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Aggression Escalated by Social Instigation or by Discontinuation of Reinforcement ("Frustration") in Mice: Inhibition by Anpirtoline: A 5-HT_{1B} Receptor Agonist

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Experiments with social instigation or the omission of scheduled reinforcement show that serotonergic mechanisms may be involved in escalated aggression in animals. 5-HT_{1B} receptor agonists have anti-aggressive effects in individuals who show moderate as well as high levels of aggression. The present study compared the effects of the 5-HT_{1B} agonist anpirtoline (0.125–1.5 mg/kg) on (1) species-typical aggressive behavior in male mice, (2) aggression "instigated" or primed by prior exposure to the opponent, and (3) aggression heightened by "frustration" caused by omission of scheduled reinforcement. The effects of anpirtoline on species-typical behavior were also assessed after pretreatment with the 5-$HT_{1B/1D}$ receptor antagonist GR127935 (10 mg/kg). Anpirtoline, like other 5-HT_{1B} agonists (CP-94,253, zolmitriptan), decreased both instigated and frustration-heightened aggression, while motor behavior was unaffected. The aggression-inhibiting effects of anpirtoline were blocked by pretreatment with GR127935. The current results indicate that the 5-HT_{1B} receptor is critically involved in the modulation of escalated aggression.

[Neuropsychopharmacology 27:171–181, 2002]

KEY WORDS: *5-HT receptors; Aggression; Anpirtoline; Social instigation; Serotonin; Frustration; GR127935; Reinforcement; Sucrose; Impulsivity*

The development of valid experimental protocols that model clinically relevant, excessive levels of aggressive behavior is a continuing challenge for pre-clinical research on aggression (Miczek 2001). The leading neurobiological hypothesis attributes such intense aggressive behavior, often related to expressions of impulsivity, to a deficit in brain serotonin (Linnoila et al. 1983; Virkkunen et al. 1989; Coccaro 1989). An early example of this research strategy was the demonstration that mice would exhibit intense aggressive behavior after prolonged isolated housing, and this isolation-induced aggression is associated with lower serotonin turnover in the brainstem (Garattini et al. 1967). Long-standing concerns with the validity and reliability of isolation-induced aggression and its limited species generality prompt the search for alternate approaches (Brain 1975; Krsiak 1975). Similarly, correlation of aggressive behavior with a single tissue or CSF measurement of serotonin or its metabolite need to be reconciled with the anti-aggressive effects of agonist

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Received August 6, 2001; revised December 18, 2001; accepted January 17, 2002.

Online publication: 1/21/02 at www.acnp.org/citations/Npp012102230.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/02/$–see front matter
PII S0893-133X(02)00291-9

and antagonist treatment of specific serotonin receptor subtypes (Olivier et al. 1995; de Almeida et al. 2001).

The exposure of an experimental subject to a potential rival for a short time prior to the actual confrontation engenders intense levels of aggression, as originally described in mice (Lagerspetz 1969; Tellegen and Horn 1972). For example, mice, rats, and hamsters initiate attacks with very short latency and at high frequency when tested with an intruder in their home cage or in an unfamiliar locale after having been provoked previously by an opponent (Potegal 1992; Fish et al. 1999). Instigation or priming is specific for increasing aggressive behavior and does not activate locomotion, feeding, or sexual behavior (Lagerspetz and Hautojarvi 1967; Potegal and Tenbrink 1984; Potegal 1992). Even after removal of the instigating or priming stimulus, high levels of aggression persist in fish and rodents, presumably from increased "aggressive arousal" or "attack readiness" (Heiligenberg 1974; Potegal and Tenbrink 1984; Potegal 1992). At the neurochemical level, animals that have been instigated to fight are characterized by a long-lasting decrease in serotonin in hypothalamus and in medial prefrontal cortex (Payne et al. 1984; van Erp and Miczek 2000).

An exceptionally efficient approach to amplify aggression uses "frustrative non-reward" as the motivational process, operationally defined by omission or discontinuation of scheduled reinforcement (see Dollard et al. 1939; Amsel and Roussel 1952; Azrin et al. 1966; Thompson and Bloom 1966; Kelly 1974). Bursts of aggression can be observed after discontinuation of scheduled rewards in many different species such as fish, birds, rodents, monkeys, and humans (Thompson and Bloom 1966; Cherek and Pickens 1970; Caprara 1982; Evenden and Ryan 1996). For example, Azrin et al. (1966) used a procedure to quantify a pigeon's attack toward an immobilized target bird after the experimental subject's food-reinforced key pecking was extinguished. Individuals differ in the way they tolerate discontinuation of scheduled reinforcement, and an individual's "frustration tolerance" is postulated to be reflected in varying amounts of aggression (Cherek and Pickens 1970). The present experiments attempt to study extinction-induced aggression in mice, a species that has become of considerable interest because of developments in molecular genetics (Miczek et al. 2001). Whether or not heightened aggression resulting from discontinued reinforcement is characterized by a distinctive profile of serotonergic activity remains to be explored.

Activation and antagonism of several 5-HT receptor subtypes in the 5-HT_1 and 5-HT_2 families suppress aggressive behavior (Sanchez et al. 1993; de Almeida and Lucion 1994; Olivier et al. 1995; de Almeida and Lucion 1997; Miczek et al. 1998; de Almeida et al. 2001). Experimental deletion of the gene coding for the 5-HT_{1B} receptor as well as pharmacological studies implicate this receptor subtype in aggressive behavior (Saudou et al. 1994; Mos et al. 1992). Agonists at postsynaptic 5-HT_{1B} receptors, such as CP-94,253, zolmitriptan, and anpirtoline, produce remarkably specific inhibition of species-typical aggression and very intense and frequent aggressive behavior at doses that do not alter the remaining behavioral repertoire (Fish et al. 1999; de Almeida et al. 2001; Miczek and de Almeida 2001). At higher 5-HT_{1B} receptor agonist doses sexual and alimentary behavior is suppressed and motor behavior stimulated (Hillegaart and Ahlenius 1998; Lee et al. 1998; Stenfors et al. 2000). Anpirtoline, a 5-HT_{1B} agonist, has been shown to have a high affinity for 5-HT_{1B} receptors (K(i)=28 nM) (Schlicker et al. 1992; Swedberg et al. 1992).

