Published in final edited form as:
Psychopharmacology (Berl). 2012 July ; 222(1): 117–128. doi:10.1007/s00213-011-2629-1.

Infralimbic and dorsal raphé microinjection of the 5-HT$_{1B}$ receptor agonist CP-93,129: attenuation of aggressive behavior in CFW male mice

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Abstract

Rationale—Aggressive behavior and impaired impulse control have been associated with dysregulations in the serotonergic system and with impaired functioning of the prefrontal cortex. 5-HT$_{1B}$ receptors have been shown to specifically modulate several types of offensive aggression.

Objective—To characterize the relative importance of 2 populations of 5-HT$_{1B}$ receptors in the dorsal raphé nucleus (DRN) and infralimbic cortex (ILC) in the modulation of aggressive behavior.

Methods—Male CFW mice were conditioned on a fixed-ratio 5 schedule of reinforcement to self-administer a 6% (w/v) alcohol solution. Mice repeatedly engaged in 5 min aggressive confrontations until aggressive behavior stabilized. Next, a cannula was implanted into either the DRN or the ILC. After recovery, mice were tested for aggression after self-administration of either 1.0 g/kg alcohol or water prior to a microinjection of the 5-HT$_{1B}$ agonist, CP-93,129 (0–1.0 μg/infusion).

Results—In both the DRN and ILC, CP-93,129 reduced aggressive behaviors after both water and alcohol self-administration. Intra-raphé CP-93,129 dose-dependently reduced both aggressive and locomotor behaviors. However, the anti-aggressive effects of intra-cortical CP-93,129 were behaviorally specific.

Conclusions—These findings highlight the importance of the serotonergic system in the modulation of aggression and suggest that the behaviorally specific effects of 5-HT$_{1B}$ receptor agonists are regionally selective. 5-HT$_{1B}$ receptors in a medial subregion of the prefrontal cortex, the ILC, appear to be critically involved in the attenuation of species-typical levels of aggression.

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Keywords

MICROINJECTION; SOCIAL BEHAVIOR; PREFRONTAL CORTEX; INFRALIMBIC CORTEX; AGGRESSIVE BEHAVIOR; SEROTONIN; DORSAL RAPHE; 5-HT

Introduction

High rates of violence and aggression occur under the influence of alcohol in a subset of humans (Virkkunen et al. 1994), primates (Miczek et al. 1993; Weerts et al. 1993) and rodents (Lister and Hilakivi 1988; Miczek et al. 1998; Miczek and O'Donnell 1980; Miczek et al. 1992) who show a consistent pro-aggressive behavioral response to moderate doses of alcohol. Those individuals who are sensitive to the aggression-heightening effects of alcohol have a distinct neurochemical, pharmacological and behavioral profile characterized by low central levels of the 5-HT metabolite-5-hydroxy-indole acetic acid (5-HIAA), and 5-HT transporter (5-HTT). In both humans and non-human primates, reduced levels of these markers of 5-HT metabolism and activity are inversely correlated with heightened aggressive and violent behavior (Brown et al. 1979; Ferrari et al. 2005; Higley et al. 1996a; Reif et al. 2007), impulsive and risky behavior (Fairbanks et al. 2001; Frankle et al. 2005; Linnoila et al. 1983; Mehlman et al. 1994; Virkkunen et al. 1996; Virkkunen and Linnoila 1993), excessive alcohol consumption (Higley and Linnoila 1997; Higley et al. 1996b) and a family history of alcoholism (Cloninger 1987; Higley and Linnoila 1997; Higley et al. 1996b). Preclinical studies in rodents have similarly found blunted levels of 5-HT metabolites in aggressive mice (Giacalone et al. 1968; van der Vegt et al. 2003), observed that genetically modified mice that lack the 5-HTT gene are less aggressive than their wild-type counterparts (Holmes et al. 2002) and, using *in vivo* microdialysis, shown that cortical levels of 5-HT in the rat are significantly blunted during and after an aggressive encounter (van Erp and Miczek 2000). Together, these data point to an impulsive, pathological phenotype that is characterized by escalated levels of aggression and dampened serotonergic activity.

Mechanistically, the neural circuitry of species-typical aggression and aggression after alcohol self-administration may share common elements in the prefrontal cortex (PFC; Giancola 2000). One key role of the ventral medial prefrontal cortex is to exert inhibitory control over impulsive behaviors; impaired function of the prefrontal cortex is a hallmark feature of individuals diagnosed with high trait impulsivity (Best et al. 2002; Davidson et al. 2000; Fineberg et al. 2010; Kable and Glimcher 2007; Sripada et al. 2010). Likewise, highly impulsive and violent individuals have reduced metabolic activity in the frontal cortex and are less able to regulate their behavioral actions (Raine et al. 1994; Volkow et al. 1994; Yang and Raine 2009). In rodents, levels of cFos immunoreactivity are significantly increased in the prefrontal cortex immediately after an aggressive encounter (Halasz et al. 2006; Haller et al. 2006) and extracellular levels of 5-HT are elevated in the medial but not orbital prefrontal cortex of rats during performance of a delay discounting task (Winstanley et al. 2006). Similarly, mice that have been selectively bred for high and low levels of aggression have increased cortical levels of 5-HT after repeated resident-intruder confrontations, although this increase is blunted in the highly aggressive animals (Caramaschi et al. 2008). Specifically, the experience of repeatedly “winning” aggressive confrontations seems to functionally alter serotonergic tone in the prefrontal cortex. Together, these studies provide evidence to suggest that engaging in acts of aggression and impulsive behavior leads to an acute increase in cortical activity yet repeated bouts of high levels of aggression downregulates basal 5-HT activity relative to non-aggressive counterparts. These data indicate that the prefrontal cortex is actively recruited during

performance of impulsive and aggressive behaviors and that prefrontal cortical 5-HT regulates both the immediate and long-lasting consequences of these behaviors.

