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Effect of 5-HT$_{1B}$ receptor agonists injected into the prefrontal cortex on maternal aggression in rats

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Abstract

Serotonin (5-HT$_{1B}$) receptors play an essential role in the inhibition of aggressive behavior in rodents. CP-94,253, a 5-HT$_{1B}$ receptor agonist, can reduce aggression in male mice when administered directly into the ventro-orbitofrontal (VO) prefrontal cortex (PFC). The objective of the current study was to assess the effects of two selective 5-HT$_{1B}$ receptor agonists (CP-94,253 and CP-93,129), microinjected into the VO PFC, on maternal aggressive behavior after social instigation in rats. CP-94,253 (0.56 µg/0.2 µL, N = 8, and 1.0 µg/0.2 µL, N = 8) or CP-93,129 (1.0 µg/0.2 µL, N = 9) was microinjected into the VO PFC of Wistar rats on the 9th day postpartum and 15 min thereafter the aggressive behavior by the resident female against a male intruder was recorded for 10 min. The frequency and duration of aggressive and non-aggressive behaviors were analyzed using ANOVA and *post hoc* tests. CP-93,129 significantly decreased maternal aggression. The frequency of lateral attacks, bites and pinnings was reduced compared to control, while the non-aggressive behaviors and maternal care were largely unaffected by this treatment. CP-94,253 had no significant effects on aggressive or non-aggressive behaviors when microinjected into the same area of female rats. CP-93,129, a specific 5-HT$_{1B}$ receptor agonist, administered into the VO PFC reduced maternal aggressive behavior, while the CP-94,253 agonist did not significantly affect this behavior after social instigation in female rats. We conclude that only the 5-HT$_{1B}$ receptor agonist CP-93,129 administered into the VO PFC decreased aggression in female rats postpartum after social instigation.

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Activation of serotonin (5-HT_{1B}) receptors can play an inhibitory role in several types of aggressive behavior in rodents when injected intraperitoneally (1). CP-94,253, anpirtoline and zolmitriptan, all 5-HT_{1B} receptor agonists, when administered systemically have anti-aggressive effects under conditions that induce moderate or escalated levels of aggression such as social instigation, self-administration of alcohol or frustration, without impairing non-aggressive activities (2,3). This 5-HT receptor subtype received considerable attention when mutant 129/Sv mice lacking the gene for the 5-HT_{1B} receptor were found to display more aggressive behavior (4,5). Among the most useful pharmacological tools employed to stimulate the 5-HT_{1B} receptors are the agonists CP-94,253 and CP-93,129 that have high affinity and selectivity for this receptor subtype (CP-94,253: Ki = 2.0 ± 0.4 nM and CP-93,129: Ki = 8.1 ± 1.6 nM) (6,7). CP-93,129 is the agonist with the highest selectivity for 5-HT_{1B} relative to other 5-HT receptor subtypes, and is most effective when administered centrally due to its poor penetration of the blood-brain barrier (8). Autoradiographic and immunohistochemical studies have localized the 5-HT_{1B} receptor in mesencephalic nuclei, basal ganglia and also in the cortex, including the ventro-orbitofrontal (VO) prefrontal cortex (PFC) (8-10). The PFC contains a considerable number of 5-HT_{1B} receptors (11) and is one of the most important sites for the anti-aggressive effects of the 5-HT_{1B} receptor agonists (12,13).

The current study focuses on the PFC, more specifically the VO area, because of its important role in the inhibitory control of behavior, and in particular in the inhibition of impulsive and aggressive behavior (14-16). Stimulation of 5-HT_{1B} receptors in the VO PFC proved to effectively reduce aggressive behavior in resident male mice confronting an intruder without discernible side effects (12). From a clinical perspective, it is important to study animal aggression that escalates above the species-typical level (2, 3). One experimental approach used to escalate aggressive behavior is to provoke an animal by exposing it to a rival, and this procedure is often referred to as social instigation (17-19). Most individuals engage in aggressive behavior at twice the usual level after being instigated than without such provocation (2,3). Nearly all evidence about 5-HT_{1B} receptors and escalated aggression has been obtained from experiments with males, and there is an urgent need to investigate this neurobiological mechanism in female aggression.

The objective of the current study was to assess the effects of two selective 5-HT_{1B} receptor agonists (CP-94,253 and CP-93,129) microinjected into the VO PFC on maternal aggressive behavior in rats that were socially instigated.