In order to assess the anti-aggressive effects of the 5-HT_{1B} receptor agonist anpirtoline, we compared its effects on the species-typical display of aggressive behavior in mice to those on heightened levels of aggression caused by instigation by a male opponent ("social instigation-induced aggression") or discontinuation of scheduled reinforcement ("frustration-induced aggression"). Based on studies with 5-HT_{1B} receptor agonists such as CP 94,235 and zolmitriptan, anpirtoline was predicted to dose-dependently reverse the behavioral enhancement of aggression in both studies. Furthermore, we postulate that these anti-aggressive effects of anpirtoline are caused by the activation on 5-HT_{1B} receptors as the anpirtoline effects were competitively antagonized by pretreatment with the 5-$HT_{1B/D}$ receptor antagonist GR127935.

MATERIALS AND METHODS

Animals

Adult male CFW mice (Charles River Laboratories, Wilmington, MA), weighing between 22–25 g on arrival, were housed as "residents" with females of the same strain in clear polycarbonate cages (28 ×17 ×14 cm). The floor of each cage was covered with wood chip bedding, and Purina rodent chow and water were freely available through the wire lid. Additional male CFW mice (n = 40) were housed in groups of 10 and served as stimulus animals. The subjects were allowed to acclimate to the laboratory environment for 7 days. All mice were housed in a room with controlled 22 ± 1°C temperature and 30–40% humidity on a 12:12 h photocycle. All procedures followed the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and were approved by the Tufts University Animal Care and Use Committee.

Apparatus and Measurements

The behavior of the mice was recorded on videotape, using a low-lux video camera and a standard videocassette recorder. Aggressive and non-aggressive behav-

iors were measured by a trained observer using a custom-designed data acquisition system similar to that described earlier (Miczek 1982). The aggressive behaviors were defined and illustrated previously (Miczek and Donnell 1978) and included anogenital contact with the intruder, pursuit, sideways threat, bite, and tail rattle, as well as non-aggressive behaviors: grooming, walking, contact, and rearing. Inter- and intra-observer reliability for encoding these behaviors were calculated using the Spearman correlation coefficient and ranged from 0.95 for the duration of walking to 0.98 for the frequency of attack bites.

Drugs

Anpirtoline hydrochloride (6-chloro-2-[piperidinyl-4-thio] pyridine) was purchased from Tocris Cookson (Ballwin, MO) and dissolved in 0.9% saline. GR127935 (N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2-methyl-4′-(methyl-1,2,4-oxadiozol-3-yl)-[1,1-biphenyl]-4-carboxamide) was donated by Glaxo Research and Development Ltd. (London, England) and was suspended in 10% BCD (hydroxypropyl-β-cyclodextrin) with the aid of sonication.

EXPERIMENTAL PROCEDURES

Experiment 1: Anpirtoline Effects on Species-typical Aggression by a Resident Toward an Intruder

According to previously established methods (Miczek and O'Donnell 1978), the females and pups were removed from the home cage before the aggressive confrontation, and an intruder was placed into the resident's home cage. Tests lasted for 5 minutes after the first attack, or for 5 minutes if no attack occurred. After the fifth or sixth confrontation with a male intruder in the resident's home cage, the rate of attack bites had stabilized at 20 bites. The mice were tested twice a week for aggression with a minimum of 72 h between each confrontation. All encounters were conducted between 1400 and 1900 h, and the behaviors were analyzed at a later date. After 10 confrontations, new intruders were used as stimulus animals.

Twelve adult male CFW mice were used to determine the effects of anpirtoline (0.125, 0.25, 0.5, and 1.0 mg/kg) on species-typical levels of aggression. Doses of anpirtoline were administered in a counterbalanced sequence 15 minutes before the confrontation with a male intruder. The drug test was alternated with a vehicle test. Thereafter, the anpirtoline dose-effect determination was repeated after pre-treatment with GR127935 (10 mg/kg). GR127935 was administered 30 minutes prior to anpirtoline, and the tests were scheduled 15 minutes after the administration of anpirtoline.

Experiment 2: Anpirtoline Effects on Aggression after Social Instigation

Suppressed Aggression in Neutral Arena. After a stable level of fighting was confirmed in confrontations between the resident and an intruder in the resident's home cage, confrontations were scheduled in an unfamiliar "neutral" cage (Miczek and O'Donnell 1980). The neutral arena was a clear polycarbonate cage that was approximately 4 times as large as the standard home cage (30 × 33 × 46 cm) with unsoiled pine shavings covering the floor. The resident was moved from its home cage and placed into one corner of the neutral cage, and the stimulus opponent was placed in the opposite corner at the same time. As in the home cage, these confrontations were 5 minutes long after the first attack or were terminated after 5 minutes if no attack occurred. At the end of each test, the neutral cage was cleaned with 20% alcohol solution and the pine shavings were changed. The levels of aggression in the neutral cage were 40% lower than in the home cage, confirming previous observations (Miczek and O'Donnell 1980).

