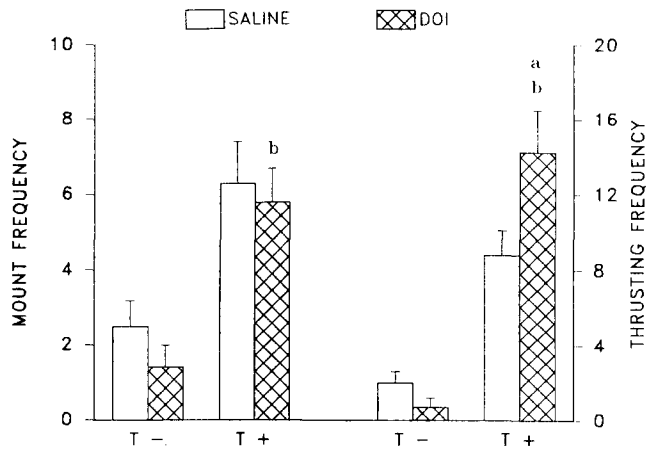

Fig. 3. Effect of i.p. injection of DOI (0.5 mg/kg) on mean frequency ± S.E.M. of mount (left side) and thrusting (right side of the figure) by castrated adult male rats (150 days old). T−: without testosterone, and T+: with testosterone substitution therapy. Each group (saline T−; saline T+; DOI T−; DOI T+) represents the average for 15 animals. [a] Significance versus saline injection in T+ animals ($P < 0.01$). [b] Significance versus DOI injection in T− animals ($P < 0.01$).

In adult male rats (180 days old), tested with a sexually receptive female, DOI increased the number of mounts, but had no effect on thrusting (Fig. 4). The plasma level of testosterone in the animals at 180 days of age was lower than in those 60 days of age (Table 1).

4. Discussion

The results showed that, in young adult male rats (60 days old), DOI (0.5 mg/kg) increased sexual interest as evaluated by the increase in the number of mounts and thrustings. Pre-pubertal animals (35 days old), in the presence of receptive females, had their frequency of genital sniffing and pursuit increased after DOI injection. If pre-pubertal males were in the presence of non-receptive females, no effect was recorded. This experiment showed that DOI can specifically increase sexual motivation in pre-pubertal rats, but it is not able to trigger the sexual behaviors of mount and thrusting. In rats at the age of 35 days, plasma testosterone is low, its peak occurs around 50–60 days of age. The age of 60–80 days is considered a transition period between puberty and the adult phase (Ojeda and Urbanski, 1988).

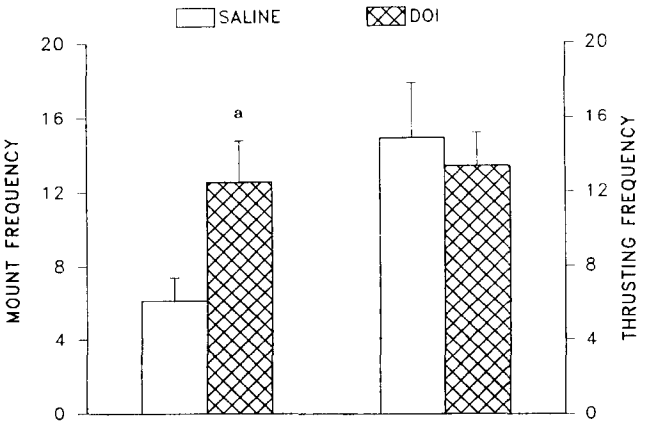

Fig. 4. Effect of s.c. injection of DOI (0.5 mg/kg) on mean frequency ± S.E.M. of mount (left side) and thrusting (right side of the figure) by adult male rats (180 days old). Saline group represents 7 animals, and DOI group 9 animals. [a] Significance versus the respective control injected with saline ($P < 0.05$).

Table 1
Mean concentration ± S.E.M. of testosterone (ng/μl) in male rats 60 ($n = 10$) and 180 ($n = 16$) days of age

Age (days)	Plasma testosterone (ng/μl)
60	1.89 ± 0.47
180	0.99 ± 0.14 [a]

[a] Significance versus the males aged 60 days ($P < 0.05$).

