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ORIGINAL INVESTIGATION

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5-HT_{1B} receptors, ventral orbitofrontal cortex, and aggressive behavior in mice

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Abstract *Rationale:* Systemic injections of 5-HT_{1B} receptor agonists have been shown to have specific anti-aggressive effects in aggressive individuals. One site of action for these drugs is the 5-HT_{1B} receptors in the ventral orbitofrontal cortex (VO PFC), an area that has been implicated in the inhibitory control of behavior and is a terminal region for 5-HT projections. *Objective:* To assess the anti-aggressive effects of the 5-HT_{1B} receptor agonist CP-94,253 when microinjected into the VO PFC (0.1, 0.56, and 1.0 μg/0.2 μl) or into the infralimbic prefrontal cortex (IL PFC; 1.0 μg/0.2 μl) in separate groups of aggressive resident male mice. To confirm the 5-HT_{1B} receptor as the critical site of action for the anti-aggressive effects, the 5-$HT_{1B/D}$ antagonist GR-127,935 was microinjected at 10.0 μg/0.2 μl into the VO PFC. After recovery from surgery, the anti-aggressive effects of microinjected CP-94,253 were studied during 5-min resident–intruder confrontations that were recorded and analyzed. *Results:* Microinjections of CP-94,253 (0.56 and 1.0 μg/0.2 μl) dose-dependently reduced the frequency of attack bites and sideways threats. This effect was behaviorally specific because non-aggressive motor activities were not significantly altered by the drug. In the IL vmPFC or in an area lateral to the VO PFC, CP-94,253 (1.0 μg/0.2 μl) did not have significant behavioral effects. *Conclusions:* The results highlight the 5-HT_{1B} receptors in the VO PFC as a particularly important site for the inhibition of species-typical aggressive behavior in male mice.

Keywords 5-HT receptors · Aggressive behavior · Male · Microinjection · CP-94,253 · GR-127,935 · Infralimbic cortex · Prefrontal cortex

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Introduction

When activated systemically, 5-HT_{1B} receptors exert an inhibitory role on several types of aggressive behavior in mice (for reviews, see Miczek et al. 2002; de Almeida et al. 2005). The 5-HT_{1B} receptor agonists such as CP-94,253, anpirtoline, and zolmitriptan administered systemically have anti-aggressive effects in individuals with moderate as well as high levels of aggression without impairing non-aggressive activities (Fish et al. 1999; de Almeida et al. 2001b; de Almeida and Miczek 2002). Further support for the significant role of this receptor subtype derives from the finding of increased aggression in mutant 129Sv mice lacking the 5-HT_{1B} receptor gene (Saudou et al. 1994; but see Bouwknecht et al. 2001 for a review).

The mouse and rat 5-HT_{1B} receptor is functionally homologous to the human 5-HT_{1B} receptor, differing by a single amino-acid (asparagine vs theonine) in the seventh transmembrane domain of the receptor (Schlicker et al. 1997; Sari 2004). The receptors are coupled to $G_{i/o}$ proteins and are located both pre- and postsynaptically in several

brain areas, with high density in areas such as the basal ganglia, striatum, and the frontal cortex, as well as in the raphe nuclei where they may act as somatodendritic autoreceptors (Boschert et al. 1994; Davidson and Stamford 1995; for a review, see Sari 2004). Activation of presynaptic 5-HT_{1B} receptors inhibits 5-HT release and decreases extracellular concentrations of 5-HT in the cortex, ventral hippocampus, striatum, and diencephalon in rats and primates (Engel et al. 1986; Hoyer and Middlemiss 1989; Hjörth and Sharp 1991; Chopin et al. 1994; Martin and Humphrey 1994; Rollema et al. 1996; Bonaventure et al. 1997; Roberts et al. 1997; Knobelman et al. 2000, for a review, see Sari 2004). Recently, in vivo microdialysis data confirmed a 30–40% suppression of extracellular 5-HT in mouse medial PFC after systemic CP-94,253 administration of an anti-aggressive 10 mg/kg dose (Faccidomo et al. 2005). CP-94,253 exhibits significantly greater binding affinity for the 5-HT_{1B} receptors (Ki=2.0±0.4 nM) than for other serotonergic receptor subtypes (Koe et al. 1992; Koe and Lebel 1995). This compound represents a useful tool for characterizing the role of 5-HT_{1B} receptors in specific terminal regions in the regulation of aggressive behavior.