5-HT_{1B} receptors are located both pre- and post-synaptically throughout the central nervous system and modulate a wide variety of behaviors including sex, feeding, drug self-administration, sleep, anxiety, stress, depression and aggression (Ahlenius and Larsson 1998; Barnes and Sharp 1999; Bouwknecht et al. 2001; Clark and Neumaier 2001; Clark et al. 2002; Clark et al. 2004; Fish et al. 1999; Lee and Simansky 1997; Moret and Briley 2000; Parsons et al. 1998; Stern et al. 1998; Zhuang et al. 1999). Genetic deletion of the 5-HT_{1B} receptor can lead to heightened aggression and impulsivity, and allelic variation of the 5-HT_{1B} receptor has been linked to impulsive aggression, antisocial personality disorder and alcoholism (Fehr et al. 2000; Lappalainen et al. 1998, but see New et al. 2001; Sinha et al. 2003). The association of this polymorphism with impulsive aggression suggests that a higher frequency of the 5-HT_{1B} receptor gene may contribute to a trait-like phenotype that predisposes individuals to engage in maladaptive patterns of aggression and alcohol drinking. Pharmacologically, 5-HT_{1B} receptor agonists selectively attenuate alcohol-heightened aggression with greater potency than non-heightened aggression, in both mice and humans (Fish et al. 1999; Fish et al. 2008; Gowin et al. 2010). Site-specific microinjection of the 5-HT_{1B} agonists, CP-94,253 and CP-93,129 into the ventral orbital prefrontal cortex (VO-PFC), potently and efficaciously reduce species-typical and escalated offensive aggression (Centenaro et al. 2008; De Almeida et al. 2006). In contrast, alcohol-heightened aggression is significantly increased after microinjection of CP-94,253 into the infralimbic (ILC) but not orbitofrontal region of the prefrontal cortex (Faccidomo et al. 2008). Collectively, these studies suggest that prefrontal 5-HT_{1B} receptors are critically involved in the modulation of both species-typical and escalated aggression, an effect that depends on the targeted sub-region.

The primary goal of this study was to compare the relative importance of two distinct populations of 5-HT_{1B} receptors, those located in the dorsal raphé nucleus (DRN) and those in the PFC, on the modulation of aggressive behavior. To achieve this goal, we used a 5-HT_{1B} receptor agonist that was highly selective for the 5-HT_{1B} receptor, relative to other 5-HT receptors (ie: 5-$HT1_A$ and 5-HT_{1D} receptors that are abundant in these regions). CP-94,253 and CP-93,129 have different dissociation constants for the 5-HT_{1B} receptor (5-HT_{1B} K_d = 2 nM vs. 55 nM, respectively; Koe et al. 1992a; Koe et al. 1992b) with CP-94,253 having a slightly greater affinity for the 5-$HT1_B$ receptor than CP-93,129. However, CP-93,129 has a much lower affinity for the 5-$HT1_A$ receptor than does CP-94,253 (5-HT_{1A} K_d = 1500 nM vs. 89 nM, respectively) and is therefore more selective for the 5-HT_{1B} receptor. Moreover, Bannai et al. 2007 previously found that intra-raphé injection of CP-93,129 decreased escalated aggression that is observed in animals when they perform an operant conditioning task that is reinforced by the opportunity to engage in an aggressive confrontation. However, the importance of this population of 5-HT_{1B} receptors in the DRN on species-typical and aggression after alcohol self-administration has not been fully characterized. Thus, we chose to use the 5-HT_{1B} agonist, CP-93,129, to preferentially activate 5-HT_{1B} receptors in the DRN and ILC, in order to assess the importance of these two populations of receptors on the modulation of aggressive behavior.

Materials and Methods

Subjects

Male “resident” Swiss Webster mice (n=23; CFW, Charles River Labs, Wilmington, MA) were pair-housed upon arrival with a conspecific female in clear, polycarbonate cages (28 × 17cm) lined with pine shavings. Purina rodent chow was freely available through the cage lid and water was available for 3 hr/day. Mouse litters were weaned at 21 days postpartum.

"Intruders" were male CFW mice (n=46) that were housed in groups of 8–12 in a large polycarbonate cage (48 × 26cm) lined with corn cob bedding, with unlimited access to food and water. The vivarium was maintained on a 12 hour light/dark photocycle (lights off at 0700h), with temperature set at 21 ± 1° C and 23% humidity. All mice were cared for according to the Guide for the Care and Use of Laboratory Animals (National Research Council 1996) and the Tufts University IACUC approved all experimental procedures.

Alcohol Self-administration Apparatus

Resident male mice were trained to emit an operant response (nose-poke) on a custom designed aluminum panel (16.5 × 3.8 × 15.9 cm) that was inserted into their home cage with thumb screws (see Miczek and de Almeida 2001 and Faccidomo et al. 2008 for details). Briefly, each side of the panel contained a cue light positioned above a drinking trough (3 × 5 cm; Med Associates; Georgia, VT). A photobeam spanned the entrance of the drinking trough and a nose-poke was recorded when the photobeam was disrupted, causing a relay click as auditory feedback. Each trough was connected to a syringe pump (Med Associates). The panel and pump were connected to an interface and computer that recorded the behavior of each mouse (MED-PC for Windows v.4.1; Med Associates). A white house light was illuminated throughout the session and a 28V fan minimized external noise.

Acquisition of Alcohol Self-administration

A modified sucrose fading procedure was used to facilitate alcohol self-administration (Faccidomo et al. 2008; Samson 1986). Prior to the 1st drinking session, the resident was acclimated to the initial reinforcing solution by presentation of a plastic bottle containing sucrose (10% w/v) solution through the cage lid for 16 hrs. The next day, fluid access was restricted for 21 hrs prior to the 1st self-administration session. Each session began by removing the female and pups, then placing the operant conditioning panel into the home cage. Initially, every nose-poke into either trough was reinforced (Fixed Ratio 1 schedule; FR 1) by a 0.05 ml delivery of a 10% sucrose solution. Each reinforcement was accompanied by a brief noise and absence of the house light, both of which served as secondary cues. Responses that occurred during delivery of the reinforcement (1.26 sec) were recorded but were not reinforced. During the second session, responding on only the "active" nose-poke ("active" side was counterbalanced across mice) was reinforced and the reinforcement schedule was increased to FR 5. Responding on the "inactive" nose-poke was recorded but held no contingencies. In subsequent sessions, alcohol was gradually added to the reinforcing solution in 1% increments up to 6%. Next, the sucrose concentration in the reinforcing solution was decreased in 1% decrements until residents were self-administering unsweetened alcohol (6% w/v). Initially, sessions lasted for 30 minutes, after which the mice were reunited with their cage mate and given access to water for 3 hrs. Also, when the mice began self-administering a 5% sucrose/6% alcohol solution, the duration of the session was shortened to prevent severe intoxication. Sessions were terminated after each resident consumed 1.0 g/kg alcohol. Drinking sessions occurred 5 days per week between 0700 – 1400 hrs.

Apparatus and Measurements

Aggressive confrontations were videotaped using a low-lux video camera (Panasonic BL-200) connected to a VCR and monitor. All confrontations were analyzed by trained observers using a custom-designed keyboard connected to a computer running The Observer software (Noldus, The Observer v.5.0; Wageningen, The Netherlands). The frequencies and durations of salient aggressive (attack bites, sideways threat, tail rattles, pursuit) and non-aggressive (grooming, rearing, walking) behaviors were quantified according to the operational descriptions provided by Grant and Mackintosh 1963 and Miczek and O'Donnell 1978.

