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Activation of 5-HT1b Receptors in the Bed Nucleus of the Stria Terminalis Attenuates the Negative/Anxiogenic Effects of Cocaine

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by

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Activation of 5-HT1b Receptors in the Bed Nucleus of the Stria Terminalis Attenuates the Negative/Anxiogenic Effects of Cocaine

Adam K. Klein

Abstract

Consistent with the Opponent Process Theory of motivated behavior, cocaine administration produces dual and opposing affective states: an initial rewarding "high" followed by a dysphoric/anxiogenic "crash". It therefore seems likely that the motivation to seek cocaine is dependent upon the organism's assessment of the positive relative to the negative consequences of its use. While the neurobiology of the reinforcing aspects of cocaine has been well established, less is known about the systems responsible for the drug's negative actions. In this context, the current study involved an assessment of serotonergic (5-HT) function, which is enhanced by cocaine administration and has been linked to the presence of anxiogenic and depressive states in human and animal studies. In particular, we investigated the role of 5-HT within the bed nucleus of the stria terminalis (BNST) – a structure within the extended amygdala that is activated during periods of stress and during the negative affective state associated with the withdrawal from drugs of abuse. The present study tested the hypothesis that increased 5-HT release in the BNST contributes to the anxiogenic effects of cocaine. A runway self-administration paradigm was employed in which animals were trained to traverse a straight alley in order to earn an infusion of IV cocaine (1.0mg/kg) delivered upon goal box entry. Testing consisted of 16 single daily trials. In this task, animals develop ambivalence

about goal box entry (reflected by the development of approach/avoidance "retreat" behaviors) that we have shown to reflect the dual positive (rewarding) and negative (anxiogenic) associations that subjects form with the cocaine-paired goal-box. To assess the involvement of 5-HT signaling within the BNST on this conflict behavior, prior to each trial rats received bilateral intracranial injections of CP94,253 (0.5μg and 1.0μg/side in 0.5μl or aCSF vehicle), a potent and selective 5-HT_{1B} agonist that inhibits local 5-HT release via activation of terminal autoreceptors. Results indicated that CP94,253 did not alter the positive incentive properties of cocaine (start latencies were unaffected) nor did it alter gross motor behavior (as revealed in subsequent locomotor activity testing). Treatments did, however, selectively attenuate the negative effects of cocaine, as indicated by a dose-dependent decrease in the frequency of approach-avoidance "retreat" behaviors. This effect was independent of whether the manipulation occurred before (10 mins prior) or after (5 mins post-infusion) cocaine self-administration. We therefore conclude that 5-HT signaling within the BNST likely contributes to the negative/anxiogenic effects of cocaine. This work supported by NIDA grant DA-033370 awarded to AE.

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Introduction

Cocaine is a stimulant drug of abuse that produces an initial state of euphoria that is typically followed by a "crash", where the user experiences dysphoria, irritability, anxiety and cravings (Resnick et al 1977; Gawin 1991; Williamson et al 1997). Both the positive reinforcing effects, as well as the aversive and anxiogenic effects of cocaine have also been demonstrated in laboratory animals (Yang et al 1992; Rogerio and Takahashi 1992; Ettenberg 2004; Hayase et al 2005). The mixed positive and negative effects of cocaine conceptually fit well with Solomon and Corbitt's "opponent process theory" of motivated behavior (Solomon and Corbit 1974). Briefly, any stimulus that causes a shift in the organism's affective state initiates two processes, a primary homeostatic shift followed by a delayed "opponent process" whose function is to return the organism to its affective baseline. Cocaine's actions would appear to adhere to this theory in that the initial response to drug administration is characterized by a positive, rewarding state, followed by a delayed anxiogenic state (the "crash"), and eventually a return to "neutral" affective homeostasis (Ettenberg and Geist 1993; Ettenberg et al 1999; Raven et al 2000; Ben-Shahar et al 2004; Ettenberg 2004). One of the key features of drug addiction involves dysregulation of these oscillations between positive and negative states due to repeated activation of a homeostatic mechanism. As tolerance is built to the initial positive effects of the drug, the delayed negative effects become

sensitized (Kreek & Koob, 1998; Koob & Le Moal, 2008; Su, Wenzel, Baird, & Ettenberg, 2011; Su et al., 2013).

A number of rodent models of anxiety have been employed in the study of the aversive effects of cocaine, including tests of defensive withdrawal (Yang et al 1992; Sorg et al 2002), defensive burying (Gill et al 2013), the Elevated Plus Maze (Rogerio and Takahashi 1992; Hayase et al 2005; Gill et al 2013), Open Field testing (Yang et al 1992; Gill et al 2013), and Conditioned Place Aversion (O'Neill et al 2013; Su et al 2013; Wenzel et al 2013). These models work by exploiting the rodent's natural aversion to novel or potentially threatening environments and aversive stimuli. Although cocaine administration alone does not seem to precipitate anxiety-like defensive behaviors, the drug does enhance these behaviors in the presence of a threatening environment (Blanchard and Blanchard 1999).