Administration of this agonist inhibits parental defense and predatory attacks, and it plays a specific inhibitory role in rodent aggression (Bell et al. 1995). Blockade of the CP-94,253 effects with the 5-$HT_{1B/D}$ receptor antagonist GR-127,935 points to the 5-HT_{1B} receptors as the relevant site of action for anti-aggressive, antidepressant, locomotor stimulating, and anxiolytic-like effects (O'Neill et al. 1997; Parsons et al. 1998; Fletcher and Korth 1999; Knobelman et al. 2000; Millan et al. 2002). The 5-HT_{1B} receptors appear to be particularly relevant to aggressive behavior, as agonists at this site reduce aggression without impairing locomotor behavior in mice and rats (Olivier et al. 1989a,b; Sijbesma et al. 1991; Sanchez et al. 1993; Fish et al. 1999; for a review, see Olivier and van Oorschot 2005). The selective agonist of the 5-HT_{1B} receptor, CP-94,253, has proven to be very effective in reducing intensely high levels of aggression engendered by administration of alcohol or social instigation in mice (Fish et al. 1999; de Almeida et al. 2001a,b). Anpirtoline, another piperidine derivative with 5-HT_{1B} agonist effects, also decreases alcohol-heightened aggression in mice without compromising motor functions (Miczek and de Almeida 2001).

Higher doses of CP-94,253 can also produce locomotor stimulating effects in different behavioral tests that do not involve aggression (O'Neill et al. 1997; Parsons et al. 1998; Fletcher and Korth 1999; Knobelman et al. 2000; Millan et al. 2002; Fish et al. 2000). The 5-HT_{1B} receptors are thus of potential interest in the treatment of aggressive outbursts in the context of depression, schizophrenia, Parkinson's disease, and impulsive disorders (Boulenguez et al. 1998; Moret and Briley 2000; Audinot et al. 2001; Millan et al. 2002).

The prefrontal cortex contains a high density of 5-HT_{1B} receptors, and it is one of the most important sites of action for these drugs. The ventral orbitofrontal cortex (VO PFC) has been implicated in the inhibitory control of conditioned behavior (Chudasama and Robbins 2003) and is related to a range of affective and motivated behaviors in rodents, primates, and humans (Morgan and LeDoux 1995; Wall and Messier 2000; Wall et al. 2003). There is evidence showing regional differences between the dorsal and ventral [ventral prelimbic and infralimbic, ventromedial orbital] areas of the PFC in the control of cognitive, affective, and autonomic processing. For example, Chudasama and Robbins (2003) found that the VO and the IL PFC mediate dissociable but complementary processes in associative and inhibitory control over classically conditioned discriminative behavior. There is considerable further evidence that rodents possess well-defined subregions such as prelimbic, infralimbic, and orbitofrontal cortices (McAlonan and Brown 2003, Morgan et al. 2003), each with different cognitive, affective, and behavioral functions (Bechara et al. 2000; Kolb and Robbins 2003).

One strategy for evaluating the role of 5-HT_{1B} receptors is to microinject a relatively selective agonist into specific VO PFC or IL PFC regions; another is to investigate differential contributions of these receptor pools to the control of aggressive behavior. To confirm the 5-HT_{1B} receptor as a critical site of action for the anti-aggressive effects, a 5-$HT_{1B/D}$ antagonist GR-127,935 was microinjected into the ventral orbital prefrontal cortex.

Materials and methods

Animals

Adult male CF1 mice (FEPPS, Porto Alegre, RS, Brazil), weighing ca. 25 g on arrival, were housed in clear polycarbonate cages (28×17×14 cm) with wood chip bedding and wire lids through which rodent chow and water were available without restriction. Male mice (n=85) were housed as "residents" in pairs with females of the same strain. Additional male mice (n=90) were housed in groups of ten and served as "intruder" mice. The subjects were allowed to acclimate to the laboratory environment for 7 days with free access to food and water. All mice were housed in an environmentally controlled room maintained at 22±1°C on a 12:12 h photo cycle, with light on at 4 a.m. The animals were tested in the light phase of the photo cycle from 1 to 4 p.m. twice a week, each test separated by at least 72 h.

Surgery and histological analysis

After anesthesia (ketamine 100 mg/kg and xylazine 10 mg/kg body weight intramuscular), male mice (n=85) were placed in a stereotaxic apparatus with a mouse frame adaptor (David Kopf; Tujunga, CA, USA). The animals were implanted with a unilateral guide cannula (27 gauge) in the left hemisphere, fixed to the skull with dental cement. The coordinates for the ventral orbital prefrontal cortex were as follows: 2.2 mm anterior to bregma, 0.8 mm lateral to the midsagittal line, and 1.0 mm below dura mater; and for the

infralimbic prefrontal cortex: 1.42 mm anterior to bregma, 0.6 mm lateral to the midsagittal line, and 1.0 mm below dura mater. The skull position was adjusted so that bregma and lambda were at the same level.

At the end of the experiment, all mice received an overdose of anesthetics. Brains were perfused with 0.9% saline solution and then with 10% formaldehyde. The brains were removed and fixed in 10% formaldehyde and later cut into 100 μm coronal sections on a vibratome. The slices were stained with Cresyl violet, and placements of the cannula tracks and tips were examined by microscopy to identify animals with an exact localization.