Resident-Intruder Confrontations

After 3 weeks of pair-housing, aggression-naïve residents were screened for aggressive behavior until stable baseline levels of aggression emerged (ca. 6–10 confrontations with less than 15% variability; (Miczek and O'Donnell 1978). Aggressive confrontations began by removing the female and pups and introducing a male "intruder" mouse into the home cage of the resident. Confrontations lasted for 5 min after the 1st attack bite or for 5 min if no attack was initiated. After the confrontation, the female and pups were reunited with their resident cage-mate. Importantly, each resident repeatedly confronted the same intruder and aggressive intruders (ca. 1 intruder/cage) were excluded as stimulus animals.

Drugs

95% Ethyl alcohol (Pharmco Products Inc.; Brookfield, CT) was diluted with tap water to 6% (w/v). The 5-HT_{1B} agonist CP-93,129 (3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrollo[3,2-b]pyrid-5-one, generously donated by Pfizer, Groton, CT) was freshly dissolved in aCSF (in mM, 147 NaCl, 1.3 anhydrous $CaCl_2$, 0.9 anhydrous $MgCl_2$, 4.0 KCl, pH = 6.7–7).

Experiment 1: intra-raphé microinjection and aggression

Residents (n=14) were anesthetized with a ketamine (100 mg/kg)/xylazine (10 mg/kg) cocktail, i.p), placed into a stereotaxic frame (Kopf Instruments; Tujunga, CA) and implanted with a 26 gauge guide cannula (Plastics One; Roanoke, VA) aimed at the DRN (AP –4.4 mm; ML ±0; DV −1.7 mm from bregma; 26° angle; Franklin and Paxinos 2001). A 33 gauge obdurator (Plastics One), extending 0.5 mm beyond the cannula tip, was inserted after surgery and moved daily to prevent blockage and scarring. An aversive tasting quinine polish (Bite It©) coated the headmount and obdurator to prevent gnawing damage by the female cagemate. After 1–2 weeks recovery, residents resumed alcohol self-administration and aggression testing.

On test days, mice consumed water or 1.0 g/kg alcohol immediately before microinjection of either artificial cerebrospinal fluid (aCSF) or CP-93,129 (0.1–1.0 μg) via a 33 gauge injector that extended 2 mm beneath the guide cannulae. Eight microinjections were conducted using a Latin Square design with a minimum of 2 tests/week. The injector was connected to a glass syringe (CMA Microdialysis, North Chelmsford, MA) and pump that infused 0.5 μl over 4 min (0.125 μl/min). The injector remained in place for 1 min after the infusion to allow for diffusion and to minimize vertical capillary action along the injection tract. Mice were unrestrained during the infusion and a 5 min aggressive confrontation commenced 10 min after the injection.

Immediately after the final aggression test, mice were deeply anesthesized (Avertin®) and intracardially perfused with 0.9% saline and 4% paraformaldehyde (PFA). To verify implant position, the brains were sliced on a sliding microtome in 60 μm coronal sections, and stained with cresyl violet. Histological verification of cannula placement revealed that 4 residents had cannulae placements outside of the DRN; these residents were excluded from the final analysis (Figure 1A & B). The cannula of 1 mouse became obstructed after completion of 4 out of 8 treatment conditions; this resident was also excluded from the final analysis.

Experiment 2: intra-cortical microinjection and aggression

A second group of residents (n=9) was anesthetized with Avertin® (2,2,2 tribromoethanol; 400 mg/kg, i.p.) and implanted with a cannula aimed at either the right or the left infralimbic cortex (ILC; AP +1.7mm; ML ±0.4mm; DV −1.2mm, from dura). They were tested for aggression 10 min after consuming water or 1.0 g/kg alcohol and a microinjection of either aCSF or CP−93,129 (0.1–1.0 μg). Eight microinjections were conducted using a Latin

Square design with a minimum of 2 tests/week. Histological verification of cannula placement revealed that 2 residents had cannulae placements within the lateral septum; these residents were excluded from the final analysis (Figure 1C & D).

Statistical Analysis

The frequencies of the aggressive behaviors and the durations of the non-aggressive behaviors were separately analyzed using a two-way repeated measures ANOVA. The Holm-Sidak post-hoc test was run when appropriate, using the aCSF and water tests as the control conditions. α was set at 0.05 for all comparisons.

Results

Experiment 1: intra-raphé microinjection and aggression

Intra-raphé administration of the selective 5-$HT1_B$ agonist, CP-93,129 produced a dose-dependent decrease in aggressive behavior after consumption of 1.0 g/kg alcohol or water. Specifically, a significant main effect of drug was found on *the frequency* of attack bites ($F(3,24) = 22.0$, $p<0.001$; Figure 2, Table 1), sideways threats ($F(3,24) = 15.8$, $p<0.001$; Table 1), and tail rattles ($F(3,24) = 9.9$, $p<0.001$; Table 1). Post-hoc tests revealed that this effect was due to a reduction of these aggressive behaviors after infusion of 0.5 and 1.0 μg CP-93,129, irrespective of whether the resident consumed water or alcohol prior to the infusion. There was also a significant main effect of CP-93,129 on the *duration* of attack bites ($F(3,24) = 14.4$, $p<0.001$; Table 1) and sideways threats ($F(3,24) = 6.8$, $p=0.002$; Table 1). Post-hoc tests revealed that this effect was due to a reduction of both behaviors after infusion of 1.0 μg CP-93,129 and a reduction in the duration of attack bites after infusion of 0.5 μg CP−93,129. Aggressive pursuit of the intruder rarely occurred and was unaffected by any treatment condition.

Multiple indices of motor activity were dose-dependently reduced by intra-raphé administration of the 5-HT_{1B} agonist, CP-93,129 (Table 1). Specifically, there was a significant main effect of drug on the *duration* of walking ($F(3,24) = 10.6$, $p<0.001$; Figure 2; Table 1) and rearing ($F(3,24) = 4.8$, $p=0.009$; Table 1). *The frequencies* of walking ($F(3,24) = 29.4$, $p<0.001$), rearing ($F(3,24) = 5.0$, $p=0.008$), and self-grooming ($F(3,24) = 4.0$, $p=0.019$) were also dose-dependently reduced by drug treatment (Figure 2, Table 1). Post-hoc tests revealed that infusion of the highest dose of CP-93,129 (1.0 μg) significantly decreased frequencies of all of these motor behaviors and walking frequency was also significantly affected by a moderate dose of CP-93,129 (0.5 μg). Social contact with the intruder is rarely observed and was not changed by any of the drug treatments. Consumption of 1.0 g/kg alcohol did not affect any of these aggressive and non-aggressive behaviors.