In order to asses a possible relationship between testosterone and DOI effects on sexual behavior of adult males, rats were castrated and some then received testosterone and others not. In those animals treated with testosterone substitution therapy, DOI (0.5 mg/kg) increased the frequency of mount and thrusting. In the group with no substitution therapy, the same dose of DOI had no effect. The results of these three experiments allow us to conclude that DOI can increase the sexual interest of male rats, but testosterone is necessary for this effect. DOI increases the social interaction of pre-pubertal males towards sexually receptive females, but does not initiate the motor activities of mount and thrusting.

In previous studies, it was shown that the administration of DOI, in the doses of 0.1 and 1.0 mg/kg s.c. in 180-day-old male Sprague-Dawley and Long-Evans rats, diminished the frequency of mount and intromission, increased the latency of ejaculation and diminished copulatory efficiency (Foreman et al., 1989; Gorzalka et al., 1990). On the other hand, Klint et al. (1992) also demonstrated a decrease in the number of intromissions, but a marked, though non-significant, increase in the number of mounts. Some methodological differences between these experiments and the 3 mentioned above should be emphasized: experience of the males, strain of rats, route of injection and age. The animals used in the present study had less sexual experience than those analyzed by the above-mentioned authors. Rats of different strains also differ as to pattern of sexual behavior. Gorzalka et al. (1990) reported that Long-Evans males have greater copulatory vigour than Sprague-Dawley ones. Age was a variable tested in the present work.

Experiment 4 aimed to analyze the role of DOI in rats in which the testosterone level was not as low as that of a castrated rat, but was lower than that of a young adult animal. Otherwise, this experiment used animals of the same age, and the drug was injected by the same route as Foreman et al. (1989) and Gorzalka et al. (1990) used for administration. First, it was shown that the level of plasma testosterone of animals at the age of 180 days was lower than that of rats at the age of 60 days. Moreover, in rats at the age of 180 days, DOI (0.5 mg/kg) increased the frequency of mount, without significantly changing the number of thrustings.

The four experiments demonstrated that DOI can increase sexual arousal, as long as there is a certain level of circulating testosterone. If the level is low, as for instance in 180-day-old rats, DOI affects only mount behavior. However, if plasma testosterone is very low, as in the castrated animals, no effect is observed. On the other hand, when the testosterone level is high, as in young adults (60 days) or after testosterone treatment of castrated animals, DOI increases thrusting behavior. The dissociation between the effects of DOI on the frequency of mount and thrusting may reflect the action of different levels of testosterone on the 5-HT receptor. It seems that testosterone modulates the action of DOI on male sexual behavior.

Serotonin is involved in various neuroendocrine functions. Serotonergic neurons have been considered mediators of some effects of hormones in the central regulation of behaviors (Long et al., 1983). The anatomical proximity of the steroid-concentrating sites in the brain and of the areas containing serotonergic receptors may help to explain the interactions between steroid hormones and the serotonergic system (Biegon et al., 1982). It was shown that, in castrated male rats without substitution therapy, the inhibition of 5-HT synthesis by *p*-chlorophenylalanine facilitates the induction of sexual behavior by testosterone (Soderstein et al., 1976). However, the mechanism of the interaction between serotonin and testosterone on sexual behavior remains unknown.

Previous work showed that DOI does not induce penile erection unless the 5-HT_2 receptors are inhibited by antagonists. It seems that DOI affects penile erection specifically through 5-HT_{1C} receptors (Berendsen et al., 1990). Therefore, in the present work, the observed behavioral changes were probably due to effects of DOI on the sexual motivation of the male and were not a consequence of a change in penile erection. Moreover, the results for the pre-pubertal rats, that showed no mounts but had increased genital sniffing and pursuit of the sexually receptive females, reinforce the idea of a sexual motivational change after DOI injection.

DOI is a 5-HT_2 receptor agonist that acts on post-synaptic excitatory receptors located in several brain areas (Wright et al., 1990; Kidd et al., 1991). However, it was reported that DOI can also act on 5-HT_{1A} receptors, although it has much greater affinity for the 5-HT_2 receptors (Darmani et al., 1990; Wright et al., 1990). 8-OH-DPAT, a 5-HT_{1A} receptor agonist, increases sexual behavior of males (Ahlenius and Larsson, 1989), even in castrated rats (Ahlenius et al., 1981). DOI, on the contrary, seems to need testosterone to affect sexual behavior of males.

In conclusion, DOI appears to act on a neural system that is modulated by testosterone. Apparently, it is a system that matures under the influence of testosterone before puberty, since pre-pubertal rats are affected by the drug. In adult males, the action of the 5-HT_2 receptor agonist requires a certain level of the hormone. Castration cancels the excitatory effect of DOI.