Drugs and injections

CP-94,253 (3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxy-pirolo[3,2-b]pyridine; Charles Pfizer, Groton, CT, USA) was dissolved with the aid of sonication in a vehicle of 5% Tween 80, 5% dimethyl sulfoxide, and 90% distilled water. GR-127,935 (*N*-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2-methyl-4′-(methyl-1,2,4-oxadiozol-3-yl)-[1,1-biphenyl]-4-carboxamide; Glaxo Research and Development, London, England) was suspended in 10% hydroxypropyl-betacyclodextrin and sonicated.

The injection needle (30 gauge) was 1.0 mm longer than the guide cannula, which was fixed 1.0 mm dorsal to the target areas. Therefore, only the inner cannula penetrated into the tissue. The solution was slowly infused over a 60-s period, using a Hamilton syringe connected by polyethylene tubing to the injection needle which stayed in situ for a further minute after the injection to allow drug diffusion. Animals were randomly assigned to be injected into the different brain regions with CP-94,253, GR-127,935, or vehicle.

One dose of CP-94,253 (0.1, 0.3, or 1.0 μg/kg for VO PFC and 1.0 μg/kg for IL PFC) or vehicle was administered in a counterbalanced sequence 10 min before the confrontation with the intruder. In the agonist dose–effect experiment, each animal received one drug treatment and one corresponding vehicle.

GR-127,935 (10.0 μg/kg) was administered 30 min before CP-94,253 (1, 3.0, 10.0 μg/kg), and all tests occurred 10 min after the administration of CP-94,253. Each dose combination was administered to separate groups of animals, and each drug test was accompanied by a vehicle test. In the antagonism experiment, the animals received maximally two microinjections, consisting of CP-94,253 and GR-127,935 on one occasion and the corresponding vehicles on the other.

Four cohorts of mice were studied as follows:

1. CP-94,253 dose–effect determination into VO PFC. Four groups of seven mice each (n=28) were used to determine the dose-dependent effects of CP-94,253 (0.1, 0.56, or 1.0 μg/0.2 μl) or vehicle on aggressive behavior into the VO PFC.
2. CP 94,253 dose–effect determination and antagonism by GR-127,935 into VO PFC. Fourty-five additional mice were used to determine the effects of GR-127,935 (10.0 μg/0.2 μl) on the dose–effect curve of CP-94,253 (1.0, 3.0, or 10.0 μg/0.2 μl) on aggressive behavior into the VO PFC. Specifically, the mice were assigned as follows:; for vehicle, n=8; GR-127,935 at the dose of 10.0 μg/0.2 μl, n=8; Vehicle + vehicle, n=8; GR-127,935 10.0 μg/0.2 μl + CP-94,253 1.0 μg/0.2 μl, n=7; GR-127,935 10.0 μg/0.2 μl + CP-94,253 3.0 μg/0.2 μl, n=6; and GR-127,935 10.0 μg/0.2 μl + CP-94,253 10.0 μg/0.2 μl, n=8.
3. CP-94,253 into the IL PFC. Six more mice were used to assess the effects of CP-94,253 (1.0 μg/0.2 μl) and the corresponding vehicle on aggressive behavior into the IL PFC.
4. For the microinjection experiment with CP-94,253 (1.0 μg/0.2 μl) into an area, more lateral to the VO PFC 6 mice were used.

Behavioral analysis

After 72 h of recovery from surgery, the effects of microinjected CP-94,253 and GR-127,935 were studied in male CF1 mice during resident–intruder confrontations. The behavioral recording began 30 min after the microinjection of GR127935 and 10 min after vehicle or CP-94,253 microinjections. The male intruder was placed in the resident's cage, and their behaviors were videotaped (Miczek and O'Donnell 1978). An investigator with proven reliability analyzed the videotapes at a later time. Each behavioral sample lasted for exactly 5 min commencing with the resident's first attack bite (i.e., the pre-attack data were not included).

The analysis of aggressive behavior comprised the measurement of salient acts and postures such as anogenital contact and sniffing the intruder, pursuit, sideways threat, bite, and tail rattle, as well as non-aggressive elements such as grooming, walking, and rearing as previously defined and illustrated (Miczek and O'Donnell 1978). Frequency and duration of all behaviors were recorded. Inter- and intra-observer reliability for encoding these behaviors was calculated using the Spearman correlation coefficient and ranged from 0.87 for the duration of walking to 0.95 for the frequency of attack bites.

Data analysis

All data for the experiments on the dose–effect determination of CP-94,253 and on the antagonism with GR-127,935 on aggression were analyzed using a one-way analysis of variance (One-Way ANOVA), and when appropriate (α<0.05), Bonferroni post hoc t tests were used with vehicle as the common control. The effect of GR-127,935 alone as compared with vehicle was analyzed using a paired t test. ED_{50}s were calculated based on linear regression of the dose–effect curve, and 95% confidence intervals were calculated based on linear regression of the confidence intervals of the individual doses.

