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Pharmacological Features of Masculine Sexual Behavior in an Animal Model of Depression

H. BONILLA-JAIME, S. RETANA-MARQUEZ AND J. VELAZQUEZ-MOCTEZUMA

Biology of Reproduction, Universidad Autonoma Metropolitana-Iztapalapa, México City, C.P. 09340, México

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BONILLA-JAIME, H., S. RETANA-MARQUEZ AND J. VELAZQUEZ-MOCTEZUMA. *Pharmacological features of masculine sexual behavior in an animal model of depression.* PHARMACOL BIOCHEM BEHAV **60**(1) 39–45, 1998.— Neonatal treatment with clomipramine induces behavioral alterations during adulthood that resemble symptoms observed in human depression. Therefore, it has been proposed as an animal model of depression. Impairment of male sexual performance is one of the main effects of this treatment. Using this model of depression, we evaluated the effects of drugs that stimulate sexual performance by acting selectively on the adrenergic, serotonergic, or cholinergic system. Yohimbine, a selective antagonist of the alpha-2 receptors; 8-OH-DPAT, a selective agonist of the 5-HT_{1A} receptors; and oxotremorine, a muscarinic agonist, were administered to male rats neonatally treated with clomipramine that showed sexual behavior impairments. Yohimbine and oxotremorine induced only a slight improvement of sexual deficiencies. 8-OH-DPAT not only restored sexual behavior to normal levels, but induced facilitation in most of the copulatory parameters. These results suggest that neonatal treatment with clomipramine induces sexual deficits acting mainly on the adrenergic and cholinergic systems, while the serotoninergic system seems to be preserved. © 1998 Elsevier Science Inc.

Masculine sexual behavior Models of depression Yohimbine Oxotremorine 8-OH-DPAT Clomipramine

SEVERAL proposals for animal models of depression have emerged during the last decade (29). Some of them try to replicate, in the laboratory animal, the main signs of human depression [i.e., anhedonia, decrease of locomotor activity, abnormalities in the sleep pattern, among others (9,27,30)]. Anhedonia, which is the core symptom of depression, is displayed in the laboratory rat as a clear decrease in the frequency of presentation of pleasure-seeking behaviors, which includes intracraneal self-stimulation (15), consumption of water with sucrose (16,31), and sexual behavior (17).

Recently, neonatal treatment with clomipramine in rats has been proposed as an animal model of depression. During adulthood, this procedure is capable of inducing, during the adulhood, behavioral changes that closely resembles the picture displayed by depressed humans (28). These behavioral abnormalities include a decrease of aggressive and pleasure-seeking behaviors such as intracranial self-stimulation (26) and sexual behavior (17), abnormalities in the sleep pattern (14,27), altered response to stressful situations (7,25), and increased immobility in the forced swim test (24).

On the other hand, it is well known that male sexual behavior in rats is regulated by several neurotransmitter systems (2). Copulatory behavior in male rats can be enhanced by the specific blockade of the $alpha_2$ receptors in the adrenergic system (4,10), the stimulation of the 5-HT_1a receptors in the serotoninergic system (1,5), and the stimulation of muscarinic receptors in the cholinergic system (8,19). In addition to their participation in the regulation of masculine sexual behavior, the serotoninergic, adrenergic, and cholinergic system have been implicated in the etiology of depression, as well [for review, see (18)].

Thus, it is possible that the alterations of masculine sexual behavior observed in this animal model of depression can be due to a failure in the monoaminergic and/or in the cholinergic system. In this study we analyze if masculine sexual behavior abnormalities induced by neonatal treatment with clomipramine can be reversed by the administration of a selective $alpha_2$ blocker such as yohimbine, a selective agonist of the 5-HT_{1A} receptors (10,21), 8-OH-DPAT (1,5), and a selective agonist of muscarinic receptors, oxotremorine (8,19).

METHOD

Wistar rats from our own vivarium were used in this study. Three days after delivery, female pups were eliminated and male pups were crossfostered, keeping the same size (n = 5)

Requests for reprints should be addressed to Dr. Javier Velázquez Moctezuma, Departamento de Biologia de la Reproducción, Universidad Autonoma Metropolitana-Iztapalapa, México City, C.P. 09340, México.