Instigation-heightened Aggression. Fourteen male mice were used in an experiment to engender very high levels of aggression caused by social instigation or provocation (Fish et al. 1999). Specifically, an experimentally naive male intruder was placed inside a protective cage, a clear perforated polycarbonate cylinder (18 × 6 cm), for 5 minutes in the center of the resident's home cage. The residents typically threatened the protected intruder and attacked the perforated cage. Thereafter, the resident was moved and confronted a stimulus opponent in a neutral cage for 5 minutes. Two confrontations with an opponent in a neutral cage were scheduled per week, one after instigation and the other without instigation. For each instigation procedure, a new naive male stimulus animal was placed inside the protective cage. The protective cage was cleaned after each test with 20% alcohol solution.

Experiment 3: Anpirtoline Effects on Extinction-heightened Aggression

Animals. Nine adult male CFW mice (Charles River Laboratories, Wilmington, MA), weighing 25–30 g, were housed in polycarbonate cages (28 ×17 × 14 cm). As before, additional male CFW mice were housed in groups of 10 (n = 30) in larger cages (44 × 24 × 14 cm), and these animals served as stimulus intruder mice.

Apparatus. The experimental set-up and conditioning procedure was identical to those described and illustrated previously (Miczek and de Almeida 2001). Briefly, at the beginning of each session, an experimental panel was inserted into the middle of the mouse cage and affixed to the side walls with two thumbscrews. The panel contained nose-poke sensors and stimulus lights on the left

and right sides, with a fluid delivery cup and a house light in the center (all devices from Med Associates, St. Albans, VT). The fluid delivery cup was connected to a syringe pump. The devices in the panel as well as the pump were connected to an interface that in turn was controlled by a PC running Windows Med PC.

Procedure. During the initial two days, the procedure consisted of reinforcing each nose poke response with the delivery of 0.05 ml sucrose (10%) (Fixed Ratio, FR 1). In successive sessions, the FR requirement was increased gradually to 5. Thereafter, the sucrose solution was decreased from 10% to 8%, 6%, and eventually 4%. Acquisition of the nose-poke response occurred within minutes of the first experimental session in mice that had been restricted to fluid intake to 8 h per day and that had been exposed to sucrose in their drinking fluid previously. During the first 3 weeks, daily experimental sessions lasted 30 minutes, during the next 4 weeks 15 minutes, and finally 5 minutes.

When response rates were stable (i.e., <10% variation across three consecutive sessions), extinction sessions were scheduled twice a week. During the extinction session nose-poke responses were reinforced only three times. After the third reinforcement delivery, the number of nose pokes continued to be counted for a period of 5 minutes. During the non-extinction session (FR 5), nose poking was reinforced by deliveries of 4% sucrose solution for the entire daily 5-minute session.

Five minutes after the end of the extinction or non-extinction sessions, each resident mouse was tested for aggression with an intruder. The response panel was kept in the cage and a male intruder was placed into the resident's home cage. These confrontations were scheduled every other day, and the latency to the first attack bite and the frequency of attacks were measured for 5 minutes. The same behavioral acts and postures were analyzed as was described before. After three further confrontations (8th, 9th, and 10th confrontation), aggressive behavior was stable (i.e., less than 15% variation in two consecutive tests) and was not affected by sucrose self-administration (attack frequency 14.57 ± 1.17 after self-administering sucrose 4% vs. 18.44 ± 4.81 in the absence of self-administration). Thereafter, extinction and non-extinction tests were each conducted once a week.

Anpirtoline (0.25, 0.50, 1.0 mg/kg) or the saline vehicle was administered intraperitoneally 15 min before the experimental session with sucrose-reinforced responding. These doses of anpirtoline were selected based on previous findings of significant anti-aggressive effects (Miczek and de Almeida 2001). Aggression tests occurred 20 minutes after the injections of anpirtoline.

Data Analysis. All data from the experiments were analyzed using a one-way repeated measure analysis of variance, and when appropriate, Dunnett's post hoc tests were used with vehicle values as control. All data for the dose-effect of anpirtoline and antagonism with GR127935 experiments were analyzed using a one-way repeated measure analysis of variance, and when appropriate, Dunnett's post hoc tests were used with vehicle values as the common control. The effect of GR 127935 alone as compared with vehicle was analyzed using a paired *t*-test. The ED_{50} was defined as the dose of anpirtoline that produced a 50% reduction in behavior as compared with the baseline and was calculated using linear regression. Non-overlapping 95% confidence intervals were considered statistically different. The data for the instigation and extinction effects were also analyzed using a one-way repeated measure analysis of variance. The α level was set at 0.05.

RESULTS

Experiment 1

Effects of Anpirtoline on Species-typical Aggression. Anpirtoline significantly decreased the frequency of attack bites ($F_{(4,11)} = 37.75$, $p < .001$), sideways threats ($F_{(4,11)} = 21.88$, $p < .001$), and tail rattles ($F_{(4,11)} = 25.31$, $p < .001$) at the 0.5 mg/kg and 1.0 mg/kg doses when compared with vehicle control (Figure 1). When administered alone, 10 mg/kg GR127,935 did not affect any aggressive behavior. When administered prior to anpirtoline, GR127,935 produced a significant rightward shift in the dose-effect curve for bite frequency (Figure 1). The ED_{50}'s and CI_{95} for the effects of anpirtoline on the frequency of attack bites and sideways threats were 0.45 mg/kg (0.29, 0.69) and 0.64 mg/kg (0.33, 1.41), respectively. In the presence of GR127,935, the ED_{50} and CI_{95} for the effects of anpirtoline on attacks bites and sideways threats were 0.84 mg/kg (0.41,1.03) and 0.98 mg/kg (0.62, 1.16), respectively. In the presence of GR127,935, 0.5 to 2.0 mg/kg anpirtoline were necessary to decrease the frequency of sideways threats ($F_{(5,11)} = 15.04$, $p < .001$). The frequency of bites ($F_{(5,11)} = 14.96$, $p < .001$) and tail rattles ($F_{(5,11)} = 4.04$, $p < .003$) were significantly decreased at the doses of 1.0 and 2.0 mg/kg anpirtoline.