Results

The histological analysis showed that 73 mice out of the total 85 animals had their cannula correctly placed in the VO PFC (Fig. 1), and six mice had their cannula correctly placed in the IL PFC (Fig. 2). An anatomical control group (n=6) was included in this experiment, comprised of animals with placements in a region more lateral to the VO PFC.

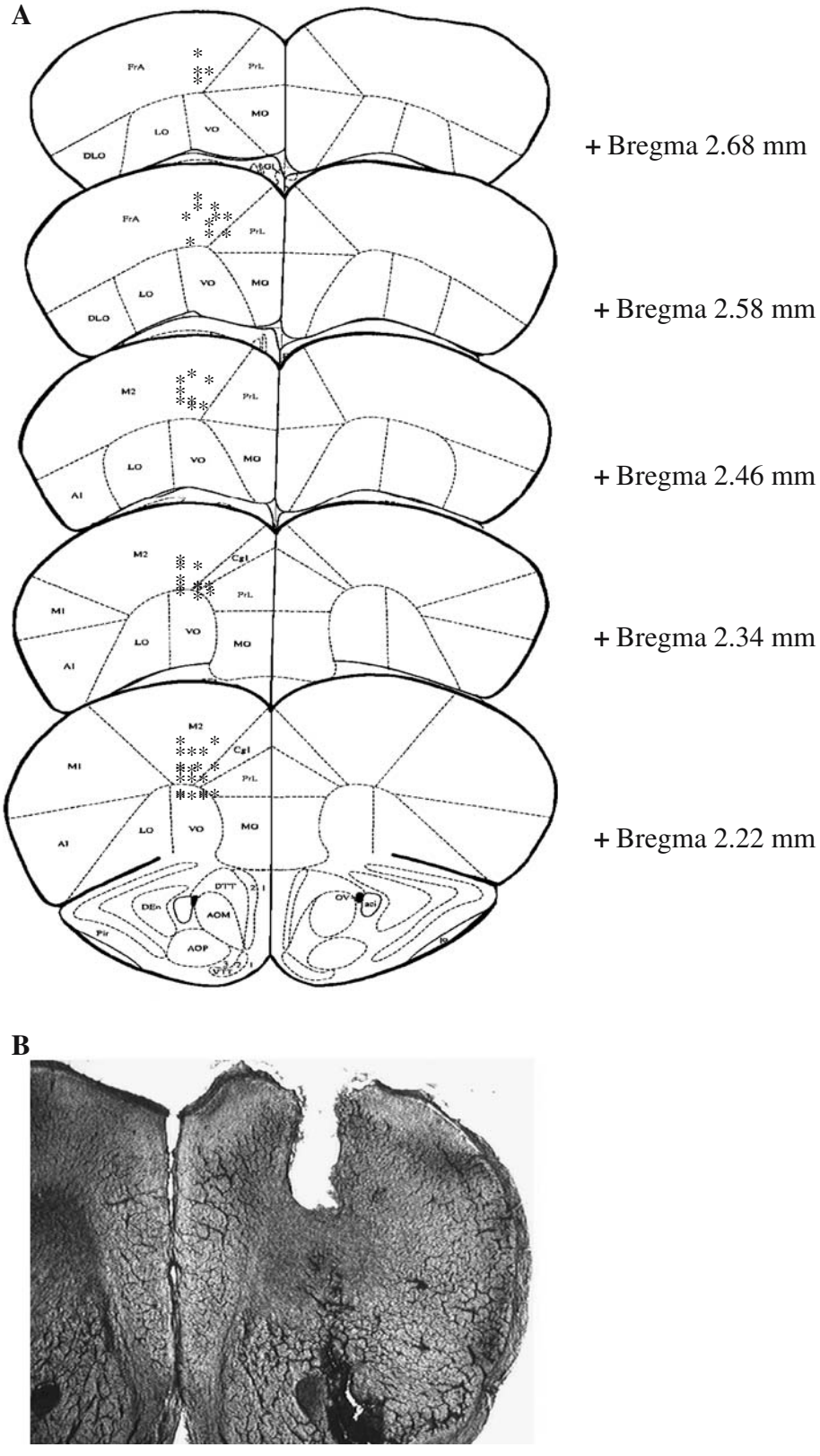

Fig. 1 **a** Schematic representation of successive coronal sections (rostral to caudal) of the mouse brain (n=73) showing the histological verification of cannula placements in the ventral orbital prefrontal cortex. * represents the site of the cannula tip. VO (ventro orbital cortex): 2.68; 2.58; 2.46; 2.34, and 2.22 mm anterior to the bregma. *Cg1* Cingulate cortex, area 1, *PrL* prelimbic cortex, *MO* medial orbital cortex, *LO* lateral orbital cortex. Images from Paxinos and Franklin 2001. **b** Photomicrograph of histological placement in the ventral orbital frontal cortex