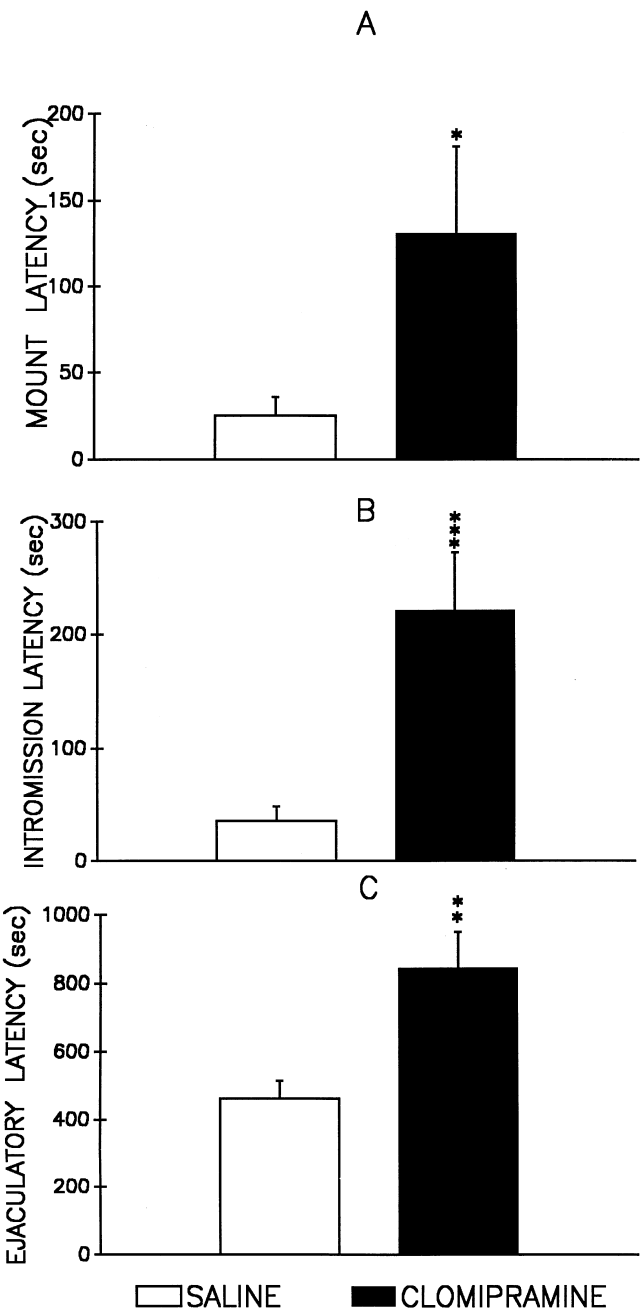

FIG. 1. Effect of neonatal administration of clomipramine or saline on mount, intromission, and ejaculation latencies displayed in a sexual behavior test performed during adulthood. Mann–Whitney U-test. $*p < 0.05$; $**p < 0.005$, $***p < 0.0005$.