Neither the duration nor the frequency of non-aggressive behavioral elements such as walking, rearing, grooming and contact were affected by administration of anpirtoline (Table 1).

Experiment 2

Aggression in Neutral Cage and after Instigation. In comparison to the level of aggressive behavior by the resident when confronting an intruder in the home cage, the frequency of attacks and sideways threats was significantly reduced from 19.9 ± 1.2 to 14.0 ± 2.0 ($F_{(2,11)} = 48.45$, $p < .001$) and 24.6 ± 1.6 to 17.3 ± 2.2 ($F_{(2,11)} = 47.76$, $p < .001$), respectively in the neutral cage. The la-

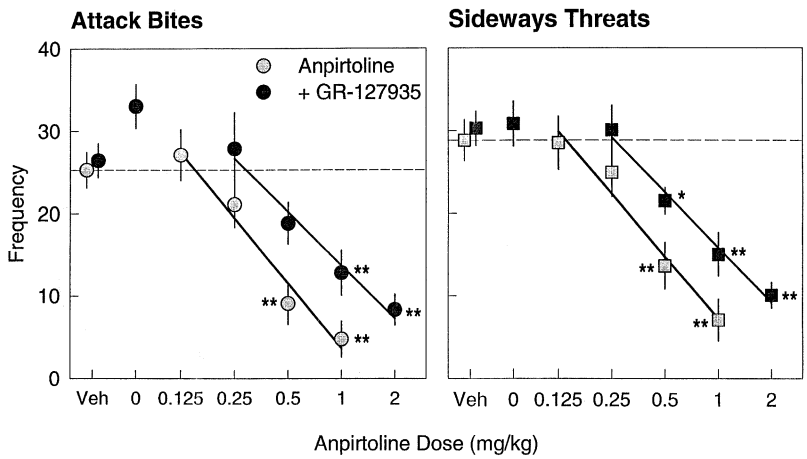

Figure 1. The effects of anpirtoline on the frequency of attack bites (circles) and sideways threats (squares) in the resident male's home cage. Open symbols represent data after treatment with the agonist alone. Filled symbols represent data from tests when the agonist was given after pretreatment with 10 mg/kg GR 127935. Data are presented as means ± 1 standard error (vertical lines). Asterisks denote statistically significant differences relative to vehicle (*=$p < .05$, **=$p < .01$).

tency to the first attack was significantly lengthened from 6.6 ± 1.82 in the home cage to 62.68 ± 16.64 sec in the neutral cage ($t_{(28)} = 23.16$, $p < .0001$; Table 2). After instigating the resident mice with a male opponent for 5 min, all elements of aggressive behaviors showed a significant increase by 120–540%. The frequency of pursuits ($F_{(2,11)} = 10.08$, $p < .001$), sideways threats ($F_{(2,11)} = 47.76$, $p < .001$), attack bites ($F_{(2,11)} = 48.45$, $p < .001$) and tail rattles ($F_{(2,11)} = 52.50$, $p < .001$) were significantly increased in comparison with the level of fighting in the absence of instigation (Figure 2, top).

Among the elements of non-aggressive behaviors, only the duration of contact was significantly reduced after instigation ($F_{(2,11)} = 4.52$, $p < .023$). Other behaviors such as grooming, rearing, and walking remained unaffected by the instigation procedure (Table 2).

Effects of Anpirtoline in Noninstigated Aggression. Anpirtoline (0.25–1.5 mg/kg) significantly reduced the frequency of attack bites ($F_{(5,12)} = 18.41$, $p < .001$) when compared with vehicle group. The frequency of sideways threats ($F_{(5,12)} = 9.54$, $p < .001$) and tail rattles were significantly decreased after administration of 0.5, 1.0, and 1.5 mg/kg anpirtoline (Figure 2, bottom). None of the items that comprise non-aggressive motor activities were affected by administration of anpirtoline (Table 3).

Table 1. Effects of Anpirtoline and GR 127935 (10 mg/kg) on Aggressive and Non-Aggressive Behaviors

Anpirtoline dose (mg/kg)	Vehicle	0	0.125	0.25	0.5	1.0	2.0
Aggressive behaviors							
Latency to first attack (s)							
Anpirtoline	9.3 ± 2.5	8.2 ± 1.1	**21.2 ± 4.7**	**18.6 ± 5.4**	**47.2 ± 26.1**	**78.8 ± 34.9**	
+ GR 127935	8.1 ± 2.0	6.7 ± 0.3		**14.0 ± 3.3**	**14.9 ± 8.2**	**34.5 ± 16.0**	**44.4 ± 36.8**
Pursuit frequency ±							
Anpirtoline	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.2	
+ GR 127935	0.8 ± 0.2	0.7 ± 0.3		0.8 ± 0.3	1.4 ± 0.6	0.1 ± 0.2	0.3 ± 0.3
Tail rattle frequency							
Anpirtoline	100.9 ± 8.0	106.7 ± 8.1	110.6 ± 12.2	91.7 ± 8.6	**51.8 ± 10.2**	**24.8 ± 7.5**	
+ GR 127935	50.9 ± 5.0	39.5 ± 8.4		49.5 ± 8.2	42.9 ± 8.2	**22.6 ± 6.2**	**23.7 ± 4.3**
Non-aggressive behaviors							
Groom duration							
Anpirtoline	14.9 ± 1.9	16.4 ± 3.4	11.5 ± 5.2	18.6 ± 5.1	16.9 ± 5.7	5.4 ± 1.8	
+ GR 127935	15.4 ± 2.6	17.3 ± 7.8		12.7 ± 2.7	9.5 ± 2.6	9.9 ± 3.2	24.8 ± 9.7
Rear duration							
Anpirtoline	46.0 ± 4.9	42.4 ± 5.1	53.7 ± 13.1	44.6 ± 7.8	45.9 ± 8.6	49.8 ± 9.0	
+ GR 127935	50.2 ± 6.2	41.2 ± 5.3		59.6 ± 6.5	63.1 ± 10.2	42.5 ±11.4	50.6 ± 12.6
Walk duration							
Anpirtoline	94.8 ± 3.8	99.2 ± 4.6	92.6 ± 8.9	90.2 ± 5.2	86.5 ± 5.7	76.1 ± 4.3	
+ GR 127935	87.3 ± 3.4	83.1 ± 6.0		86.2 ± 5.0	77.1 ± 5.3	70.1 ± 6.7	60.9 ± 3.3
Contact duration							
Anpirtoline	3.5 ± 1.8	4.4 ± 1.4	7.0 ± 2.9	8.5 ± 4.1	11.2 ± 5.6	15.2 ± 5.6	
+ GR 127935	4.2 ± 1.4	4.3 ± 0.8		2.4 ± 0.8	3.9 ± 1.5	4.3 ± 1.4	5.8 ± 2.0