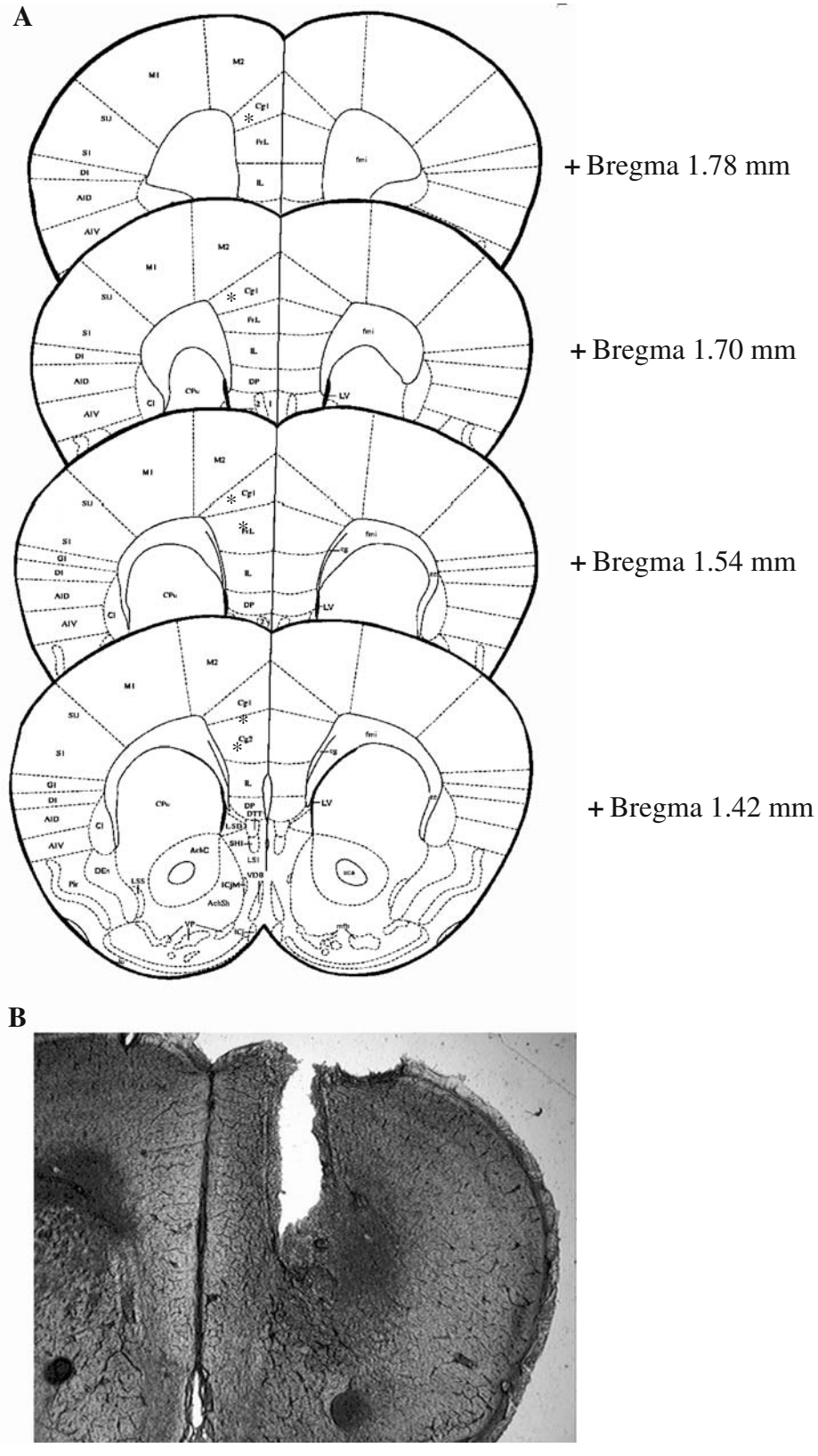

Fig. 2 **a** Schematic representation of successive coronal sections (rostral to caudal) of the mouse brain (n=6) showing the histological verification of cannula placements in infralimbic PFC. * represents the site of the cannula tip. IL: (infralimbic cortex): 1.78, 1.70, 1.54, and 1.42 mm anterior to the bregma. *Cg1* cingulate cortex, area 1, *M2* secondary motor cortex; *PrL* prelimbic cortex. Images from Paxinos and Franklin 2001. **b** Photomicrograph of histological placement in the infralimbic prefrontal cortex

Behavioral effects of CP-94,253 into ventral orbitofrontal prefrontal cortex

CP-94,253 exerted significant anti-aggressive effects when injected into the VO PFC. Specifically, CP-94,253 (0.56 and 1.0 μg/0.2 μl) reduced the frequency of attack bites [$F(3,24)=9.889$, $p<0.001$; Fig. 3a) and sideways threats [$F(3,24)=16.877$, $p<0.001$; Fig. 3b). The ED_{50} for the anti-aggressive effects of CP-94,253 (0.1–1.0 μg/0.2 μl) was 0.42 μg (CI_{95}=0.21, 0.91). Microinjections of 1 μg CP-94,253 into the far lateral VO PFC (n=6) did not alter any of the salient elements of aggressive behavior (Table 2).