Female Wistar rats (N = 42), born and bred at Universidade do Vale do Rio dos Sinos (UNISINOS) 3-4 months old and weighing 250-350 g were maintained on a 12:12-h light:dark cycle, lights on at 4:00 am. After delivery, each litter was randomly culled to 8 pups. To test aggressive behavior, the experimental female rats confronted male Wistar rats (N = 42), which served as stimulus intruders. Some of these males were used as opponents for the social instigation. On the 3rd postpartum day, females were selected for aggressiveness and only those displaying more than 2 bites against the intruder during a 10-min session were used as subjects. The social instigation procedure was implemented on the 5th postpartum day. The social instigation consisted of placing a clear perforated glass cylinder (28 cm long, 10 cm in diameter) containing an opponent male in the center of the female residents' home cage for 5 min. The residents typically threatened the protected opponent and attacked the perforated glass cylinder. In general, rodents initiate attacks with very short latency and at high frequency when tested with an intruder in their home cage after

having been provoked previously by an opponent (18). Social instigation specifically increases aggression and does not trigger other activities such as feeding, sexual behavior or locomotion (18,19), presumably due to increased "aggressive arousal" or "attack readiness" (17,18). During the social instigation and the behavioral test with the intruder the pups remained inside the cage together with their dams.

On the 6th day postpartum, each female was anesthetized with 100 mg/kg ketamine and 100 mg/kg xylazine intraperitoneally, placed in a stereotaxic frame (David Kopf, Tujunga, CA, USA), and implanted with a guide cannula (22 gauge) fixed with dental cement to the skull. The cannula was aimed at the VO PFC in the right hemisphere: 4.3 mm anterior to the bregma, 0.6 mm lateral to the sagittal line, and 2.1 mm below the dura mater. The parameters were based on the Paxinos and Watson Atlas for Rats. Experiments were performed in accordance with the NIH Guide for Animal Care and Use and were approved by the Research Committee of the University. Females remained separated from the pups for 2 h.

On the 9th postpartum day, the microinjections were followed by the resident-intruder test. The naive male intruder was placed inside the female's cage and immediately thereafter the behaviors were videotaped for 10 min. The behavioral recordings began 15 min after the injection of CP-93,129, CP-94,253 or vehicle. The drugs used in this experiment were donated by Pfizer. CP-93,129, a 5-HT_{1B} receptor agonist (Pfizer, Groton, CT, USA), was diluted in 0.9% saline and CP-94,253 (Pfizer) was diluted in 90% distilled water containing 5% dimethylsulfoxide and 5% Tween 80. Each animal received only one injection per brain area of CP-93,129 (1.0 μg, N = 9) and vehicle (N = 8) or CP-94,253 (0.56 or 1.0 μg, N = 8 for each group) or vehicle (N = 8) and the injection volume was 0.2 μL. The solution was slowly infused using a Hamilton syringe connected by tubing to the injecting needle, that was left *in situ* for 1 additional min after the microinjection.

After the completion of all behavioral tests, the dams were deeply anesthetized with an overdose of sodium thiopental. Brains were perfused with 0.9% saline followed by 10% formaldehyde. The brains were removed and fixed in 4% formaldehyde and later cut into 50-mm coronal slices on a vibratome. The slices were placed on gelatinized slides and stained with cresyl violet. Locations of the cannula tips were determined by microscopy and only data from the animals with the correct location were used.

The frequency of each behavior is reported as mean ± SEM. The effects of CP-93,129 microinjections on the frequency of the behavioral measures were assessed using the Student *t*-test, and those of CP-94,253 by analysis of variance (ANOVA) followed by the *post hoc* Newman-Keuls test. In all cases, the alpha level was set at 0.05.

As shown in Figure 1, microinjection of CP-93,129 (1.0 μg/0.2 μL) significantly reduced the frequency of lateral attacks ($P = 0.005$), lateral threats ($P = 0.01$) and pinnings ($P = 0.02$) relative to saline control. In contrast to the decrease in all offensive behaviors (lateral attack, lateral threat and pinning) by the resident female rats, the concurrently assessed frequency of non-aggressive behaviors such as walking, grooming and pup care was unchanged after CP-93,129 microinjection (Table 1). The duration of sniffing decreased ($P = 0.03$) after microinjection of CP-93,129, and the rearing behavior increased ($P < 0.05$; Table 1).

Neither dose of CP-94,253 (0.56 or 1.0 μg) altered the frequency of any salient aggressive behaviors (lateral attack ($F(2.21) = 0.54$; $P = 0.5$), lateral threat ($F(2.21) = 0.26$, $P = 0.7$), pinning ($F(2.21) = 0.15$; $P = 0.8$) (Figure 1). Furthermore, most of the non-aggressive behaviors (walking, grooming, pup care, sniffing, and rearing) were not affected by microinjections with CP-94,253

(Table 1).