Serotonin (5-HT) has been strongly implicated in the development and expression of anxiety like behaviors (Watson and Man 2000; Sena et al 2003; Abrams et al 2005) and selective 5-HT reuptake inhibitors (SSRIs) have been well established as the first line of treatment for anxiety disorders (Baldwin et al 2005). Additionally, although cocaine is often considered to primarily work through enhanced dopamine signaling, it also has significant affinity for the 5-HT transporter (Cunningham et al 1992a; Cunningham et al 1992b; Walsh and Cunningham 1997; Filip et al 2004), implicating 5-HT in the behavioral effects of cocaine. Although the effects of cocaine on the serotonergic system have not been as well characterized, the drug's high affinity for the 5-HT transporter and the known role of 5-HT in

anxiety suggest that these circuits may be important for the anxiogenic/aversive effects of cocaine.

Previous work from our laboratory has demonstrated the anxiogenic role of 5-HT in rats running an alleyway for cocaine, with inactivation of the dorsal raphé (Ettenberg et al 2011) or treatment with the anxiolytic 5-HT_{1A} agonist buspirone reducing the anxiogenic "retreat" response (Ettenberg and Bernardi 2006). Buspirone was also effective at selectively attenuating the delayed negative effects of cocaine in a Conditioned Place Test, without modifying the drug's initial positive effects (Ettenberg and Bernardi 2007).

In order to investigate the mixed appetitive and aversive effects of cocaine, our lab has employed a runway model of drug self-administration that, in contrast to typical methods of lever-press operant self-administration, is uniquely suited to assess the behavior of an undrugged animal as it makes the decision to consume a drug. Rats running for cocaine develop a unique pattern of "retreat behavior" that emerges over trials (Ettenberg and Geist 1991; Ettenberg and Geist 1993; Ettenberg 2004). This retreat behavior provides a quantifiable measure of the animal's approach-avoidance conflict (See Fig. 1) and has been shown to result from the subject's mixed positive and negative associations with the goal box that in turn stem from cocaine's rewarding and anxiogenic effects (Ettenberg 2004; Ettenberg 2009).

The Bed Nucleus of the Stria Terminalis (BNST) is a part of the extended amygdala that has been strongly implicated in anxiety due to stress (Ventura-Silva

et al 2012; Jennings et al 2013; Kim et al 2013; Sparta et al 2013) and addiction (Koob 2003; Koob 2008; Stamatakis et al 2014). The BNST appears to be important in the regulation of sustained anxiety due to stress, rather than specific cue induced fear (Sullivan et al 2004; Davis et al 2010). As previously mentioned, cocaine seems to potentiate anxiety-like behaviors without inducing fear or anxiety on its own, thus, the BNST is an ideal candidate brain region that could mediate the anxiogenic properties of cocaine.

The BNST also receives dense projections of fibers from the dorsal raphé nucleus and expresses multiple inhibitory and excitatory 5-HT receptor subtypes (Guo, Hammack, Hazra, Levita, & Rainnie, 2009; Hammack et al., 2009; Hazra, Guo, Dabrowska, & Rainnie, 2012). Due to the dynamic and heterogeneous nature of expression of 5-HT receptor subtypes within BNST neurons, these cells show a highly variable response to serotonin (Guo et al., 2009; Hammack et al., 2009; Hazra et al., 2012). The 5-HT_{1B} receptor serves as the terminal autoreceptor whose activation serves to decrease 5-HT release from the pre-synaptic serotonergic cell at the synapse (Sari et al 1997; Adell et al 2001). Whole-tissue samples of BNST show relatively high levels of expression of 5-HT_{1B} mRNA, whereas single cells from the same region do not express this transcript (Guo et al 2009). This finding indicates that 5-HT_{1B} receptors within the BNST are likely localized presynaptically and so manipulations targeted at these receptors should be selective to afferents into the BNST. In order to investigate the role of 5-HT signaling within the BNST on the anxiogenic actions of cocaine, this study employed the use of a selective 5-HT_{1B}

agonist infused directly into the BNST in an attempt to cause a local inhibition of 5-HT release, without disrupting 5-HT effects in other brain regions.

Materials and methods

Subjects

The subjects were 86 male Sprague–Dawley rats (Charles River Labs, Hollister, CA) weighing approximately 300g at the time of surgery. Of the 86 animals, 69 were used in experiment one, and 17 were used in experiment two. Rats were pair housed within a temperature-controlled (22°C) vivarium maintained on a reverse 12-h light/dark cycle (lights on at 2000 hours) and had *ad libitum* access to both food (Purina Rat Chow) and water throughout the duration of the experiment. Animals were handled daily for at least 7 days prior to surgery. All methods were conducted in strict adherence to the *NIH Guide for the Care and Use of Laboratory Animals* and were approved by the UCSB Institutional Animal Care and Use Committee.