Data for each behavior are means ± SEM. Values that are significantly different from baseline (avg. vehicle) are printed in **boldface** ($p<0.05$).

Table 2. Effects of Anpirtoline after instigation on Aggressive and Non-Aggressive Behaviors

Anpirtoline dose (mg/kg)	0	0.125	0.25	0.5	1.0	1.5
Aggressive behaviors						
Latency to first attack (s)	**27.7 ± 16.6**	**30.5 ± 12.0**	**34.5 ± 6.6**	**37.6 ± 10.6**	55.4 ± 17.1	130.7 ± 27.6
Pursuit frequency	**4.5 ± 0.95**	**2.4 ± 0.7**	**5.4 ± 2.4**	**2.2 ± 0.7**	**2.4 ± 1.2**	**1.5 ± 0.8**
Threat frequency	44.8 ± 3.5	25.5 ± 4.5	17.5 ± 2.3	12.2 ± 2.3	**12.1 ± 3.4**	**10.7 ± 3.5**
Bite frequency	44.7 ± 4.8	**22.0 ± 4.3**	**14.4 ± 2.1**	**9.1 ± 1.9**	**9.6 ± 3.4**	**6.5 ± 2.9**
Tail rattle frequency	44.7 ± 4.8	**22.0 ± 4.3**	**14.4 ± 2.1**	**9.1 ± 1.9**	**9.6 ± 3.4**	**6.5 ± 2.9**
Non-aggressive behaviors						
Groom duration	10.8 ± 2.3	4.6 ± 1.0	5.5 ± 1.6	4.7 ± 1.4	9.3 ± 4.5	4.8 ± 1.3
Rear duration	41.8 ± 3.3	48.9 ± 4.4	56.3 ± 8.6	47.9 ± 8.2	36.4 ± 8.7	33.1 ± 6.9
Walk duration	100.9 ± 3.9	100.7 ± 5.1	97.1 ± 7.4	93.6 ± 6.4	88.9 ± 3.4	96.9 ± 7.7
Contact duration	8.8 ± 2.7	4.4 ± 1.1	5.4 ± 1.4	7.2 ± 2.6	11.3 ± 1.9	11.1 ± 2.9

Data for each behavior are mean ± SEM. Values that are significantly different from baseline (avg. vehicle) are printed in **boldface** ($p < 0.05$).

Effects of Anpirtoline on Heightened Aggression after Instigation. Anpirtoline significantly reduced the frequency of attack bites ($F_{(5,12)} = 39.38$, $p < .001$) and sideways threats ($F_{(5,12)} = 36.88$, $p < .001$) across all doses studied when compared with vehicle group. The frequency of tail rattles was significantly decreased after 1.5 mg/kg anpirtoline (Figure 2, bottom).

None of the non-aggressive motor behaviors were affected by treatment with anpirtoline (Table 2).

Experiment 3

Aggression after Extinction ("Frustration"). The mean frequency of attack bites ($t_{(7)} = 3.1$, $p < .02$) and sideways threats ($t_{(7)} = 2.4$, $p < .05$) in extinction tests were significantly increased when compared with non-extinction tests (Figure 3, top). None of the elements of non-aggressive behavior such as walking, rearing, grooming, and contact showed any significant alteration in both tests (Table 4).

Effects of Anpirtoline on Heightened Aggression after Extinction. Anpirtoline (0.25–1.0 mg/kg) significantly reduced the frequency of attack bites ($F_{(3,24)}=71.27$, $p < .001$), sideways threats ($F_{(3,24)}=27.75$, $p < .001$), and tail rattles ($F_{(3,24)}=13.18$, $p < .001$) across all doses studied (Figure 3, bottom).

Anpirtoline (0.5–1.0 mg/kg) significantly increased the duration of contact after the extinction test ($F_{(3,24)}= 4.05$, $p < .018$) and 0.25–0.5 mg/kg of anpirtoline decreased the duration of rearing after the non-extinction test ($F_{(3,24)}= 3.36$, $p < .035$) when compared with group control. The other non-aggressive behaviors such as walking and grooming were not affected by anpirtoline in either the extinction or non-extinction tests (Table 4).