Non-aggressive motor activities such as walking, rearing, and grooming were not significantly altered by CP-94,253 in the presently studied dose range in either the VO PFC (Table 1) or the far lateral VO PFC (Table 2).

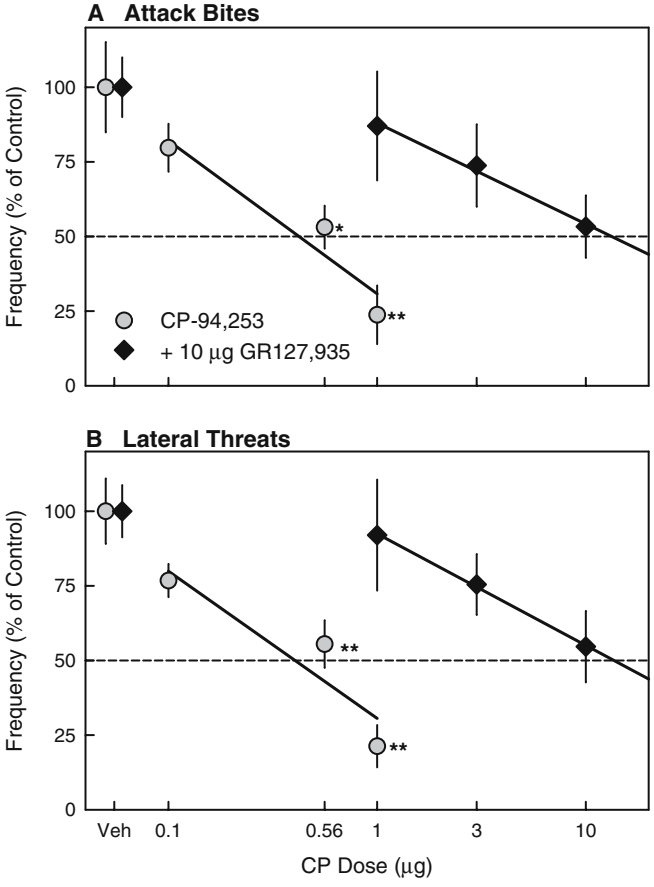

Fig. 3 Effects of CP-94,253 on the frequency of attack bites (**a**) and lateral threat postures (**b**) by resident male mice. CP-94,253 was microinjected into VO PFC either alone (*circles*) or 30 min after microinjection of 10.0 μg GR-127,935 (*diamonds*). Data points are means±SEM, with *$p<0.05$ and **$p<0.01$ compared to vehicle. Correlation coefficients (r^2) for the linear regression lines ranged from 0.839 to 0.998

Behavioral effects of CP-94,253 into infralimbic prefrontal cortex

No significant effects on aggressive or non-aggressive behaviors were found after the microinjection of 1.0 μg CP-94,253 into the IL PFC (Fig. 4) as compared to the vehicle group (Table 2).

Effects of GR-127935 on aggression

No significant effects on aggressive and non-aggressive behaviors were found after microinjection of GR-127,935 (10.0 μg/0.2 μl) or after vehicle injections.

Effects of CP-94,253 on aggression: antagonism by GR-127,935

Pretreatment with GR-127,935 antagonized the reduction of aggressive behaviors such as frequency of bites and sideways threats (Fig. 3). After pretreatment with GR-127,935, the ED_{50} for the anti-aggressive effects of CP-94,253 was shifted from 0.42 to 13.33 μg (CI_{95}=1.40–31.33).

Discussion

The key finding of the present studies is that the 5-HT_{1B} receptor agonist CP-94,253 microinjected directly into the prefrontal cortex, particularly into the ventral orbital area, decreases aggression in male mice in a pharmacologically and behaviorally specific manner. When injected into the VO PFC, CP-94,253 (0.56 and 1.0 μg/0.2 μl) significantly reduced the frequency of attack bites and sideways threats

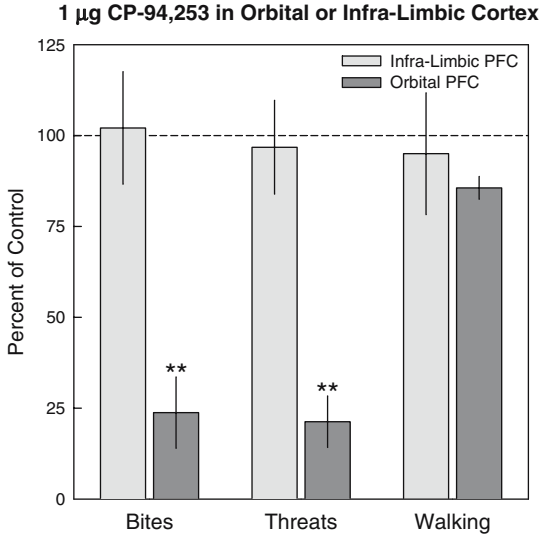

Fig. 4 Effects of 1.0 μg CP-94,253 microinjected into infralimbic or orbital PFC on the frequency of attack bites and lateral threat postures, and on walking duration. *Bars* show means±SEM, with **$p<0.01$ compared to vehicle control in the same target site