Experiment 2: intra-cortical microinjection and aggression

Intra-cortical administration of CP-93,129 produced a significant decrease in aggressive behavior after consumption of 1.0 g/kg alcohol or water. Specifically, a significant main effect of drug was found on *the frequency* of attack bites ($F(3,18) = 3.2$, $p=0.049$; Figure 3, Table 2) and on *the frequency* ($F(3,18) = 3.9$, $p=0.026$; Table 2) and *duration* of sideways threats ($F(3,18) = 3.7$, $p=0.026$ Table 2;). Post-hoc tests revealed that microinjection of 0.1 and 1.0 μg CP-93,129 significantly decreased the frequency of attack bites and that microinjection of 0.1 and 0.5 μg CP-93,129 significantly reduced the frequency and duration of sideways threats. Tail rattle *frequencies* ($F(1,18) = 8.5$, $p=0.027$; Table 2) and *durations* ($F(1,18) = 7.7$, $p=0.033$; Table 2) were significantly suppressed after self-administration of 1.0 g/kg alcohol, but not water, irrespective of CP-93,129 dose. Aggressive pursuit of the intruder rarely occurred and was unaffected by any treatment condition.

The predominant motor activity behaviors, walking, rearing and contact with the intruder, were unaffected by alcohol or CP-93,129 (Figure 3; Table 2). However, the *duration* of self-grooming was significantly decreased after microinjection of 1.0 μg of CP-93,129 ($F(3,18) = 3.3$, $p=0.046$; Table 2) and *the frequency* of grooming was significantly reduced after microinjection of 0.1 and 1.0 μg of CP-93,129 ($F(3,18) = 7.0$, $p=0.002$; Table 2).

Discussion

The relationship between serotonin and aggressive behavior has been studied for several decades and our understanding of how serotonin acts continues to be refined. Of the many 5-HT receptor subtypes, the 5-HT_{1B} receptor is especially interesting because systemic administration of 5-HT_{1B} receptor agonists has been found to decrease aggressive behavior without non-selective effects on motor behaviors (for review see Olivier and van Oorschot 2005). The current studies extend these previous studies to brain regions that contain 5-HT_{1B} receptors and that are thought to be necessary for aggressive behavior, the infralimbic cortex and the dorsal raphé nucleus. Infusion of the 5-HT_{1B} receptor agonist CP−93,129 into the infralimbic cortex and the dorsal raphé nucleus similarly decreased aggression that occurred after consumption of both water and 1.0 g/kg alcohol. Nonetheless, the behavioral specificity of the anti-aggressive effect of CP-93,129 differed between brain regions. When injected into the dorsal raphé, CP-93,129 decreased both aggressive and motor behavior; on the other hand, infralimbic injection decreased aggression without affecting motor behavior. Furthermore, CP−93,129 was more efficacious at reducing aggressive behavior when infused into the ILC − the lowest tested dose (0.1 μg CP− 93,129) significantly reduced aggression after intra-ILC but not intra-DRN microinjection. This dissociation is consistent with the literature indicating blunted serotonergic tone in the PFC of highly aggressive mice and suggests a behaviorally specific role for the infralimbic region of the prefrontal cortex in the modulation of aggression, whereas the dorsal raphé may control both aggressive and motor behaviors (Caramaschi et al. 2008). These studies further elucidate the importance of the serotonergic system in the modulation of aggression and highlight the infralimbic cortex as a critical region of interest.

The neural circuitry of aggressive behavior is becoming more widely understood and developed. Early studies correlated levels of activation or immediate early gene expression with a history of violent and/or aggressive behavior and found that the amygdala, prefrontal cortex and hypothalamus are key regions that modulate and are activated in association with aggressive behavior (Caramaschi et al. 2008; Halasz et al. 2006; Haller et al. 2006; Kollack-Walker and Newman 1995; Lin et al. 2011; Veening et al. 2005). Recently, a distinction has emerged between cortical subregions that are activated in response to social interaction versus aggressive confrontations. Specifically, the infralimbic and medial orbital cortices show greater c-fos immunoreactivity 1 hr after an aggressive, but not social, encounter. The ventral and lateral orbital cortices were only activated by social encounters demonstrating a specific role of the ILC in the modulation of aggressive behavior (Halasz et al. 2006). The ILC contains the 5-HT_{1B} receptors that are hypothesized to be important for 5-HT's anti-aggressive effects. In our studies, these receptors were targeted by microinjecting the selective 5-HT_{1B} receptor agonist, CP-93,129. Administration of this agonist reduced aggressive behaviors without affecting motor behaviors such as walking, rearing, and grooming. The behaviorally specific reduction in aggression confirms results from other microinjection studies showing that 5-HT_{1B} activation in the ventral orbitofrontal cortex (V-OFC) selectively reduces species-typical, maternal and aggression escalated by social instigation (Centenaro et al. 2008; da Veiga et al. 2011; De Almeida et al. 2006). However, they oppose the results of microinjection of a different 5-HT_{1B} receptor agonist, CP-94,253, that was shown to increase alcohol-heightened aggression and decrease extracellular 5-HT

(Faccidomo et al. 2008). Nonetheless, these studies provide evidence that cortical 5-HT_{1B} receptors are essential for aggressive behavior.

The medial prefrontal cortex is richly innervated with 5-HT projections originating from DRN neurons (O'Hearn and Molliver 1984). 5-HT_{1B} receptors are inhibitory G-protein coupled receptors and function both as pre-synaptic autoreceptors in the PFC located at the terminals of serotonergic neurons, and as heteroreceptors on non-serotonergic interneurons (Bruinvels et al. 1994; Bruinvels et al. 1993; Hoyer et al. 1985; Sari et al. 1999). Recent evidence suggests that this population of receptors might be vulnerable to alcohol-induced functional adaptations. Specifically, Chiavegatto et al. 2010 found that prefrontal 5-HT_{1B} mRNA levels were significantly reduced in mice who repeatedly engaged in alcohol-heightened aggression vs. those mice whose aggressive behavior did not escalate after alcohol consumption. This result suggests that there is a functional difference between AHAs and ANAs in their serotonergic system, which is inversely correlated with a heightened aggressive response to acute alcohol. Our prior study on CP-94,253 found that extracellular 5-HT levels following reverse perfusion in the PFC were blunted in mice with a history of alcohol consumption (Faccidomo et al. 2008). Local infusion of CP-93,129 into the PFC has been shown to significantly reduce levels of extracellular glutamate suggesting that 5-HT_{1B} receptors are likely located on glutamatergic neurons in the PFC (Golembiowska and Dziubina 2002). The glutamate neurons may in turn project to the subcortical structures involved in the expression of aggression and similar impulsive-like behaviors. Together, these data suggest a neurochemical mechanism by which 5-HT in the PFC may regulate aggressive behaviors.