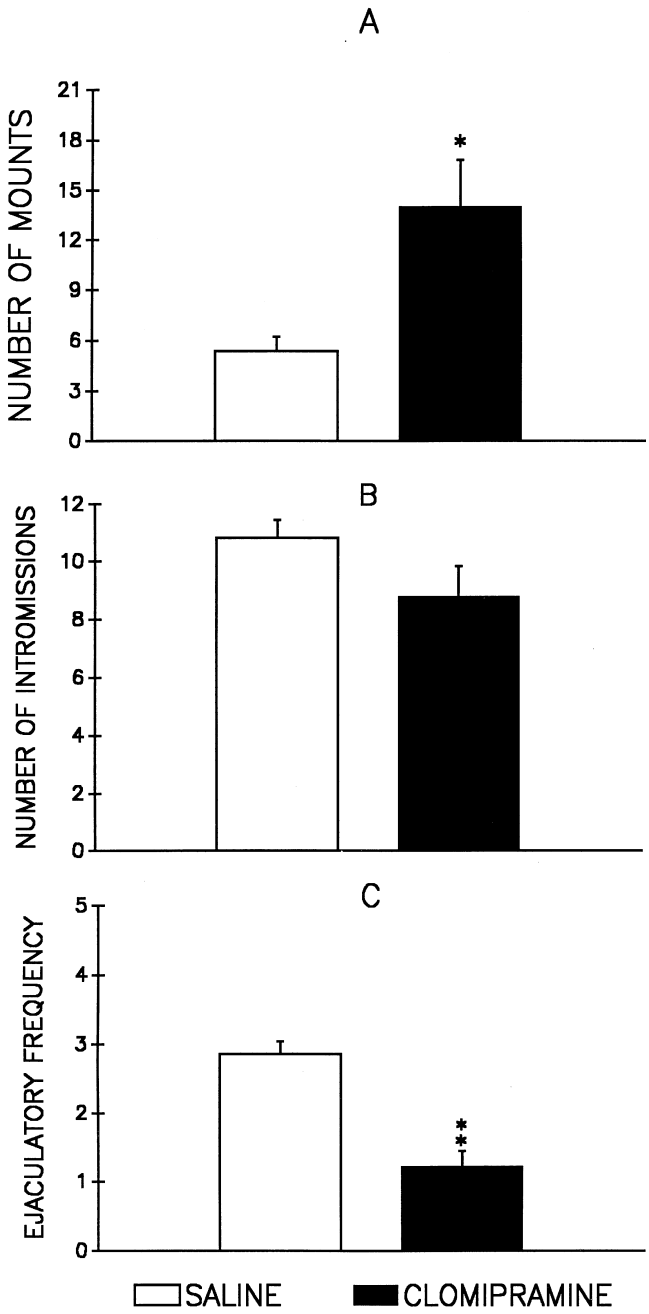

FIG. 2. (A–C) Effect of neonatal administration of clomipramine or saline on the number of mounts and intromissions preceding ejaculation as well as on ejaculatory frequency. Mann–Whitney U-test. $*p < 0.005$, $**p < 0.0005$.

in all the litters. From day 8 to day 21 of age, all the pups were injected twice daily (0800 and 1800 h). One group ($n = 22$) received clomipramine (15 mg/kg ; 0.1 ml SC) in each injection, while the control group ($n = 11$) received saline in the same volume. At 25 days of age, pups were separated from their foster mothers and were housed in groups (five per cage with the same treatment) in acrylic cages (52 × 47 cm). Animals were kept in a room with inverted light cycle (lights on 2100 h; off 0900 h.). Food and water were available ad lib.

At 5 months of age, animals were tested three times for spontaneous masculine sexual behavior, with an interval of 1 week between observations. Behavioral tests were made during the dark phase of the cycle and under a dim red light. In brief, the procedure consists in placing the male in a circu-