The lack of effect of CP-94,253 microinjections into the VO PFC, at the doses studied, on the aggressive behavior of lactating rats contrasts with the highly effective anti-aggressive effects of 0.56 and 1.0 µg of microinjections into the same brain region in aggressive resident male mice (12). Sys-

Table 1. Duration of non-aggressive behaviors after microinjection of CP-93,129 and CP-94,253.

Non-aggressive behavior	Saline (N = 8)	CP-93,129 (1.0 µg; N = 9)	Vehicle (N = 8)	CP-94,253 (0.56 µg; N = 8)	CP-94,253 (1.0 µg; N = 8)
Walking duration	98.4 ± 14.5	91.6 ± 12.3	68.1 ± 11.3	100.3 ± 17.7	93.8 ± 14.2
Grooming duration	53.3 ± 13.0	37.2 ± 10.8	42.5 ± 16.7	39.3 ± 7.5	25.7 ± 12.3
Pup care duration	6.9 ± 4.1	16.3 ± 10.6	52.2 ± 38.2	10.0 ± 10.0	70.9 ± 2.4
Sniffing duration	170.4 ± 30.7	84.2 ± 20.7*	147.2 ± 21.0	145.8 ± 40.9	158.5 ± 36.0
Rearing duration	11.5 ± 5.5	43.4 ± 14.0*	18.9 ± 6.2	30.4 ± 12.0	12.1 ± 4.5

Data are reported as means ± SEM in seconds.
*P < 0.05 compared to control (Student *t*-test).