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References

Ahlenius, S. and K. Larsson, 1989, New aspects on the serotonergic modulation of male rat sexual behavior, in: Behavioural Pharmacology of 5-HT, eds. P. Bevan, A.R. Cools and T. Archer (Lawrence Erlbaum, Hillsdale) p. 35.

Ahlenius, S., K. Larsson, L. Svensson, S. Hjorth, A. Carlsson, P. Lindberg, H. Wikstrom, D. Sanchez, L.E. Arvidsson, U. Hacksell and J.L.G. Nilsson, 1981, Effects of a new type of 5-HT receptor agonist on male rat sexual behavior, Pharmacol. Biochem. Behav. 15, 785.

Berendsen, H.G., F. Jenck and C.L.E. Broekkamp, 1990, Involvement of 5-HT_{1C} receptors in drug-induced penile erections in rats, Psychopharmacology 101, 57.

Biegon, A., T.C. Rainbow and B.S. McEwen, 1982, Quantitative autoradiography of serotonin receptors in the rat brain, Brain Res. 242, 197.

Clemens, L.G., 1974, Neurohormonal control of male sexual behavior, Reprod. Behav. 11, 23.

Darmani, N.A., B.R. Martin, U. Pandey and R.A Glennon, 1990, Do functional relationships exists between 5-HT_{1A} and 5-HT_2 receptors?, Pharmacol. Biochem. Behav. 36, 901.

Foreman, M.M., J.L. Hall and R.L. Love, 1989, The role of the 5-HT_2 receptor in the regulation of sexual performance of male rats, Life Sci. 45, 1263.

Gorzalka, B.B., S.D. Mendelson and N.V. Watson, 1990, Serotonin receptor subtypes and sexual behavior, Ann. NY Acad. Sci. 600, 435.

Hillegaart, V., 1991, Functional topography of brain serotonergic pathways in the rat, Acta Physiol. Scand. 598, 2.

Justo, S.N., G.L. Rossano, B. Szwarcfarb, M.C. Rubio and J.A. Moguilevsky, 1989, Effect of serotoninergic system on FSH secretion in male and female rats: evidence for stimulatory and inhibitory actions, Neuroendocrinology 50, 382.

Kidd, E.J., J.C. Garratt and C.A. Marsden, 1991, Effects of repeated treatment with 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) on the autoregulatory control of dorsal raphe 5-HT neuronal firing and cortical 5-HT release, Eur. J. Pharmacol. 200, 131.

Klint, T., I.L. Dahlgren and K. Larsson, 1992, The selective 5-HT_2 receptor antagonist amperozide attenuates 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane-induced inhibition of male rat sexual behavior, Eur. J. Pharmacol. 212, 241.

Long, J.B., W.W. Youngblood and J.S. Kizer, 1983, Effects of castration and adrenalectomy on in vitro rates of tryptophan hydroxylation and levels of serotonin in microdissected brain nuclei of adult male rats, Brain. Res. 277, 289.

Mendelson, S.D. and B.B. Gorzalka, 1985, Serotonin antagonist pirenperone inhibits sexual behavior in the male rat: attenuation by quipazine, Pharmacol. Biochem. Behav. 22, 565.

Ojeda, S.R. and H.F. Urbanski, 1988, Puberty in the rat, in: The Physiology of Reproduction, eds. E. Knobil and J.D. Neill (New York Raven Press, New York) p. 1699.

Olivier, B. and J. Mos, 1988, Effects of psychotropic drugs on sexual behaviour in male rats, in: Depression, Anxiety and Aggression, Preclinical and Clinical Interfaces, eds. B. Olivier and J. Mos (Netherland Medical Didactic Systems, Houten) p. 121.

Soderstein, P., K. Larsson and S. Ahlenius, 1976, Sexual behavior in castrated male rats treated with monoamine synthesis inhibitors and testosterone, Pharmacol. Biochem. Behav. 5, 319.

Watson, N.V. and B.B. Gorzalka, 1991, DOI-induced inhibition of copulatory behavior in male rats: reversal by 5-HT_2 antagonists, Pharmacol. Biochem. Behav. 39, 605.

Wright, I.K., J.C. Garratt and C.A. Marsden, 1990, Effects of a selective 5-HT_2 agonist, DOI, on 5-HT neuronal firing in the dorsal raphe nucleus and 5-HT release and metabolism in the frontal cortex, Br. J. Pharmacol. 99, 221.