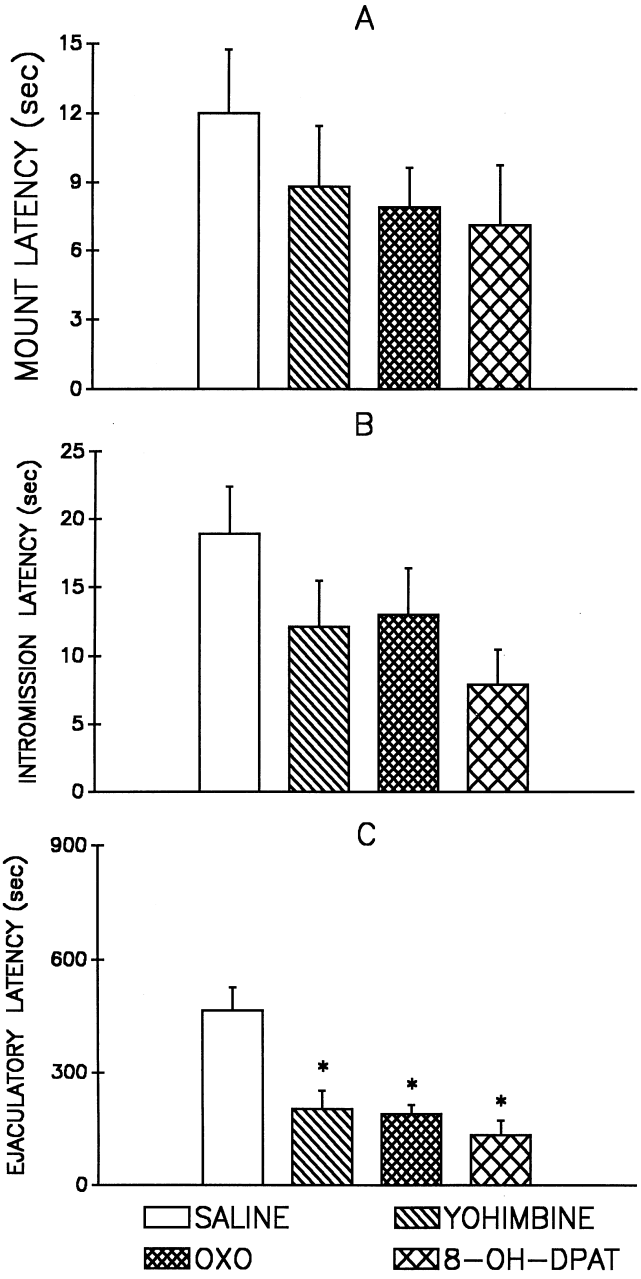

FIG. 3. Effects induced by the administration of saline or specific adrenergic, cholinergic, or serotonergic drugs on copulatory latencies of male rats neonatally injected with saline. Kruskal–Wallis test followed by Dunn test. Compared to saline administration: $*p < 0.001$.

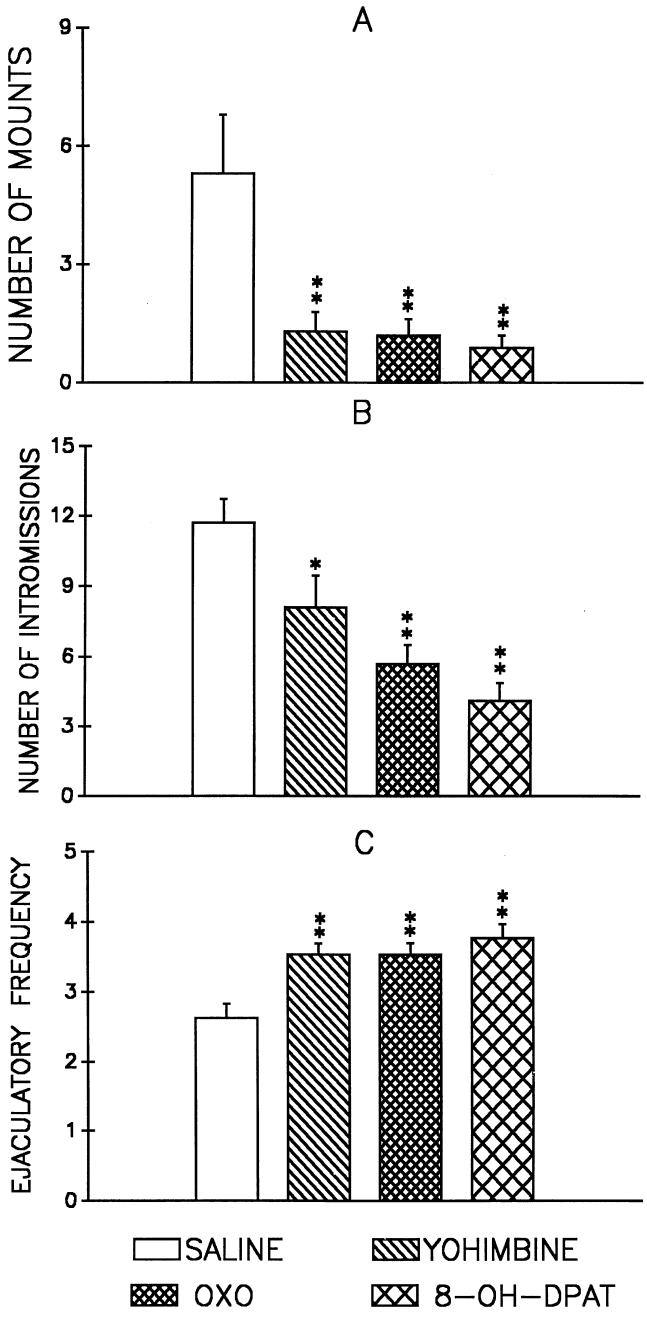

FIG. 4. (A–C) Effects induced by the administration of saline or specific adrenergic, cholinergic, or serotonergic drugs on mounts and intromissions preceding ejaculation as well as on ejaculatory frequency in male rats neonatally injected with saline. Kruskal–Wallis test followed by Dunn test. Compared to saline administration: $*p < 0.005$; $**p < 0.001$.