Surgery

Rats were deeply anesthetized with an intramuscular injection of ketamine and xylazine (56.25 and 7.5 mg/kg, respectively; Abbott Laboratories) and fitted with an indwelling intravenous catheter (13 mm of Silastic tubing, 0.3 mm inner diameter, 0.64 mm outer diameter; Dow Corning). Catheters were inserted into the right jugular vein, secured in place by silk sutures, and subcutaneously passed to a

threaded guide cannula (catalog #313G; Plastics One) that exited though a 2 mm hole on the animal's back. The guide cannula was in turn cemented to a 3 cm square piece of Mersiline mesh (Bard) that was laid flat subcutaneously on the animal's back where it was sutured in place. All animals were also fitted with bilateral intracranial guide cannulae (22 gauge, 9 mm; Catalog #313GA/SPC; Plastics One) stereotaxically aimed 1 mm above the BNST using the following coordinates relative to bregma: AP -0.4, ML ±3.5, and DV -6.2 from skull surface with a lateral inclination of 15° (Paxinos and Watson, 2005). Cannula were secured to the skull surface with 4 screws sealed in dental acrylic. During surgery, each animal received the non-opiate analgesic flunixin meglumine, (2mg/kg s.c. at a concentration of 5 mg/ml in saline) to control for post-surgical pain and saline for rehydration (3.0 ml s.c.). The catheters were flushed with Timentin (50mg/0.25ml i.v.) and heparinized saline (6.25IU, 0.1 ml i.v.). Intracranial guide cannula were protected with aluminum dust caps (catalog #303DCA; Plastics One) while i.v. catheter access ports were sealed with a small section of PE50 tubing that had one side melted shut placed over the catheter tip and protected with stainless steel stand-offs (McMaster Carr).

After surgery, catheter patency was maintained through daily flushing with 0.1 ml of Timetin antibiotic (10 mg in 0.1 ml, i.v.), followed by 0.1 ml of heparinized 0.9% physiological saline. Animals were allowed to recover for at least 7 days prior to behavioral testing and were handled daily during their recovery. Catheter patency was assessed periodically through observation of the loss of the righting reflex after intravenous injection of the fast-acting barbiturate, methohexital

(Brevital, 2.0 mg/kg/0.1 ml). Animals that were found to be unresponsive to Brevital prior to the start of behavioral testing were re-implanted with a new catheter using the left jugular vein and given an additional 4 days for recovery. If catheter patency failed during the course of behavioral testing, that animal was removed from data analysis; a total of twelve animals were removed due to catheter failure.

Drugs

Cocaine hydrochloride (provided by the National Institute on Drug Abuse) was dissolved in 0.9% physiological saline and sterile filtered through a 0.2μm filter (ThermoScientific). Cocaine was diluted to a dose of 1 mg/kg delivered in a volume of 0.1 ml over a period of 4.3 s via a 10ml syringe nested in a motorized syringe pump (Razel Scientific Instruments). The dose of 1 mg/kg intravenous cocaine was chosen based upon previous work from our laboratory showing its ability to consistently produce optimal runway behavior and consistent results in the Place Conditioning test (Raven et al 2000; Ettenberg 2004; Ettenberg and Bernardi 2006; Wenzel et al 2011; Wenzel et al 2013; Wenzel et al 2014).

The 5-HT_{1B} agonist, CP 94,253 dihydrochloride (Sigma-Aldrich) was prepared in a vehicle solution of aCSF (l-Ascorbic Acid 0.35g/L, NaCl 8.47g/L, KCl .20g/L, $MgCl_2$ .20g/L, $CaCl_2$ .18g/L, NaH_2PO_4 .276g/L, Na_2HPO_4 .5362g/L) for intracranial infusion at a concentration of 2μg/μl for the high dose and 1μg/μl for the low dose. Each subject was assigned to a single dose condition and administered 0.5μl/side of either 0μg, 0.5μg or 1.0μg per side. CP 94,253 was chosen for this

study, as it shows the greatest degree of selectivity for 5-HT_{1B} over other receptors in the 5-HT_1 family (Koe et al 1992). The doses of agonist selected were chosen based on studies that have shown behavioral effects with intracerebral administration of this compound (De Almeida et al 2006; Veiga and Miczek 2007). In experiment two, only the high dose (1.0µl) and vehicle were tested.

Apparatus

Operant testing was conducted in two identical wooden straight-arm runways. Each apparatus measured 155cm (L) x 15cm (W) x 40cm (H). On opposite ends of the straight alley were identically sized start and goal boxes (each measuring 24cm X 25cm X 40cm) separated from the middle runway section of the apparatus by retractable doors. Along the interior length of the alley were 13 infrared photodetector-emitter pairs positioned in the walls 16 cm apart from one another. Input from these photocells was fed through an Any-Maze interface (Stoetling) to a laptop computer running AnyMaze software, which recorded the subjects' location in the runway in real time throughout each trial. In addition, above each runway were two magnetic rails that ran in parallel down the entire length of the apparatus. Seated between the rails was a flow-through plastic swivel (375–22PS; Instech Laboratories) that connected the animal's intravenous catheter to a syringe in the drug delivery pump via plastic tubing (PE50). The swivel was fitted with to a wide/flat Plexiglas collar that prevented it from falling through between the two magnetic rails. Affixed to the bottom of the collar was a small disc magnet whose polarity was aligned opposite to that of the rails. The opposing polarities of

the swivel and the rails served to slightly levitate the swivel assembly above the rails thereby allowing the animal to freely move about the apparatus with minimal resistance from the drug line. For a more detailed description of the runway apparatus see Geist and Ettenberg (1990).