Table 1 Behavioral effects of CP-94,253 microinjected into VO prefrontal cortex

		Frequencies				Durations (seconds)			
CP-94,253 dose		Vehicle	1.0 μg	3.0 μg	10.0 μg	Vehicle	1.0 μg	3.0 μg	10.0 μg
Grooming	Mean	19.29	12.14	15.43	14.29	28.00	25.43	21.14	26.14
	SEM	3.83	2.33	2.41	2.79	4.48	4.64	3.88	8.17
Rearing	Mean	33.14	22.71	22.29	8.29*	32.00	34.00	31.71	24.86
	SEM	5.47	3.21	1.69	1.57	5.02	7.30	4.89	4.98
Walking	Mean	64.71	63.57	66.71	44.00*	98.29	99.14	90.43	84.14
	SEM	6.37	4.22	5.48	4.61	5.62	7.18	7.70	3.08
Sniffing	Mean	22.29	21.14	13.57	18.43	25.14	7.57	21.43	22.29
	SEM	2.74	2.93	2.48	2.81	4.23	2.78	8.38	7.81

*$p<0.05$

and did not have significant effects on the non-aggressive behavioral elements. The decrease in aggression can be considered as behaviorally specific because the non-aggressive motor activities were not significantly altered by the drug. By contrast, when injected into the IL PFC, CP-94,253 (1.0 μg/0.2 μl) did not have significant behavioral effects on either species-typical aggression or on the non-aggressive behavioral elements. Similarly, microinjections into the region lateral of the VO PFC at the same 1.0 μg dose remained without significant behavioral effects.

Evidence for the 5-HT_{1B} receptor as an important site of action for CP-94,253 originates from the observation that administration of GR-127,935, an antagonist with high affinity (ca. 10 nM) for 5-$HT_{1B/D}$ receptors (Skingle et al. 1996), produced a rightward shift of the dose–effect curve for CP-94,253. Systemic injections of GR-127,935 have also been shown to antagonize the effects of 5-HT_{1B} agonists on various behaviors such as locomotor activity, alcohol intake, drug reinforcement, and aggressive behavior (Maurel et al. 1998; Parsons et al. 1998; Fish et al. 1999; Castanon et al. 2000; Tomkins and O'Neill 2000; de Almeida et al. 2001b; Miczek and de Almeida 2001; Fletcher et al. 2002).

The 5-HT_{1B} receptors have been implicated in several physiological functions and behavioral and psychiatric disturbances including migraine, locomotor hyperactivity, drug reinforcement, depression and anxiety states, in addition to aggressive behavior (for a review, see Sari 2004). For example, stimulation of 5-HT_{1B} receptors can result in a suppression of migraine (Humphrey et al. 1991), an increase in locomotor activity (Rempel et al. 1993; Scearce-Levie et al. 1999), increased satiety (Lee and Simansky 1997), a decrease of anxiety-like behavior, increased antidepressant effects (Lucas et al. 1997; Matzen et al. 2000) as well as enhancement of some of the reinforcing effects of cocaine (Parsons et al. 1996;1999) but a decrease in alcohol intake (Maurel et al. 1999; Tomkins and O'Neill 2000). It appears that this receptor subtype represents an important target for several potential therapeutic interventions, and it remains to be discovered whether the mechanisms for the anti-aggressive effects can

Table 2 Behavioral effects of CP-94,253 microinjected into infralateral (IL) PFC or lateral ventral orbitofrontal cortex (LVOC)

Site		IL PFC		IL PFC		LVOC	
Dose		Vehicle		1.0 μg		1.0 μg	
		Frequency	Duration (seconds)	Frequency	Duration (seconds)	Frequency	Duration (seconds)
Bites	Mean	18.2		17.3		15.0	
	SEM	2.1		2.1		1.8	
Threats	Mean	19.7	13.8	17.8	10.2	15.1	12.5
	SEM	2.4	2.2	1.8	4.9	1.3	1.1
Tail rattle	Mean	17.0	56.3	17.8	30.2	13.8	17.0
	SEM	2.5	6.3	3.4	6.1	1.9	5.9
Grooming	Mean	11.7	27.8	15.8	33.2	9.1	23.6
	SEM	2.7	5.9	3.9	7.8	2.4	2.7
Rearing	Mean	13.8	21.3	19.8	28.7	17.1	31.0
	SEM	4.0	5.7	4.7	10.3	2.1	4.6
Walking	Mean	49.8	108.8	51.5	103.2	39.3	93.3
	SEM	9.6	11.1	10.4	18.7	7.3	11.3
Sniffing	Mean	16.5	46.5	19.0	46.0	18.7	26.3
	SEM	2.8	8.8	1.5	10.7	2.4	3.6

be dissociated from those on other behavioral and physiological functions.