lar Plexiglas arena (45 cm diameter) during 5 min for habituation. Thereafter, a stimulus female artificially brought to estrous with sequential treatment of 10 μg of estradiol benzoate plus 1 mg of progesterone 44 h later, was presented before the male 4 h after the administration of progesterone. To avoid the Coolidge effect (32), stimulus females were changed every 5 min. The tests lasted for 30 min and the parameters recorded were latencies and frequencies of mounts, intromissions and ejaculations, postejaculatory interval, intercopulatory and interintromission interval, and the hit rate (number of intromissions/number of intromissions + number of mounts). The full definition of each parameter can be found elsewhere (20).

Once basal sexual activity was obtained, another four weekly tests were done in both groups, each of them after one of the following four treatments: 1) saline; 2) yohimbine–HCl

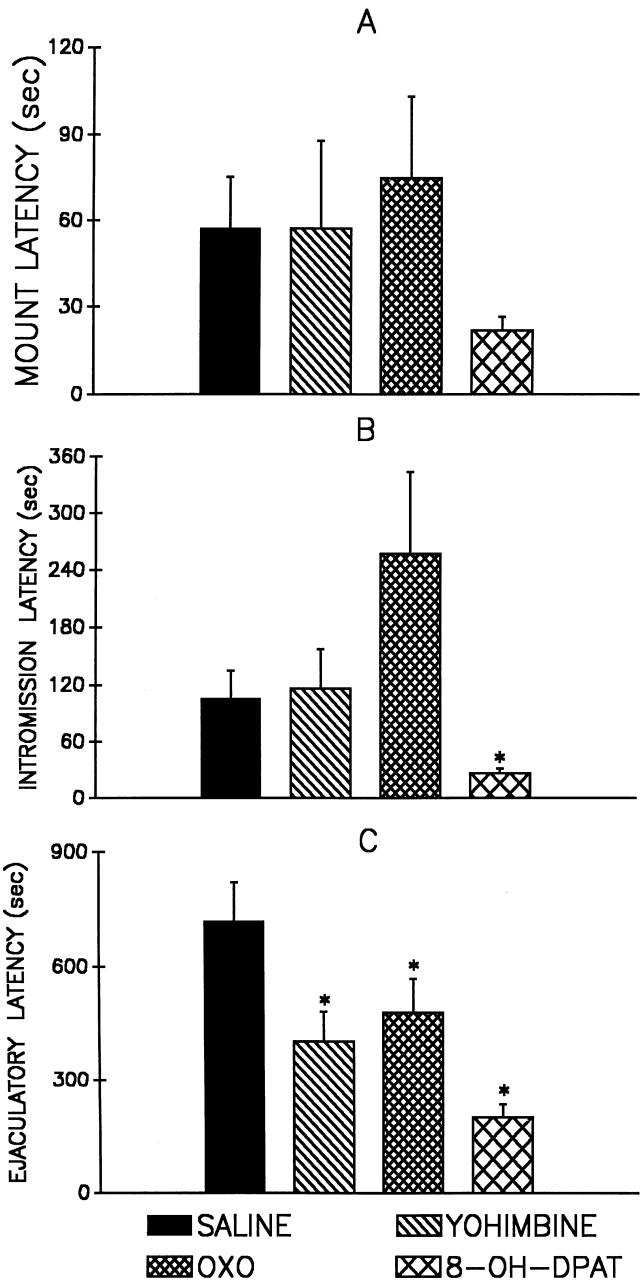

FIG. 5. (A–C) Effects induced by the administration of saline or specific adrenergic, cholinergic, or serotonergic drugs on copulatory latencies in male rats neonatally treated with clomipramine. Kruskal–Wallis test followed by Dunn test. Compared to saline administration: $*p < 0.01$.