Procedures

One day before the initiation of runway testing, subjects were acclimated to the apparatus by placing them individually into the start box and permitting them to freely explore the apparatus for 10 min (the goal door remained closed to prevent entry into the goal box).

After acclimation, 16 single daily runway trials were conducted. Two separate experiments were performed. In experiment one, CP94,253 was delivered as a Pretreatment (10 mins prior to each runway trial). Experiment two was conducted in the same manner, except CP94,253 was delivered as a post-treatment (5 minutes after each runway trial). In the pretreatment condition, subjects were individually removed from their home cages, internal infusion cannula were inserted into their head-mounted intracranial guide cannula, and slow bilateral infusions (0.5 µl/side) of one of the two doses of CP 94,253 (0.5 or 1.0µg/side) or vehicle was applied directly into the BNST. Infusions were delivered over 120s via a 25µl Hamilton syringe seated in a motorized syringe pump (KD Scientific), connected to 28 gauge injectors (catalog #313LI/SPC; Plastics One) that were securely screwed into the guide cannula and left in place for an additional 60 s following infusion to permit diffusion of the drug away from the injection tip.

Animals remained in the infusion cages for a total of 10 min prior to the start of runway testing. Each subject was then attached to the IV drug delivery system and placed into the start box of one of the two runways where, after 5 s, the start door was opened and the trial initiated. All trials for a given subject were conducted in the same runway. Animals were free to traverse the runway until they entered the goal box at which point the goal door was automatically closed behind them and an IV infusion of 1.0 mg/kg cocaine (in 0.1 ml) was administered over 4.3 s. Subjects remained in the goal box for 5 min after the infusion and were then disconnected from the drug delivery system and returned to their home cage. If an animal did not enter the goal box within 10 minutes on a given trial, they were gently pushed into the goal box and left in place for 5 minutes. To maintain catheter patency, animals were flushed with 10mg/0.1ml timentin followed by 0.1ml heparinized saline after removal from the apparatus.

The animals in experiment two (post-treatment control) were treated identically, with the exception of being immediately placed into the runway and receiving their intracranial infusion 5 minutes after entering the goal box and getting their cocaine infusion. Additionally, only the 1.0μg dose and vehicle were used in the second experiment. This condition was included to serve as a control for any non-specific anxiolytic effects of the treatment.

Three dependent measures were recorded on every trial. “Start latency” was defined as the time required for the animal to leave the start box (i.e., time to break the first infrared photodetector-emitter in the alley) once the start door was

opened. “Total Run Time” was defined as the amount of time required for the rat to enter the goal box, *after* it had left the start box (i.e., the difference between the first photobeam break in the alley and entry into the goal box). “Retreats” were counted as the number of times an animal halted its forward motion and retreated back at least the length of two photodetector-emitters (i.e., at least 32cm). On trials where an animal was pushed into the goal box, the end latency used to calculate “Total Run Time” was recorded as 600s.

Spontaneous locomotor activity

To ensure that central application of CP 94,253 treatment did not produce nonspecific alterations in the response capacity of the subjects, animals from the runway experiment were examined in a test of spontaneous locomotor activity. Locomotor behavior was measured in 12 identical Plexiglas chambers each measuring 20 cm(L) x 40 cm(W) x 20 cm(H) (Kinder Scientific). Chambers were lined with 15 infrared photodetector-emitter pairs evenly spaced along their long axis and 7 along their narrow axis, each located 8 cm from the floor of the chamber. Movement within the chamber produced photobeam interruptions that were recorded by a desktop computer running custom software (Kinder Scientific). At the start of testing, all animals were allowed to acclimate to the locomotor chambers for 60 min. Rats were then removed from their testing chambers and received bilateral microinjections of either the high or low dose of CP94,253. Each animal received the same dose of the drug or vehicle as in runway testing. After intracranial injections,

animals were immediately returned to the locomotor chamber for a 60 min test session.

Histology

After completion of behavioral testing, animals were euthanized with an overdose of sodium pentobarbital and phenytoin sodium solution (Euthasol; Virbac) and perfused with 60mL PBS followed by 60mL 4% PFA. Brains were removed and post-fixed in 4% PFA for 48h before being transferred to 30% sucrose for at least 72h to cryoprotect, then sliced on a cryostat (CM1800; Leica) into 40μm frozen sections which were then mounted on 1.5% gelatin-coated slides and stored at -20°C before staining. Slides were Nissl stained in a thionin solution and viewed under magnification to determine cannula placement within the BNST. A subject's inclusion in this experiment required strict histological confirmation of bilateral placements directly above the target brain areas under investigation (see Fig. 5). This necessitated the removal of any subjects from the study with cannula that were not both appropriately situated in brain and any animals exhibiting significant damage (necrosis) around the injection site; this decision was made by an individual (A.E.) blind to the group assignment of the animal.

Statistical Analysis

The data for each dependent measure (Start Latency, Run Time and Retreat Frequency) were individually subjected to two-factor (Group X Trial) analyses of variance (ANOVA) conducted using SPSS 23 (IBM). Separate analyses were conducted for each experiment. Twelve animals were removed due to catheter

failure, four were removed due to clogged cannulae, and six due to illness/infection. An additional seventeen animals were removed due to improper cannulae placements. A total of 31 animals were included in the analysis for experiment one, and 16 animals for experiment two.