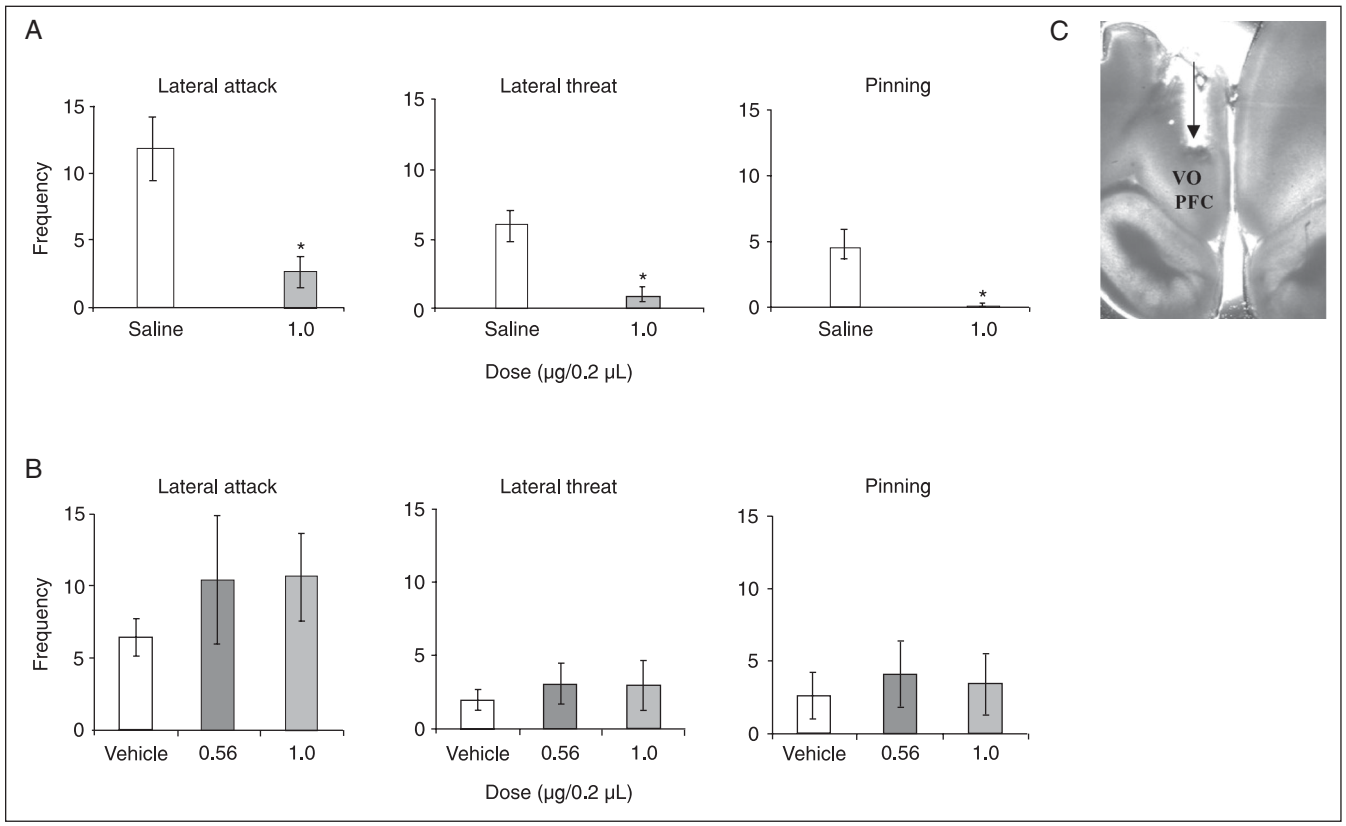

Figure 1. *A*, Effects of CP-93,129, a 5-HT_{1B} receptor agonist, microinjected into the ventro-orbitofrontal prefrontal cortex (VO PFC) at the dose of 1.0 µg/0.2 µL (N = 9) relative to the saline group. *P < 0.05 compared to control (Student *t*-test). *B*, Effects of CP-94,253, a 5-HT_{1B} receptor agonist, and vehicle (N = 8) microinjected into the VO PFC at the dose of 0.56 µg/0.2 µL (N = 8) and 1.0 µg/0.2 µL (N = 8; one-way ANOVA). Data are reported as the mean frequency ± SEM of lateral attack, lateral threat and pinning during a 10-min test against an intruder male by lactating female rats; *C*, Coronal slice from the female brain showing the site of microinjection into the VO PFC. The arrow indicates the trajectory of the cannula.

temic injections of this compound are also very effective in decreasing species-typical aggressive behavior of male mice in the resident-intruder model and in alcohol-heightened aggressive behavior in mice (2) as well as in suppressing the motivation to fight in male mice (13). CP-93,129 and CP-94,253 are highly selective agonists for the 5-HT$_{1B}$ receptor subtype.

CP-93,129 exhibits much higher binding affinities for the 5-HT$_{1B}$ receptor than for the 5-HT$_{1A}$ receptor relative to the 5-propoxy compound CP-94,253 (185-fold higher selectivity for CP-93,129 *vs* 45- to 60-fold higher selectivity for CP-94,253 (7). CP-93,129 is the most selective ligand for the 5-HT$_{1B}$ receptor, but it does not penetrate the blood-brain barrier readily (7). Another aspect to be considered is that female rats were used in the present study. Most of the previous studies with 5-HT$_{1B}$ receptor manipulations used male rodents to test aggressiveness. The female rats studied here were tested on the 9th postpartum day in both experiments with the 5-HT$_{1B}$ receptor agonists, ruling out any endocrine factor as being responsible for the differential effects of the two compounds.

In the current experiments, microinjections of CP-93,129 were very effective, and it is likely that these specific anti-aggressive effects are mediated by the 5-HT$_{1B}$ receptors, although future experiments using antagonists will be needed to confirm this mechanism. Moreover, CP-94,253 is a propoxy derivative of CP-93,129 and consequently a less polar compound, which is able to diffuse more readily throughout the microinjected area than CP-93,129. It may be possible that a higher dose of CP-94,253 have been shown to be effective. The prefrontal cortex contains a considerable number of 5-HT$_{1B}$ receptors (16) and is one of the most important sites for the anti-aggressive effects of the 5-HT$_{1B}$ receptor agonists (12,13). It is important to note that only the offensive components of the female aggressive behavior such as lateral sideways threat, aggressive posture and pinning the intruder were affected by the 5-HT$_{1B}$ receptor agonist, CP-93,129, while the defensive behaviors involved in maternal aggression were not affected. It can be hypothesized that the VO PFC is an area that probably stimulates offensive rather than defensive components of behavior in female rats. CP-93,129 reduces lateral attack, which can be characterized as an offensive element of aggression due to its similarity to male territorial behavior. On the other hand, the frontal attack (jump attack) seems to be an element of defensiveness or fear in the reaction of a female to an intruder. CP-93,129 had no effect on this latter type of behavior.

Confirming previous results obtained with other models of aggression, sex and species, the 5-HT$_{1B}$ receptors in the VO PFC have effects on the aggressive behavior of female rats. CP-93,129 decreased maternal aggression and CP-94,253 had no behavioral effect on postpartum aggression in Wistar rats, in contrast to its reduction of aggressive behavior in males.

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