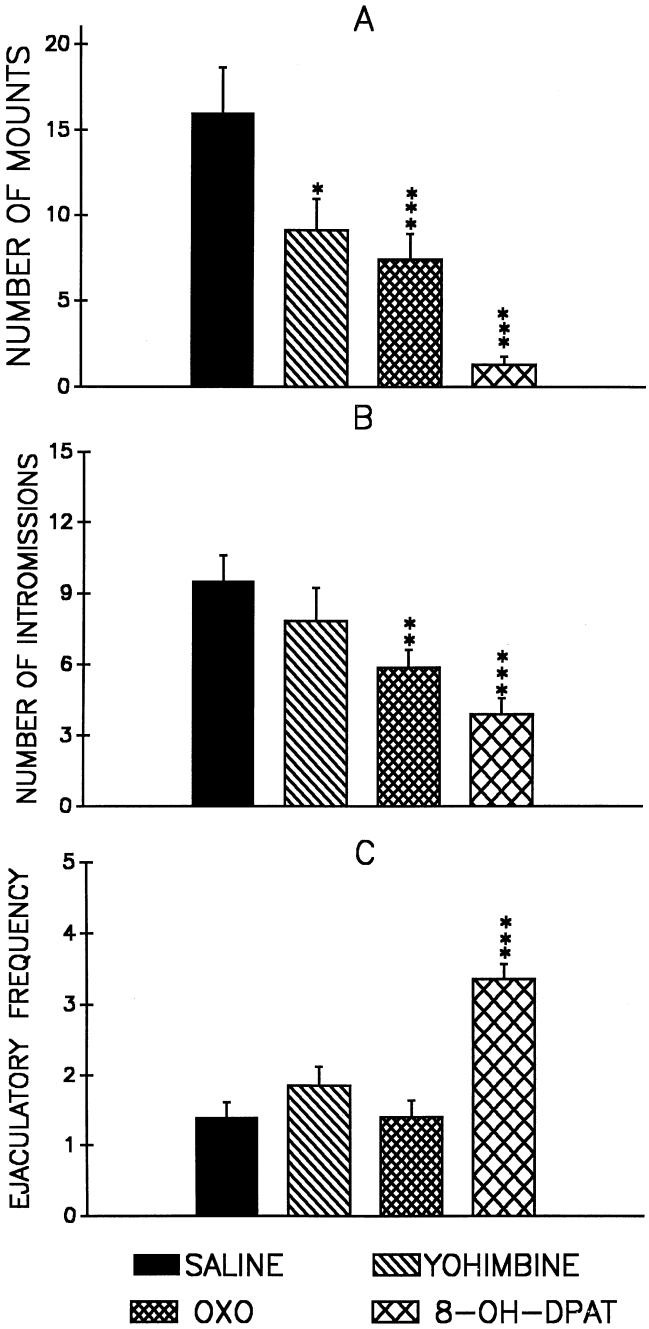

FIG. 6. (A–C) Effects induced by the administration of saline or specific adrenergic, cholinergic, or serotonergic drugs on mounts and intromissions preceding ejaculation as well as on ejaculatory frequency in male rats neonatally treated with clomipramine. Kruskal–Wallis test followed by Dunn test. $*p < 0.01$, $**p < 0.005$, $***p < 0.001$.

(Sigma Co., St. Louis, MO) 2 mg/kg b.wt., 30 min before the onset of the test; 3) 8-hydroxi-2-dimetil-m-propilamino tretalin (8-OH-DPAT) (Res. Biochem. Inc.) 0.125 mg/kg b.wt., 15 min before the test; and 4) oxotremorine sesquifumarate (OXO) (Sigma Co.) 0.4 mg/kg b.wt., 30 min before test. All the injections were intraperitoneal in a volume of 0.3 ml OXO and 8-OH-DPAT were disolved in saline. Yohimbine was dissolved in destilled water. Fifteen minutes before OXO administration, subjects received peripheral muscarinic blockage with scopolamine-methyl bromide in a dose of 3 mg/kg b.wt. The subjects received all the injections in a random sequence following a Latin square design. The dose selected for each drug has been reported as reliable to induce a clear stimulatory effect on male sexual performance (1,4,19).