DISCUSSION

The currently developed experimental protocols successfully engender escalated levels of aggressive behavior in the mouse that approximate several behavioral features of clinical concern. In order to assess heightened aggression, the species-typical pattern of aggressive behavior by resident Swiss-Webster mice toward intruders served as the standard for comparison. This random bred strain of mice has been most frequently selected for psychopharmacological studies of aggres-

Table 3. Effects of Anpirtoline on Aggressive and Non-Aggressive Behaviors in Neutral Cage

Anpirtoline dose (mg/kg)	0	0.125	0.25	0.5	1.0	1.5
Aggressive behaviors						
Latency to first attack (s)	57.7 ± 17.0	60.3 ± 11.1	65.1 ± 17.4	83.3 ± 21.5	**103.3 ± 32.8**	**141.5 ± 35.5**
Pursuit frequency	3.7 ± 1.0	3.3 ± 1.1	6.1 ± 1.4	3.3 ± 1.5	3.3 ± 2.8	1.5 ± 0.5
Threat frequency	20.5 ± 3.1	18.9 ± 3.5	18.8 ± 3.3	**10.0 ± 2.3**	**6.1 ± 1.7**	**8.1 ± 2.6**
Bite frequency	19.9 ± 1.3	17.1 ± 2.8	**12.5 ± 2.6**	**8.3 ± 2.2**	**3.0 ± 0.8**	**2.0 ± 0.7**
Tail rattle frequency	59.8 ± 4.3	59.9 ± 8.6	46.8 ± 9.1	**32.5 ± 7.8**	**17.9 ± 4.7**	**18.9 ± 4.0**
Non-aggressive behaviors						
Groom duration	2.9 ± 0.5	6.1 ± 3.6	4.3 ± 1.9	0.3 ± 0.2	3.3 ± 1.3	2.7 ± 1.0
Rear duration	43.4 ± 5.6	48.2 ± 6.8	41.1 ± 8.2	47.8 ± 11.1	42.0 ± 10.9	20.8 ± 6.2
Walk duration	93.5 ± 3.1	95.8 ± 7.8	93.3 ± 7.3	80.8 ± 4.6	89.1 ± 8.0	81.0 ± 7.3
Contact duration	8.1 ± 2.2	4.2 ± 1.0	12.0 ± 3.1	11.1 ± 4.7	19.1 ± 6.8	8.8 ± 3.0

Data for each behavior are mean (± SEM). Values that are significantly different from baseline (avg. vehicle) are printed in **boldface** ($p < 0.05$).

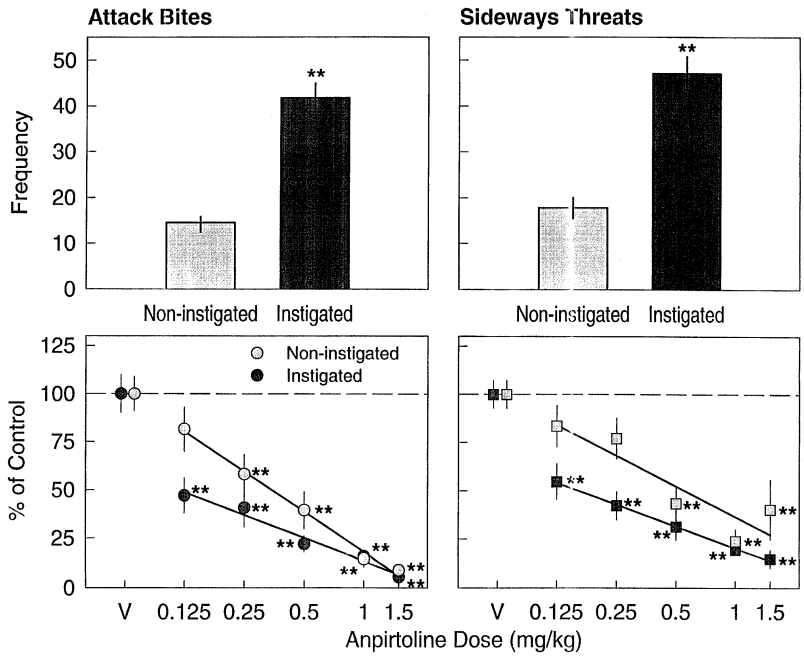

Figure 2. Top: The frequency of attack bites and sideways threats by non-instigated and instigated male mice confronted by a male opponent. Bars indicate means ± 1 standard error (vertical lines). Bottom: The effects of anpirtoline on attack bites (circles) and sideways threats (squares). Open symbols represent non-instigated mice, and filled symbols represent instigated mice. Data are presented as mean percent change from baseline ± 1 standard error (vertical lines). Asterisks denote statistically significant differences relative to vehicle (**=$p < .01$).

sion under controlled laboratory conditions (Valzelli et al. 1967; Krsiak 1979; Miczek et al. 2001). Both the social instigation protocol as well as the "frustration" procedure more than doubled the frequency of attacks and threats toward a stimulus opponent, and these experimental protocols proved repeatable over several months. In strains of mice such as DBA/2J, C57BL/6, and Balb/cJ in which many individuals exhibit low levels of aggressive behavior, the social instigation procedure engendered increased fighting in confrontations with intruders (Faccidomo et al., in preparation). The escalated levels of aggression in socially instigated or "frustrated" mice provided a direct test of the putative anti-aggressive effects of anpirtoline (Rilke et al. 2001). The potent and efficacious inhibition of heightened aggressive behavior by anpirtoline adds to the evidence from studies with CP 94,253 and zolmitriptan implicating the 5-HT$_{1B}$ receptor as a promising target for anti-aggressive medication (Fish et al. 1999; de Almeida et al. 2001).