Results

Experiment One

Figure 2 depicts the mean (± SEM) start latencies of all groups across the 16 runway trials. The ANOVA revealed a significant main effect of trial ($F_{(15,420)}$=7.044, p<.001), but no significant effect of group ($F_{(2,28)}$=.255, p = .776) and no significant Group x Trial interaction ($F_{(30,420)}$=.605, p = .952, indicating that all animals reliably decreased their start latencies over the course of the experiment.

Analysis of retreat frequencies (Fig. 3) revealed a significant main effect of Group ($F_{(2,28)}$=5.069, p = .013) and a significant main effect of trial ($F_{(15,420)}$=3.283, p< .001). The was no significant Group x Trial interaction ($F_{(30,420)}$=1.432, p = .093). Post-hoc analysis of retreat data using Fischer's Least Significant Difference showed that both the low dose (p = .008) and high dose (p = .010) were different than vehicle, while the differences between doses of the agonist were not significant (p = .935). This effect is also apparent from representative spatio-temporal records as, compared to vehicle, the treated groups reliably entered the goalbox with minimal retreats and shorter latencies (see Figure 1).

The ANOVA conducted on runtime data similarly showed an effect of treatment (Fig 4.). There was a main effect of group ($F_{(2,28)}$=3.509, p = .044). The effect of Trial did not reach significance ($F_{(15,420)}$=1.429, p = .130), and there was no Group x Trial interaction ($F_{(30,420)}$=1.223, p = .197).

Spontaneous Locomotor Activity

Two factor ANOVAs (Group x Time) were also computed on data obtained from locomotor testing. The analysis of scores obtained during baseline testing showed a main effect of time ($F_{(11,19)}$=61.041, p<.001) but no effect of group ($F_{(2,29)}$=.826, p = .448) or group x time interaction ($F_{(22,319)}$=1.086, p = .360) indicating that all animals showed normal and comparable habituation to the novel locomotor-chamber environment.

The data from the 5-HT_{1B} agonist challenge, using the same dose each animal received during runway testing, similarly showed a main effect of time ($F_{(11,18)}$=22.69, p<.001) but no effect of group ($F_{(2,28)}$=1.051, p = .363) and no significant group x time interaction ($F_{(22,308)}$=1.451, p = .089). These results indicate that treatment with the agonist does not produce any non-specific motoric effects that could have confounded the interpretation of the behavioral measures obtained in the runway.

Histology

Slides were examined under a projection light microscope to verify correct placements of the intracranial guide cannula over the anterior segments of the

BNST. Due to the extreme heterogeneity of BNST subnuclei (Kim et al., 2013; Stamatakis et al., 2014; Ventura-Silva et al., 2012), very strict histological confirmation of placements was necessary. The highlighted portion of figure 5 indicates the location of placements considered to be correctly located. Any animal that could not be verified to have both its cannula located in the highlighted regions was removed from analysis. A total of 17 animals were removed from the study either due to cannula being located in the wrong region or the inability to accurately verify correct placement of one or both cannula.

Experiment Two

The second experiment was conducted to test for non-specific anxiolytic effects of treatment with CP94,253 into the BNST. Just like in experiment one, treatment with the 5-HT_{1B} agonist 5 mins after receiving the cocaine infusion was able to attenuate retreats, as shown in Fig 8. There was a significant main effect of Group ($F_{(1,14)} = 7.963$, $p = .014$), and a significant main effect of Trial ($F_{(15,210)} = 6.155$, $p < .001$), as well as a significant Group x Trial interaction ($F_{(15,210)} = 2.586$, $p = .001$). Since only one dose and vehicle were used, no post-hoc analysis was conducted.

Similar to experiment one, the analysis for Start Latency showed a significant main effect of Trial ($F_{(15,210)} = 2.865$, $p < .001$), and no main effect of Group ($F_{(1,14)} = 4.202$, $p = .060$), as depicted in Fig 7. There was also no Group x Trial interaction ($F_{(15,210)} = 1.120$, $p = .340$).

The analysis of Total Run Time data (Fig. 9) revealed a significant main effect of Trial ($F_{(15,210)} = 4.458$, $p<.001$) and a significant main effect of Group ($F_{(1,14)} =$

5.246, p = .038), with no significant Group x Trial interaction ($F_{(15,210)}$ = 1.287, p =.213).

Discussion

Cocaine has been shown to produce both rewarding and aversive effects (see introduction for references). The present study sought to explore the role of 5-HT signaling within the BNST in modulating cocaine's aversive and anxiogenic effects. The impact of 5-HT_{1B} activation was examined in a runway model of drug self-administration, which is uniquely capable of evaluating both the positive and negative effects within the same animal on the same trial and in the same apparatus. Runway results confirm findings from previous studies that show that animals running for IV cocaine delivered upon goal-box entry develop a characteristic pattern of retreats, reflecting an approach/avoidance conflict about receiving a stimulus having mixed positive and negative effects (see Ettenberg et al 1999; Ettenberg 2004; Raven et al 2000). Pretreating animals with a selective 5-HT_{1B} agonist into the BNST significantly decreased expression of retreat behaviors, suggesting a role for BNST-serotonin in modulating the aversive/anxiogenic properties of cocaine. Importantly, treatment with the agonist did not have a significant effect on the animals' spontaneous locomotor activity, indicating that the reduction in retreats was not due to a nonspecific sedative effect of the treatment.