Statistical analysis of spontaneous sexual activity was done using the Mann–Whitney U-test. The Kruskal–Wallis test was used to analyze the effect of the different injections. When significant, it was followed by the Dunn test.

RESULTS

After three sexual behavior tests, all the subjects displayed activity. Figure 1 shows the effects of neonatal treatment with clomipramine on mount, intromission, and ejaculation latencies. As can be seen, when compared to the saline group, a significant increase of mount, intromission, and ejaculation latencies is present. In addition, the number of mounts (Fig. 2A) in the clomipramine group showed a huge increase. Ejaculatory frequency (Fig. 2C) displayed a significant reduction. The number of intromissions preceding ejaculation did not showed differences between saline and clomipramine treatment (Fig. 2B).

Figure 3 shows the effect of the administration of yohimbine, oxotremorine, and 8-OH-DPAT in the copulatory latencies of the group neonatally treated with saline. As can be seen, mount and intromission latencies showed a trend to decrease. All the treatments induced a significant shortening of the ejaculatory latency. In addition, the number of mounts (Fig. 4A) and the number of intromissions preceding ejaculation (Fig. 4B) are drastically reduced. The final corroboration of the stimulatory effect of these treatments is related to the ejaculatory frequency that presented a significant increase when compared to the effect of the administration of saline (Fig. 4C).

Regarding the effect of yohimbine administered to the rats neonatally treated with clomipramine, there was a significant reduction of the ejaculatory latency (Fig. 5C). There was an important trend to decrease in the number of mounts that did not reach statistical significance (Fig. 6A). After OXO administration, mount and intromission latencies showed a large dispersion with a mean higher than the mean obtained with saline (Fig. 5A and B). The ejaculatory latency showed an important reduction; however, it did not reach statistical significance (Fig. 5C). OXO administration elicited a significant reduction in the number of mounts and intromissions preceding ejaculation (Fig. 6A and B).

On the other hand, 8-OH-DPAT administration induced a clear stimulation in almost all parameters. As shown in Fig. 5B and C, intromission and ejaculation latencies were significantly reduced. The same effect was observed in the number of mounts and intromissions preceding ejaculation (Fig. 6A and B), which were drastically reduced. Ejaculatory frequency displayed a significant increase compared with the rest of the treatments in the neonatally clomipramine treated group (Fig. 6C). All the effects induced by 8-OH-DPAT were also statistically significant when compared with the data obtained in the animals neonatally injected with saline, revealing not only a normalization of the sexual impairments induced by neonatal clomipramine, but a stimulatory effect in sexual performance. Figure 7A and B shows the effect of the different treatments on the hit rate in both groups. As can be noted, only the injection of 8-OH-DPAT in rats neonatally treated with clomipramine elicited a significant increase.