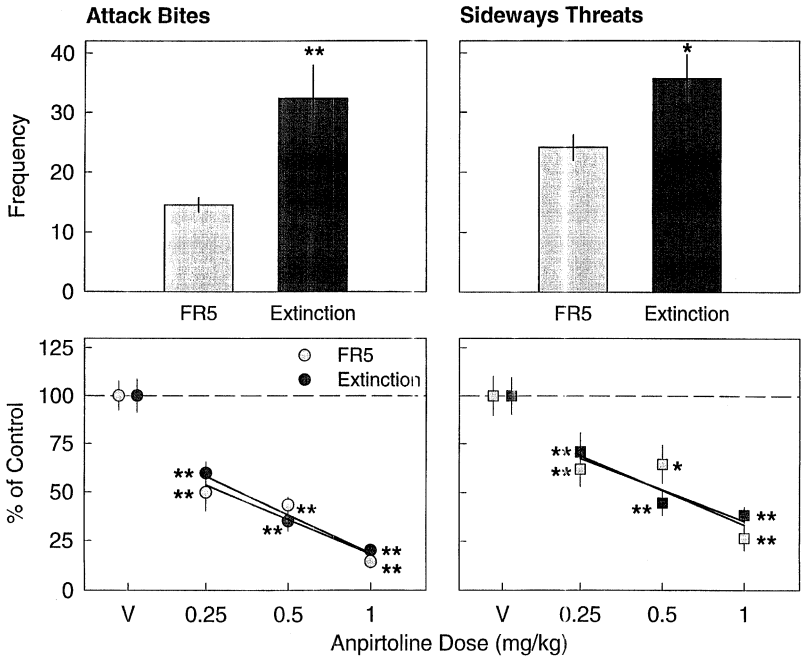

Figure 3. Top: The frequency of attack bites and sideways threats on FR5 (fixed ratio) non-extinction and extinction test. Bars indicate means ± 1 standard error (vertical lines). Bottom: The effects of anpirtoline on attack bites (circles) and sideways threats (squares). Open symbols represent non-extinction, and filled symbols represent extinction. Data are presented as mean percent change from baseline ± 1 standard error (vertical lines). Asterisks denote statistically significant differences relative to vehicle (*=$p < .05$, **=$p < .01$).

Table 4. Effects of Anpirtoline on Aggressive and Non-Aggressive Behaviors after Non-extinction and Extinction Tests

	Non-extinction				Extinction			
Anpirtoline dose (mg/kg)	**0**	**0.25**	**0.5**	**1.0**	**0**	**0.25**	**0.5**	**1.0**
Aggressive behavior								
Latency to attack (s)	7.1 ± 3.1	**20.6 ± 5.8**	**44.4 ± 10.2**	**80.8 ± 24.9**	4.8 ± 3.5	**15.1 ± 5.1**	**30.3 ± 10.8**	**55.2 ± 22.2**
Pursuit frequency	0.1 ± 0.1	0.0 ± 0.0	0.4 ± 0.2	0.0 ± 0.0	0.8 ± 0.5	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.2
Threat frequency	21.8 ± 2.2	**12.2 ± 1.3**	**14.4 ± 3.1**	**5.3 ± 1.2**	34.8 ± 3.1	**23.7 ± 2.6**	**16.2 ± 3.0**	**12.9 ± 11.7**
Bite frequency	14.4 ± 1.1	**7.1 ± 1.5**	**6.3 ± 0.7**	**2.1 ± 0.3**	32.6 ± 2.8	**19.1 ± 2.1**	**12.2 ± 2.3**	**6.4 ± 0.9**
Tail rattle frequency	48.8 ± 6.6	**35.9 ± 5.4**	**26.9 ± 5.2**	**8.3 ± 1.4**	69.2 ± 9.2	49.8 ± 5.8	35.0 ± 9.7	34.6 ± 4.4
Non-aggressive behavior								
Grooming duration	6.9 ± 1.5	1.1 ± 0.6	5.0 ± 1.9	4.2 ± 2.6	2.5 ± 0.8	10.7 ± 4.9	1.4 ± 1.2	4.0 ± 1.5
Rearing duration	64.6 ± 11.1	**34.3 ± 6.9**	**35.4 ± 3.5**	36.2 ± 11.1	27.8 ± 4.8	47.4 ± 10.8	38.9 ± 8.4	37.8 ± 9.0
Walking duration	69.0 ± 4.7	68.1 ± 8.7	70.6 ± 10.9	78.2 ± 13.4	69.2 ± 4.3	71.7 ± 5.5	58.6 ± 5.8	72.9 ± 8.2
Contact duration	29.9 ± 7.2	36.6 ± 6.8	36.1 ± 8.4	46.7 ± 3.8	18.7 ± 3.8	33.3 ± 11.7	**54.7 ± 12.5**	**58.0 ± 12.7**

Data for each behavior are mean (± SEM). Values that are significantly different from baseline (avg. vehicle) are printed in **boldface** ($p<0.05$).

Social instigation in the form of exposure to a protected male opponent in the resident's home cage generated very high levels of attacks, and this intense form of aggression has been postulated to involve high "aggressive arousal" (Potegal 1991). Similar procedures in laboratory rats and hamsters demonstrated significant increases specifically in aggressive behavior , but not in feeding or sexual behavior (Thor and Carr 1979; Thor and Flannelly 1979; Potegal 1991). In the present sample, the effect of instigation increased specifically the salient elements of the aggressive behavioral repertoire but did not extend to non-aggressive behavioral elements. Fish et al. (1999) have demonstrated that exposure to adult male mice increased aggression, whereas other stimuli such as a juvenile or a female mouse were insufficient to escalate the levels of aggression. Whereas no significant relationship between the magnitude of the instigation effect and an individual's non-instigated baseline level of aggression was apparent, the size of the escalation in aggression varied substantially across individuals, ranging from 120–540% above baseline. The source for these persistent individual differences remains to be determined. During the aggression tests, a high incidence of wounding in the intruders was observed, but it was not analyzed. It will also be interesting to learn whether or not the intensity of the instigation effect is related to other forms of escalated aggression.