The dorsal raphé is also a site of action for the 5-HT_{1B} receptor agonists. Microinjection of CP-93,129 decreased both species-typical and aggression after alcohol self-administration, but also disrupted motor behavior. This non-specific decrease in aggression is consistent with previous findings of dorsal raphé microinjection of 5-HT_{1A} or 5-HT_{1B} receptor agonists (Bannai et al. 2007; De Almeida and Lucion 1997; Faccidomo et al. 2008; Mos et al. 1993) and may be the result of slowing of 5-HT neuronal activity. Functionally, local administration of 5-HT_{1B} receptor agonists generally decreases DRN and MRN cell firing, though regulation of 5-HT cell firing and release is chiefly mediated by somatodendritic 5-HT_{1A} receptors (Adell et al. 2001; Evrard et al. 1999; Sprouse 1991; Sprouse and Aghajanian 1987; 1988; Verge et al. 1985). Several key findings argue against an essential and specific role for dorsal raphé 5-HT_{1B} receptors in the anti-aggressive effects of 5-HT_{1B} receptor agonists. First, systemic administration of anti-aggressive 5-$HT_{1B/D}$ agonists does not decrease motor activity; the behavioral effects are more similar to cortical microinjection (de Almeida et al. 2001; Fish et al. 1999). Second, the anti-aggressive effects do not appear to depend on somatodendritic 5-HT_{1B} receptors because neurotoxic lesions of dorsal raphé 5-HT cell bodies using 5,7-dihydroxytryptamine do not prevent the anti-aggressive effects of systemically administered 5-$HT_{1B/D}$ agonists (de Almeida et al. 2001; Sijbesma et al. 1991). Although the dorsal raphé does not appear to be essential for the anti-aggressive effects of 5-HT_{1B} receptor agonists, the dorsal raphé does appear to be essential for the actions of other neuromodulators. Intra raphé $GABA_A$, CRF_1 and $GABA_B$ receptor manipulations regulate alcohol-heightened and species-typical aggression (Takahashi et al. 2010a; Takahashi et al. 2011; Takahashi et al. 2010b) and GABAergic interneurons regulate extracellular release of 5-HT in the prefrontal cortex (Takahashi et al. 2010b). Thus, GABA serves to functionally regulate serotonergic tone throughout the critical pathway for the regulation of aggressive behavior.

In summary, these findings confirm and further demonstrate the importance of prefrontal cortex 5-HT_{1B} receptors in the modulation of species-typical levels of aggression. Future studies aim to expand these findings to address whether these 5-HT_{1B}-mediated effects on

aggression can be mimicked by intra-cortical glutamatergic antagonism. Furthermore, given the ability of alcohol to down regulate 5-HT_{1B} receptors (Chiavegatto et al. 2010)and the demonstration that 5-HT_{1B} overexpression in the nucleus accumbens is linked to excessive alcohol self-administration (Furay et al. 2011; Hoplight et al. 2006), a key direction for future studies would be to investigate the neural circuitry that regulates 5-HT_{1B} receptor activity, escalated aggressive behavior and excessive alcohol intake.

Acknowledgments

The authors would like to thank J Thomas Sopko for his outstanding technical assistance. All research was supported by AA13983 (KAM)

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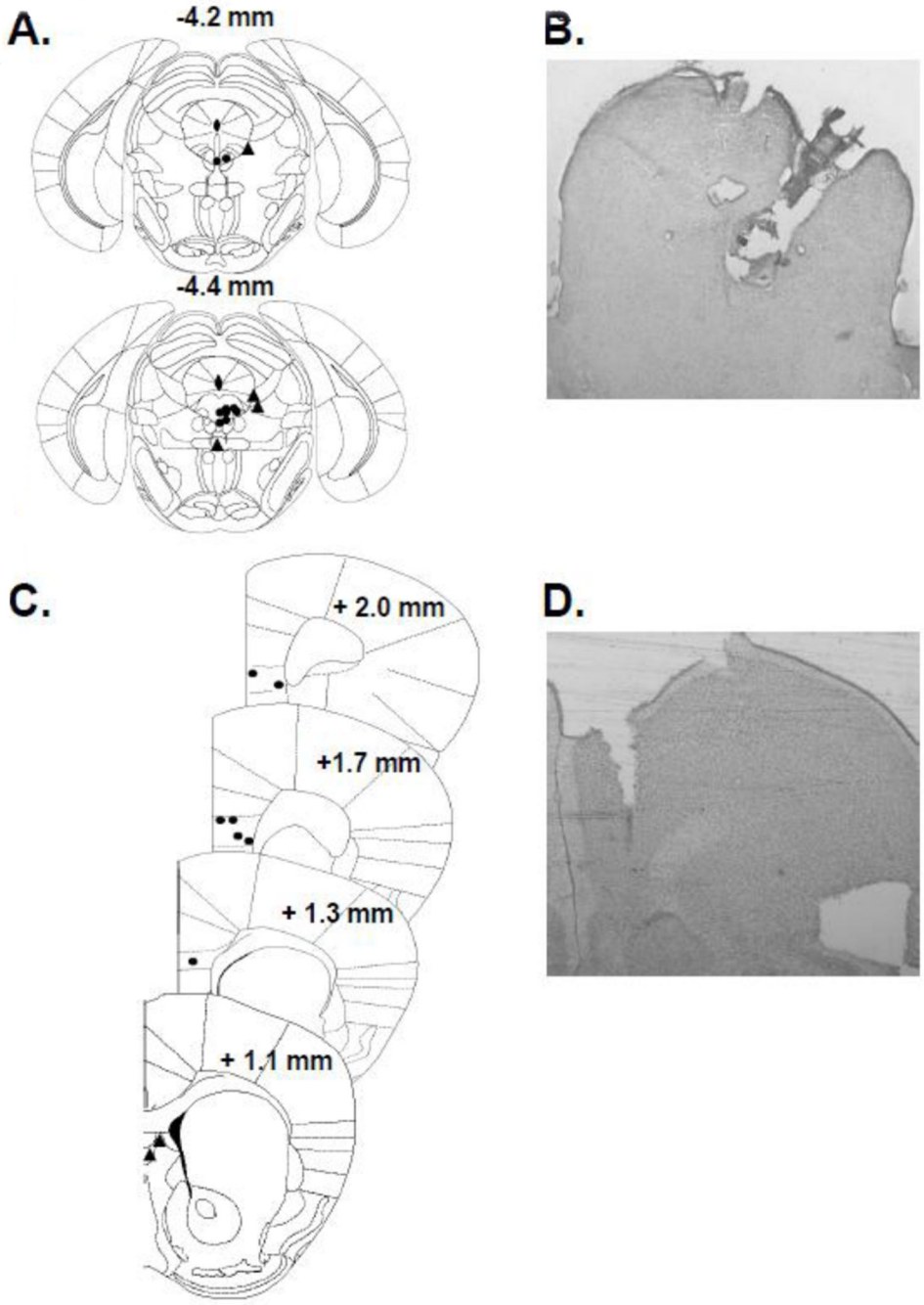

Figure 1.