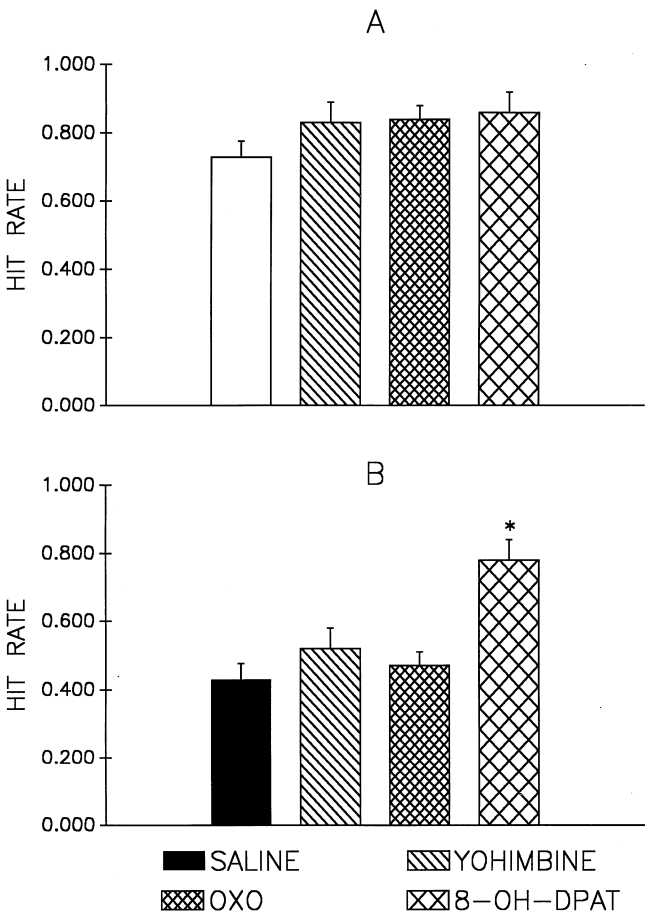

FIG. 7. Effect on the hit rate of the administration of saline or specific adrenergic, cholinergic, and serotonergic drugs in rats neonatally injected with saline (A) or clomipramine (B). Kruskal–Wallis test followed by Dunn test. $*p < 0.001$.

DISCUSSION

The present results corroborate previous reports regarding the impairment effect of neonatal treatment with clomipramine on masculine sexual performance in rats (17,23). Percentages of active subjects displaying mounts and intromissions both in control and in clomipramine-treated males are similar to those reported by Mirmiran et al. (13), but not by Neill et al. (17), who reported lower percentages of males with mounts and intromissions. Regarding the percentages of males that ejaculate, our results are very similar with those previously reported. We observed that 60% of the clomipramine-treated males displayed ejaculation, while the percentages reported by Mirmiran et al. as well as Neill et al. were 50 and 20%, respectively.

The analysis of sexual parameters showed remarkable differences between our results and those reported by Neill et al. In that study, control Wistar rats displayed much longer mount latencies (1101 s) than in our study (15 s) as well as a lower number of ejaculations [0.5 in that study and three in our study in the same testing period (30 min)]. These differences could be due to the housing conditions or to the hormonal manipulation of stimulus females.

On the other hand, it is well known that yohimbine has a stimulatory effect on masculine sexual performance in rats and humans (3,6,10). It has been claimed that these effects are due to selective blockage of the alpha-2 presynaptic receptors,

which results in an increased availability of cathecolamines at synaptic levels (4,22). Supporting this notion, another alpha-2 blocker, Idazoxan, has proved to have the same stimulatory effect on sexual behavior (11,21). In our study, blockade of alpha-2 receptors showed a stimulatory effect in the group neonatally treated with saline, but in the group neonatally treated with clomipramine, yohimbine administration induced a significant decrease of ejaculatory latency. Thus, the data indicate that this drug failed to stimulate sexual performance, which suggests that the cathecolaminergic system has been permanently altered by the neonatal administration of clomipramine.

Concerning the cholinergic system, it has been reported that the stimulation, mainly of the muscarinic receptors, elicits a facilitation of masculine sexual performance in the rat (8,19). Oxotremorine readily induces a decrease in the number of mounts and intromissions that precede ejaculation, shortening of the ejaculation latency and a significant increase of ejaculation frequency (8,19). In the rats neonatally treated with clomipramine, the stimulatory effect of OXO is limited to a decrease in the number of mounts and intromissions. Thus, the failure of oxotremorine to fully enhance sexual performance in rats neonatally treated with clomipramine suggests that the cholinergic system, and specifically the muscarinic receptors, have been permanently modified.

Early studies on the serotonergic regulation of sexual behavior indicated an inhibitory role on masculine performance [for review, see (2,12)]. However, an outstanding facilitation of masculine sexual performance can be seen after the administration of more specific agents, such as 8-OH-DPAT, which selectively stimulates the 5-HT_{1A} serotonergic receptor subtype, revealing a more complex participation of serotonin in the regulation of masculine sexual behavior (1,5). In this study, the administration of 8-OH-DPAT to neonatally treated animals resulted not only in the normalization of masculine sexual deficiencies, but in the stimulation of sexual parameters that very closely resembles the facilitation observed in normal rats. These results suggest that the serotoninergic system, especially the part linked to the 5-HT_{1A} receptor subtype, was not affected by neonatal administration of clomipramine.

Finally, the notion that neonatal clomipramine administration could induce a depressive-like behavior during the adulthood is supported by the results concerning masculine sexual behavior. The pharmacological evidence in the present study suggests that these behavioral changes are due to permanent changes mainly in the adrenergic and cholinergic systems, without the participation of the serotoninergic system.

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