After discontinuation of the scheduled delivery of sucrose, nearly all individuals in the present sample immediately showed bursts of operant responding and subsequently heightened aggression, and these rapid and high levels of aggression persisted during semiweekly confrontations over several months. Earlier studies in pigeons and monkeys have demonstrated enhanced levels of aggression after discontinuation of scheduled reinforcement (Azrin et al. 1966; Cherek and Pickens 1970; Kelly 1974; Looney and Cohen 1982). Whereas increased response rates after discontinued reinforcement deliveries are readily seen in many animal species (e.g., Amsel and Roussel 1952), it has been difficult to study increased aggressive behavior in laboratory rats and mice (Thompson and Bloom 1966). It will be important to learn how increased aggression is related to other extinction-induced behaviors, in order to assess whether or not this phenomenon is specific to aggressive behavior. Prior to the presentation of the stimulus intruder animals, the experimental mice left bite marks on the response panels in their cage. Increased biting after discontinuation of scheduled reinforcement may represent a form of adjunctive behavior (Falk 1971). It has been postulated that adjunctive behavior or displacement activity has a stabilizing function on agonistic, mating, parental, and intermittent feeding behavior when any of these activities are variable (Falk 1977). It is possible that individuals with low "frustration" tolerance, who rapidly and frequently exhibit intense aggressive behavior, are also likely to engage in other forms of impulsive behavior.

If, in fact, some individuals readily escalate their aggressive behavior as a result of being socially instigated or "frustrated" by the omission of scheduled reinforcement, then it may be informative to characterize them neurochemically. Neuropharmacologic and neurochemical assay data point to serotonin synthesis, release, metabolism, and receptor action as particularly distinctive in impulsively aggressive individuals (Olivier et al. 1995; Higley and Bennett 1999; van Erp and Miczek 2000; Lesch and Merschdorf 2000). The present experiments focused on the effects of anpirtoline because 5-HT_{1B} receptor agonists have been demonstrated to have potent and selective anti-aggressive effects. It was particularly noteworthy in individuals that show high levels of aggression as a result of social instigation or alcohol treatment (Fish et al. .1999; de Almeida et al. 2001; Rilke et al. 2001). In addition, anpirtoline inhibits anxiety-like and depression-like behaviors, pain, and

sexual behavior (Schlicker et al. 1992; Metzenauer et al. 1992; Hillegaart and Ahlenius 1998; Bouwknecht et al. 2001; O'Neill and Conway 2001).

The reduction in aggression was antagonized by GR127935, indicated by a rightward shift in the dose-effect curves of anpirtoline, demonstrating the 5-HT$_{1B}$ receptors as the key site of action. There was a tendency for GR127935 to increase species-typical aggressive behavior, but this effect was not statistically significant. GR127935 has also been shown to antagonize the effects of 5-HT$_{1B}$ agonist on locomotion, anxiolytic-like and antidepressant-like responses, cocaine self-administration, and food and alcohol intake (O'Neill et al. 1996; Pauwels et al. 1997; Maurel et al. 1998; Parsons et al. 1998; O'Neill and Conway 2001).

Converging evidence has identified a key role for the 5-HT$_{1B}$ receptors in aggression and violent behavior. Mice lacking the 5-HT$_{1B}$ receptor (1BKO) were found to be more aggressive than their wild-type counterparts (Saudou et al. 1994; Ramboz et al. 1995; Bouwknecht et al. 2001). A number of polymorphisms have been identified in the human 5-HT$_{1B}$ gene (Huang et al. 1999). One of these polymorphisms appears to be associated in two independent populations with antisocial personality disorder and alcoholism (Lappalainen et al. 1998).

The 5-HT$_{1B}$ receptor is found primarily at sites of serotonin release (i.e., synaptic terminals or axonal varicosities), and activation of these receptors inhibits serotonin release (Gothert 1992; Boschert et al. 1994; Davidson and Stamford 1995). On non-serotonergic neurons, the 5-HT$_{1B}$ receptor also is found near points of transmitter release and appears to inhibit the release of other transmitters such as acetylcoline, glutamate, and GABA (Gothert 1992). Based on the present systemic injections with anpirtoline, it is not possible to identify whether the anti-aggressive effects were mediated via pre- or postsynaptic mechanisms. Earlier, we showed that after lesions of the presynaptic and somatodendritic receptors with 5,7-DHT into the raphé nuclei, zolmitriptan or CP-94,253 remained effective to decrease aggressive behavior in male mice (de Almeida et al. 2001). These data suggest that the inhibitory effects of these 5-HT$_{1B}$ receptor agonists involved postsynaptic sites. Furthermore, microinjection studies in rats have shown that the anti-aggressive effects of 5-HT$_{1B}$ receptor agonists are primarily caused by activation of postsynaptic sites (Mos et al. 1993).

In conclusion, our data suggest an essential role of 5-HT$_{1B}$ on heightened aggression in animals. 5-HT$_{1B}$ agonists such as zolmitriptan, CP-94,253, and anpirtoline decreased aggression in individuals that showed moderate and, in particular, high levels of aggressive behavior after social instigation or omission of scheduled reinforcement. These anti-aggressive effects are interpreted as being caused by activation of postsynaptic 5-HT$_{1B}$ receptors, which contribute significantly to the specific modulation of aggression.

ACKNOWLEDGMENTS

This research was supported by USPHS research grant AA05122 and DA02632 (KAM, PI). RMMdA was supported by a fellowship from CAPES (Brazil) and UNISINOS (Brazil). We thank Mr. J. Thomas Sopko for outstanding technical assistance,.and Mr. E. Fish for valuable advice.

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