Previous studies on the role of 5-HT_{1B} receptors in cocaine addiction have demonstrated an involvement of this receptor in increasing the reward value of

cocaine (Parsons et al 1998; Filip et al 2010; Miszkiel et al 2011). The study by Filip et al, showed that intra-VTA injections of a 5-HT_{1B} antagonist impaired cocaine discrimination in dose dependent manner, while application of a 5-HT_{1B} agonist enhanced discrimination of lower doses of cocaine. The agonist alone also showed partial substitution for cocaine, suggesting an involvement of these receptors in the subjective effects of cocaine. In the study by Parsons et al., both systemic and intra-ventricular treatment with multiple different 5-HT_{1B} agonists produced operant behaviors that mirror what is seen when increasing the unit dose of cocaine under both a fixed-ratio and progressive-ratio schedule of reinforcement, suggesting 5-HT_{1B} activation produces an increase in the reward value of cocaine. Additionally, viral vector mediated overexpression of 5-HT_{1B} receptors in the efferent projections from the nucleus accumbens to the VTA enhanced locomotor sensitization, as well as the strengthening the development of conditioned place preferences to low doses of cocaine (Neumaier et al 2002).

The current study suggests a possible explanation for such results in that one would expect the "net" reward value of cocaine to reflect the relative strengths of the drug's positive and negative actions. If agonist activity at 5-HT_{1B} receptors within the BNST is in fact contributing to the aversive/anxiogenic effects of cocaine, then would expect the "net" positive experience for the animals to be enhanced. Indeed, the fact that the animals' start latencies were unaltered by the infusion of CP94,253 into the BNST, suggests that the motivation to seek the cocaine was unaffected, while the reduction in retreats suggests that the negative consequences

of cocaine were reduced. A decrease in the negative properties of cocaine even when combined with no change in the reward value of the drug, should nevertheless predict a more positive experience for the subject.

There are a number of important limitations that must be considered in the interpretation of these findings. First, and perhaps most importantly, is that the 5-HT_{1b} receptors are not exclusively located on 5-HT pre-synaptic elements -- they also exist as heteroreceptors on the terminals of glutamatergic neurons that synapse within the BNST (Guo and Rainnie 2010). Thus, it's possible that the observed behavioral effects are due to alterations in glutamate transmission within the BNST. The inhibition of excitatory glutamatergic inputs into the BNST via 5-HT_{1B} activation could provide an alternative explanation of the present results -- that is, if the glutamtergic projections are necessary to activate BNST cells and produce an anxiogenic state, then it is not possible to conclusively determine whether the observed reduction in retreat behavior was due to the agonist's effects on 5-HT or to impaired functioning of an excitatory "driver" input. Future studies to address this question could employ local lesions, viral and/or optogenetic manipulations of 5-HT fibers in combination with 5-HT_{1B} agonist treatment, to see if the drug is still effective when applied to this region in absence of any serotonergic input.

To provide insight onto the mechanisms of reduction of the animal's approach avoidance conflict, the second experiment was conducted in order to test whether the compound was acting as a simple anxiolytic (drugs like diazepam have also been shown to reduce approach-avoidance anxiety, see Ettenberg & Geist,

1991), or was able to selectively attenuate the negative state produced by the cocaine "crash". Since the agonist treatment was delivered after each runway trial, and therefore could not have influenced the animal's behavior during that trial, the observed results suggest that the treatment was indeed able to selectively attenuate the negative effects of cocaine. Thus, it could not be argued that the 5-HT treatment simply reduced the animal's anxiety prior to being placed in the runway, and hence the reductions in retreats reflect a reduced anxiogenic/conflict state independent of any interaction with cocaine.

Follow up studies could incorporate other tests of anxiety-like or depressive-like behaviors to determine whether activation of 5-HT_{1B} within the BNST produces an anxiolytic effect in the absence of cocaine.

Additionally, future studies are planned to investigate the role of 5-HT signaling in the BNST using optogenetic methods to stimulate or inhibit release of serotonin with precise temporal control. By targeting the DRN-BNST pathway with the use of a cre-inducible opsin in combination with a cre-retrograde tracer, 5-HT signaling within the BNST can be selectively manipulated at different times and locations within the apparatus to gain a better understanding of how this neurochemical projection is able to modulate both the appetitive and aversive effects of cocaine. Furthermore, this strategy will also allow for specific modulation of 5-HT systems without the "off-target" effects that are ubiquitous to pharmacological manipulations. This greater degree of both temporal and

neurochemical specificity will allow for the precise dissection of the circuits involved with all aspects of cocaine's behavioral effects.