A schematic representation of mouse dorsal raphé (DRN; **A**) and infralimbic cortex (ILC; **C**) coronal sections adapted from Franklin and Paxinos (2001). *Filled circles* indicate the approximate site of an accurately placed injection (n=9 for DRN; n=7 for ILC). *Filledtriangles* indicate the approximate site of missed placements (n=4 for DRN; n=2 for ILC). Panels **B & D** are representative photomicrographs of mouse brain coronal sections (2X) that were stained with cresyl violet to visualize the injection site.

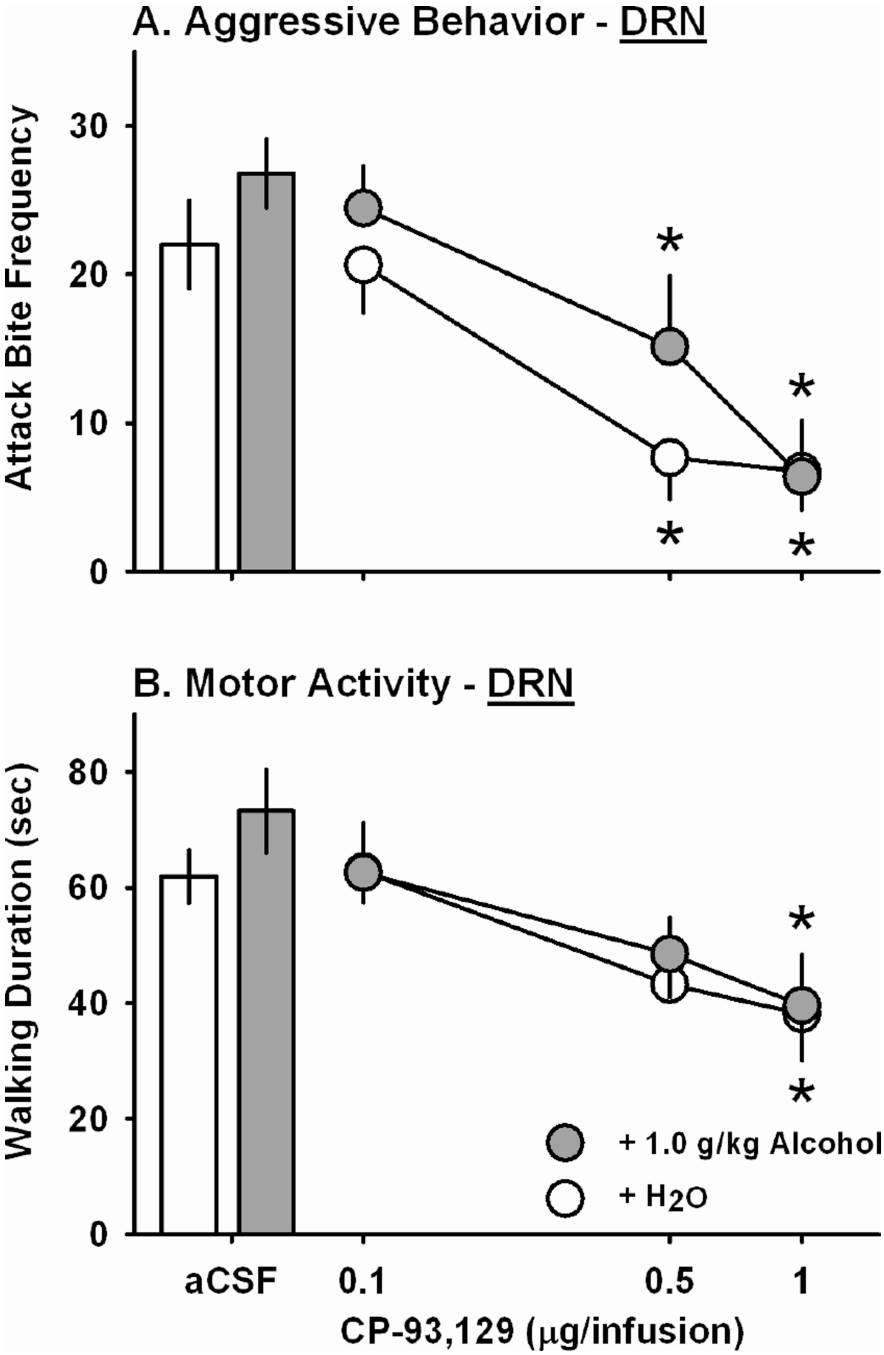

Figure 2.

Microinjection of 5-HT_{1B} receptor agonist CP-93,129 into the dorsal raphé nucleus. **Panel A** shows the effect of CP-93,129 on the mean (± SEM, *vertical lines*) frequency of attack bites after the consumption of water (+ H_2O, *open circles and bar*) or 1.0 g/kg alcohol (+ 1.0 Alc, *filled circles and bar*). **Panel B** shows the effects of CP-93,129 on the duration of walking after the consumption of water (+ H_2O, *open circles and bar*) or 1.0 g/kg alcohol (+ 1.0 Alc, *filled circles and bar*). N=7; ***** denotes a significant decrease from aCSF and $p<0.05$ for all comparisons.