Several pharmacological studies have shown that the activation of 5-HT_{1B} receptors reduces aggression in male mice and rats after systemic injections (Olivier et al. 1989a,b; Sanchez et al. 1993; Mos et al. 1992, 1993; Fish et al. 1999; de Almeida et al. 2001b; Bannai et al. 2006). A nonselective agonist eltoprazine, acting on 5-$HT_{1A/1B/2C}$ receptors, decreases aggression by resident male rats toward an intruder (Schipper et al. 1990). CGS 12066B, CP-94,253, and other 5-HT_{1B} receptor agonists such as anpirtoline and zolmitriptan also reduce aggression in male mice (Bell et al. 1995; Fish et al. 1999; Miczek and de Almeida 2001; de Almeida et al. 2001a,b).

CP-94,253 is one of the most selective agonists for 5-HT_{1B} receptors (Ki=2.0±0.4 nM; Koe et al. 1992; Koe and Lebel 1995). When microinjected into neural areas which contain a high density of 5-HT_{1B} receptors such as the frontal cortex (Bruinvels et al. 1993; Sari et al. 1999; Millan et al. 2002), CP-94,253 decreases aggressive behavior by modulating serotonergic transmission either at presynaptic or postsynaptic receptors.

Increased levels of serotonin (5-HT) are often related to a decrease in aggression in individuals with a high aggressive trait (Chiavegatto and Nelson 2003; Mehlman et al. 1994; Higley et al. 1996; Ferrari et al. 2005; de Almeida et al. 2005). For example, SSRIs increase brain 5-HT and exert anti-aggressive effects (Olivier et al. 1989a; Ferris and Delville 1994). These data are interesting and suggestive but at the same time limited because they do not provide sufficient information on the time course and anatomical specificity of the precise serotonergic changes that are linked to aggressive behavior. Contrary to the serotonin deficiency hypothesis, it remains to be resolved how agonists at 5-HT_{1A} and 5-HT_{1B} receptors decrease serotonin in corticolimbic terminals via action at somatodendritic or presynaptic sites, at the same time significantly decrease aggressive behavior (de Almeida and Lucion 1997; Miczek et al. 1998; Bannai et al. 2006; de Boer and Koolhaas 2005). There are only a few studies that try to establish a parallel between specific brain areas rich in 5-HT_{1B} receptors and changes in aggressive behavior upon activation of a discrete receptor pool (e.g., Faccidomo and Miczek 2004; Faccidomo et al. 2005).

5,7-DHT neurotoxic lesions of the raphe nuclei do not appear to prevent the anti-aggressive effects of CP-94,253 when injected systemically (de Almeida et al. 2001b). These data suggest that the effects of CP-94,253 originate from action on 5-HT_{1B} receptors that are localized postsynaptically because destruction of the 5-HT containing neurons in the dorsal raphe did not alter the effects of CP-94,253. However, because the neurotoxic lesions were only partial, a presynaptic site of action for CP-94,253 cannot be excluded.

Recently, the prefrontal cortex, more specifically the orbitofrontal region, has been assigned an important role in the inhibitory control of behavior, mainly impulsive and aggressive behavior (Blair 2001; Seguin 2004; Cardinal et al. 2004; Spinella 2004; Kheramin et al. 2005). The infralimbic area has been involved more in anxiety-like effects and cognitive functions such as attention and memory than in aggressive behavior (Wall and Messier 2002; Dalley et al. 2004). Faccidomo et al. (2005) found a 30% decrease of 5-HT in the infralimbic area after intraperitoneal CP-94,253 in male mice. Moreover, it is important to point out that the samples were collected from single mice in the absence of aggressive behavior. Fighting itself can lower levels of 5-HT in prefrontal cortex of resident rats (van Erp and Miczek 2000), and the lowered tonic activity of 5-HT may influence how 5-HT_{1B} agonists achieve their effects.

The present results highlight the 5-HT_{1B} receptors in the ventral orbital prefrontal cortex as a particularly important site for the inhibition of species-typical aggressive behavior. Future studies will have to determine whether this effect is achieved via pre- or postsynaptic 5-HT_{1B} receptor pools. Ongoing work assesses the role of more specific 5-HT_{1B} agonists and antagonists microinjected into amygdaloid nuclei, striatum, hypothalamic nuclei, dorsal periaqueductal area, and raphe nuclei to delineate the serotonergic circuit mediating male aggression.

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