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Figures

Figure 1. Sample Spatio-Temporal Records

Fig. 1- Sample Spatio-Temporal Records

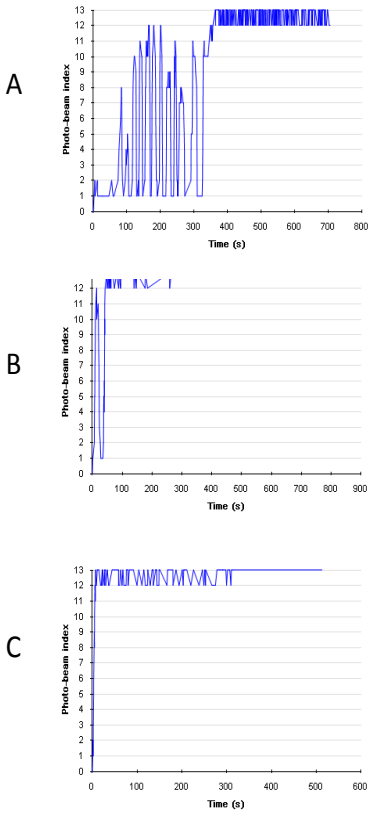


Fig. 1 Sample Spatio-Temporal Records depict the animal's location within the alley over time. Photobeam 1 was located in the start box, while photobeam 12 and 13 were located in the goal box. Crossing photobeam 13 in the goal box trigged infusion of 1.0mg/kg cocaine and closing of the door, confining the animal to the goal box.

5-HT1B ACTIVATION IN THE BSNT ATTENUATES THE NEGATIVE EFFECTS OF COCAINE

Animals were pretreated with (A.) Vehicle (B.) 0.5µg CP94,253 or (C.) 1.0µg CP94,253. All sample graphs were acquired on Trial 14, once fairly stable retreat behavior had developed. Peaks in the graph correspond to retreats, i.e., a reversal in direction away from the goal box. Retreats were defined as at least two consecutive photobeams crossed in the negative direction after at least 3 crossed moving forward.

Figure 2. Start Latencies- Experiment One

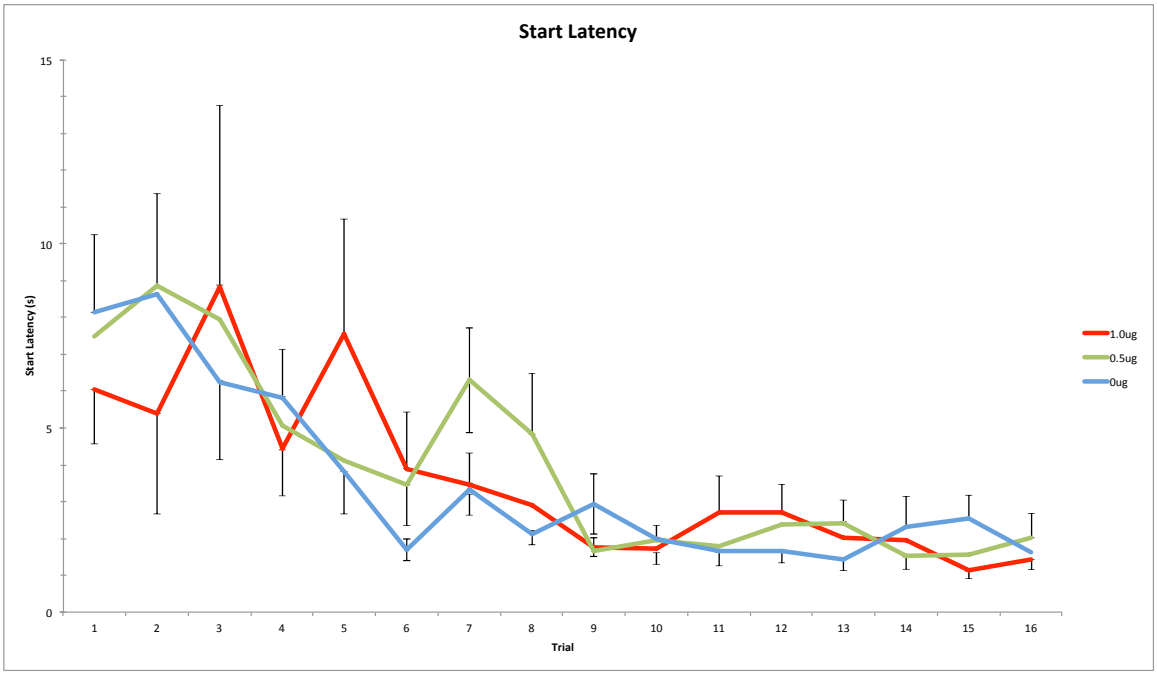


Fig 2. Group Mean (±SEM) start latencies of animals running a straight alley for single daily infusions of 1.0mg/kg cocaine after pretreatment with CP94,253. A total

of 32 animals were included in the analysis. All groups showed faster start latencies over the course of the experiment.