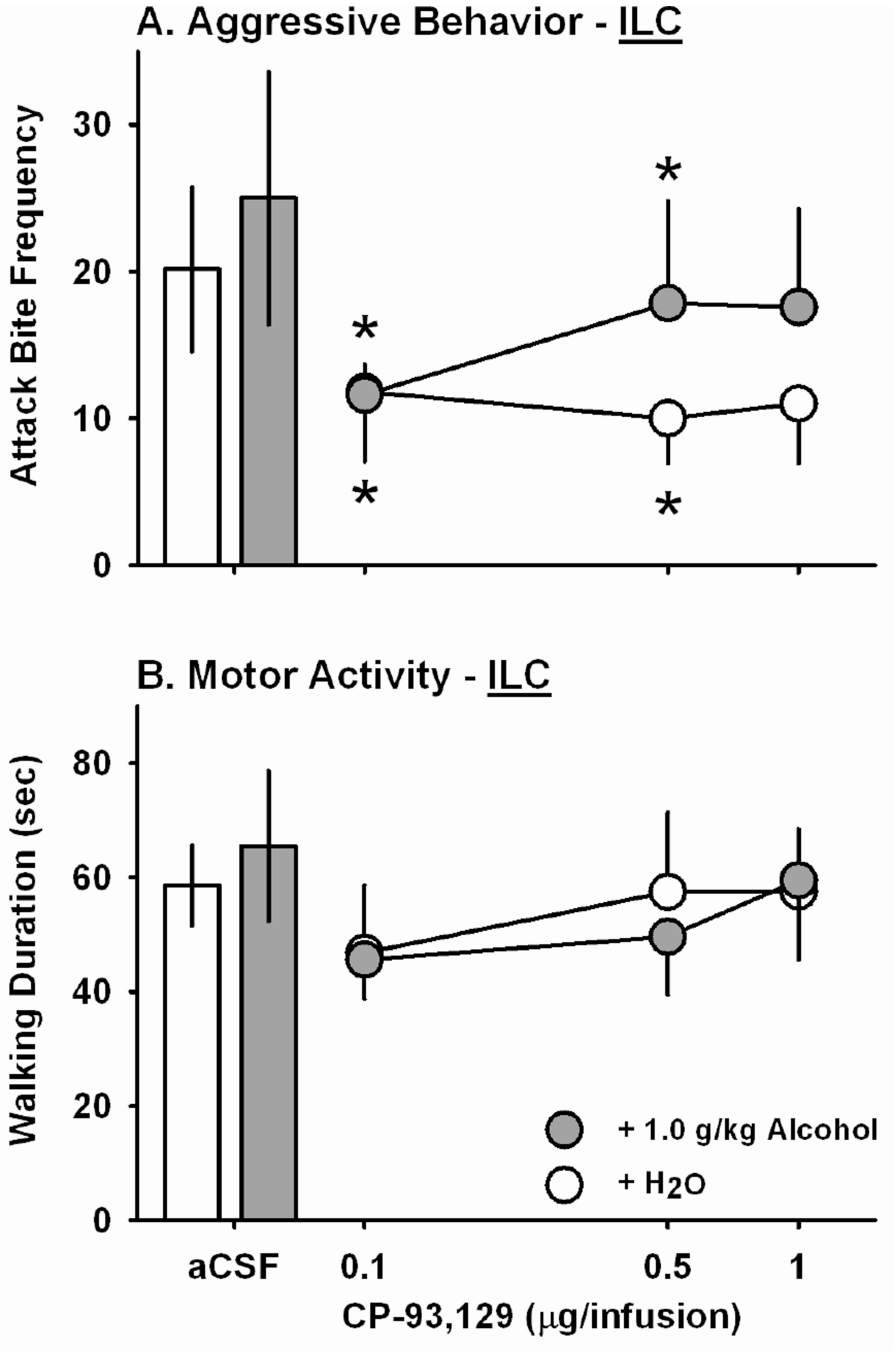

Figure 3.

Microinjection of the 5-HT_{1B} receptor agonist CP-93,129 into the infralimbic region of the prefrontal cortex. **Panel A** shows the effect of CP-93,129 on the mean (± SEM, *vertical lines*) frequency of attack bites after the consumption of water (+ H_2O, *open circles and bar*) or 1.0 g/kg alcohol (+ 1.0 Alc, *filled circles and bar*). **Panel B** shows the effects of CP-93,129 on the duration of walking after the consumption of water (+ H_2O, *open circles and bar*) or 1.0 g/kg alcohol (+ 1.0 Alc, *filled circles and bar*). N=9; * denotes a significant decrease from aCSF and $p<0.05$ for all comparisons.

Table 1

Effect of *CP-93,129* microinjection into the dorsal raphé nucleus on aggressive and non-aggressive behaviors after self-administration of alcohol or water

AGGRESSIVE	**_____+ H_2O_____**				**_____+ 1.0g/kg Alcohol_____**			
BEHAVIORS DOSE (μG)	**aCSF**	**0.1**	**0.5**	**1.0**	**aCSF**	**0.1**	**0.5**	**1.0**
Attack Bite Frequency	22.0 ± 2.9	20.6 ± 3.2	**7.7 ± 2.8**	**6.8 ± 2.6**	26.8 ± 2.3	24.4 ± 2.8	**15.1 ± 4.8**	**6.4 ± 3.8**
Attack Bite Duration (sec)	5.1 ± 0.9	5.9 ± 1.6	**1.6 ± 0.6**	**1.3 ± 0.6**	5.7 ± 0.7	5.0 ± 0.8	**3.0 ± 1.0**	**1.3 ± 0.7**
Sideways Threat Frequency	22.9 ± 3.7	21.1 ± 2.2	**12.9 ± 4.3**	**6.9 ± 3.0**	25.7 ± 3.9	22.1 ± 2.5	**14.8 ± 4.5**	**5.1 ± 2.8**
Sideways Threat Duration (sec)	29.7 ± 5.0	28.4 ± 5.0	19.6 ± 6.9	**14.0 ± 6.8**	27.9 ± 4.6	24.1 ± 3.8	17.6 ± 5.9	**4.9 ± 2.7**
Tail Rattle Frequency	33.2 ± 5.6	28.3 ± 3.4	**22.3 ± 5.6**	**11.8 ± 5.0**	28.7 ± 4.6	26.6 ± 3.6	**17.5 ± 5.8**	**11.9 ± 3.7**
Tail Rattle Duration (sec)	24.3 ± 3.9	23.6 ± 3.5	26.3 ± 9.0	15.8 ± 7.1	16.3 ± 3.1	17.9 ± 3.3	13.9 ± 4.0	12.3 ± 4.0
Pursuit Frequency	2.3 ± 0.8	1.6 ± 1.1	1.3 ± 0.9	0.3 ± 0.1	2.0 ± 1.1	2.6 ± 1.3	0.5 ± 0.2	0.8 ± 0.5
Pursuit Duration (sec)	1.8 ± 0.6	0.9 ± 0.6	0.8 ± 0.7	0.1 ± 0.1	1.1 ± 0.7	1.6 ± 0.9	0.3 ± 0.1	0.3 ± 0.1
NON-AGGRESSIVE	**_____+ H_2O_____**				**_____+ 1.0g/kg Alcohol_____**			
BEHAVIORS DOSE (μG)	**aCSF**	**0.1**	**0.5**	**1.0**	**aCSF**	**0.1**	**0.5**	**1.0**
Walking Frequency	41.9 ± 3.3	41.0 ± 4.2	**26.2 ± 5.5**	**15.8 ± 3.8**	47.6 ± 4.8	39.2 ± 3.7	**30.1 ± 5.2**	**21.9 ± 4.2**
Walking Duration (sec)	61.9 ± 4.5	62.8 ± 8.4	**43.2 ± 11.5**	**38.1 ± 10.2**	73.2 ± 7.2	62.6 ± 5.2	**48.5 ± 7.5**	**39.6 ± 9.5**
Grooming Frequency	5.1 ± 0.9	6.9 ± 1.1	4.1 ± 1.2	**5.5 ± 1.9**	5.9 ± 0.9	7.3 ± 1.1	3.0 ± 0.7	**2.3 ± 0.8**
Grooming Duration (sec)	17.9 ± 4.6	17.4 ± 4.3	10.3 ± 2.2	20.6 ± 7.6	21.1 ± 4.8	22.6 ± 3.8	22.8 ± 11.8	12.3 ± 5.9
Rearing Frequency	14.3 ± 3.1	11.9 ± 3.5	8.0 ± 2.9	**5.5 ± 2.0**	9.8 ± 2.2	11.1 ± 2.8	4.9 ± 1.5	**2.5 ± 0.8**
Rearing Duration (sec)	28.4 ± 7.9	26.1 ± 8.7	16.0 ± 7.0	**13.4 ± 6.5**	15.6 ± 3.8	26.6 ± 7.2	8.5 ± 2.5	**3.3 ± 1.4**
Contact Frequency	1.1 ± 0.6	0.8 ± 0.3	2.0 ± 1.0	0.8 ± 0.7	0.6 ± 0.4	0.6 ± 0.4	1.8 ± 1.0	0.3 ± 0.1
Contact Duration (sec)	1.1 ± 0.8	2.9 ± 2.1	3.0 ± 2.0	10.9 ± 9.6	3.3 ± 3.2	0.8 ± 0.6	7.8 ± 4.6	1.0 ± 0.7

Frequencies and durations of non-aggressive behaviors are represented as Mean ± SEM(N=9) **EMBOLDENED** values indicate a significant main effect of CP-93,129 as compared to ACSF $p<0.05$

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Table 2

Effect of *CP-93,129* microinjection into the infralimbic region of the prefrontal cortex on aggressive and non aggressive behaviors after self-administration of alcohol or water

AGGRESSIVE	**_____+ H_2O_____**				**_____+ 1.0g/kg Alcohol_____**			
BEHAVIORS DOSE (μG)	**aCSF**	**0.1**	**0.5**	**1.0**	**aCSF**	**0.1**	**0.5**	**1.0**
Attack Bite Frequency	20.1 ± 5.6	**11.8 ± 4.7**	10.0 ± 3.1	**11.0 ± 4.1**	25.0 ± 8.6	**11.6 ± 2.1**	17.8 ± 7.0	**17.6 ± 6.7**
Attack Bite Duration (sec)	5.9 ± 1.7	3.7 ± 1.5	2.8 ± 0.8	3.2 ± 1.2	7.1 ± 2.4	3.3 ± 0.7	5.3 ± 2.1	5.0 ± 2.1
Sideways Threat Frequency	28.3 ± 7.0	**21.0 ± 8.1**	**17.3 ± 4.3**	20.3 ± 7.2	35.1 ± 9.8	**17.0 ± 2.7**	**22.3 ± 8.7**	26.4 ± 9.2
Sideways Threat Duration (sec)	8.61 ± 2.1	**6.7 ± 2.6**	**5.7 ± 1.4**	6.6 ± 2.3	12.2 ± 3.5	**5.6 ± 0.9**	**6.8 ± 2.7**	8.4 ± 3.1
Tail Rattle Frequency	31.0 ± 5.7	30.0 ± 8.7	29.9 ± 9.5	43.2 ± 11.8	*22.0 ± 5.5*	*21.0 ± 3.4*	*13.3 ± 4.5*	*17.0 ± 7.5*
Tail Rattle Duration (sec)	10.4 ± 2.6	15.9 ± 6.1	10.0 ± 3.2	14.7 ± 4.2	*7.1 ± 1.9*	*7.3 ± 1.5*	*5.0 ± 1.9*	*6.6 ± 3.0*
Pursuit Frequency	0.1 ± 0.1	0.0 ± 0.0	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Pursuit Duration (sec)	0.2 ± 0.2	0.0 ± 0.0	0.3 ± 0.2	0.1 ± 0.1	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NON-AGGRESSIVE	**_____+ H_2O_____**				**_____+ 1.0g/kg Alcohol_____**			
BEHAVIORS DOSE (μG)	**aCSF**	**0.1**	**0.5**	**1.0**	**aCSF**	**0.1**	**0.5**	**1.0**
Walking Frequency	96.6 ± 11.0	**72.2 ± 18.5**	84.8 ± 20.7	**84.0 ± 17.8**	93.2 ± 16.0	**66.6 ± 8.8**	80.8 ± 18.4	**80.1 ± 19.9**
Walking Duration (sec)	58.6 ± 7.0	46.8 ± 11.9	57.5 ± 13.9	**57.6 ± 10.9**	65.5 ± 13.1	45.6 ± 6.9	49.6 ± 10.2	**59.5 ± 14.0**
Grooming Frequency	8.7 ± 1.9	3.4 ± 0.8	8.5 ± 1.7	4.2 ± 1.6	10.0 ± 1.8	4.8 ± 1.1	9.0 ± 2.7	3.4 ± 1.4
Grooming Duration (sec)	24.1 ± 6.8	12.7 ± 4.7	12.9 ± 4.0	9.1 ± 4.0	17.0 ± 5.2	14.2 ± 2.7	29.9 ± 9.3	11.6 ± 5.1
Rearing Frequency	7.0 ± 1.4	3.2 ± 2.0	5.8 ± 2.2	5.7 ± 2.2	4.7 ± 1.4	2.2 ± 1.0	4.8 ± 1.1	5.3 ± 2.9
Rearing Duration (sec)	18.5 ± 3.0	5.1 ± 3.3	7.0 ± 2.7	11.1 ± 5.0	9.4 ± 5.3	2.8 ± 1.3	8.7 ± 2.7	4.6 ± 2.2
Contact Frequency	0.3 ± 0.3	1.0 ± 0.7	0.7 ± 0.4	1.0 ± 0.7	0.6 ± 0.6	0.8 ± 0.6	0.2 ± 0.1	0.9 ± 0.9
Contact Duration (sec)	0.7 ± 0.7	2.1 ± 1.4	2.5 ± 1.4	2.1 ± 1.1	0.9 ± 0.9	2.2 ± 1.6	0.5 ± 0.4	7.0 ± 7.0

Frequencies and durations of non-aggressive behaviors are represented as Mean ± SEM (N=9) **EMBOLDENED** values indicate a significant main effect of CP-93,129 as compared to ACSF, ***ITALICISED*** values indicate a significant main effect of alcohol $p<0.05$

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