Figure 3. Retreat Frequency- Experiment One

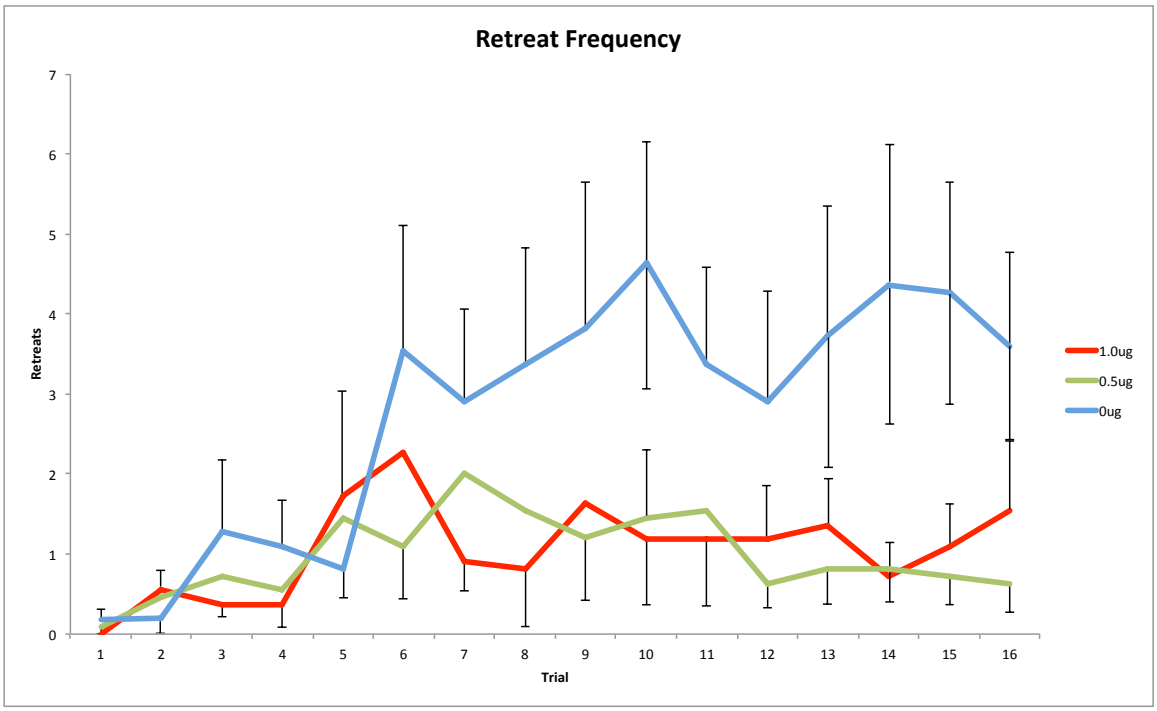


Fig 3. Group Means (±SEM) of retreat frequency data. Retreats were defined as crossing 2 photobeams back after moving forward at least 3 photobeams. A total of 33 animals (n=11 per treatment group) were included in the analysis. Both high and low doses of the 5-HT_{1B} agonist (CP94,253) were able to attenuate retreat behavior.

Figure 4. Total Run Time- Experiment One

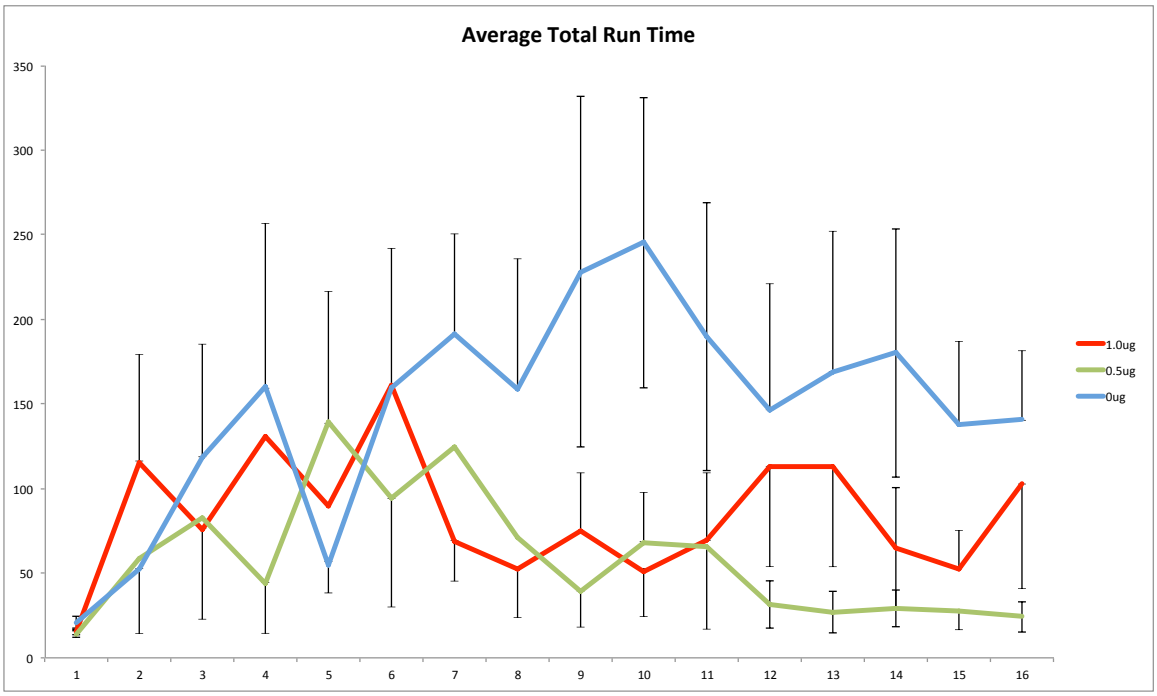
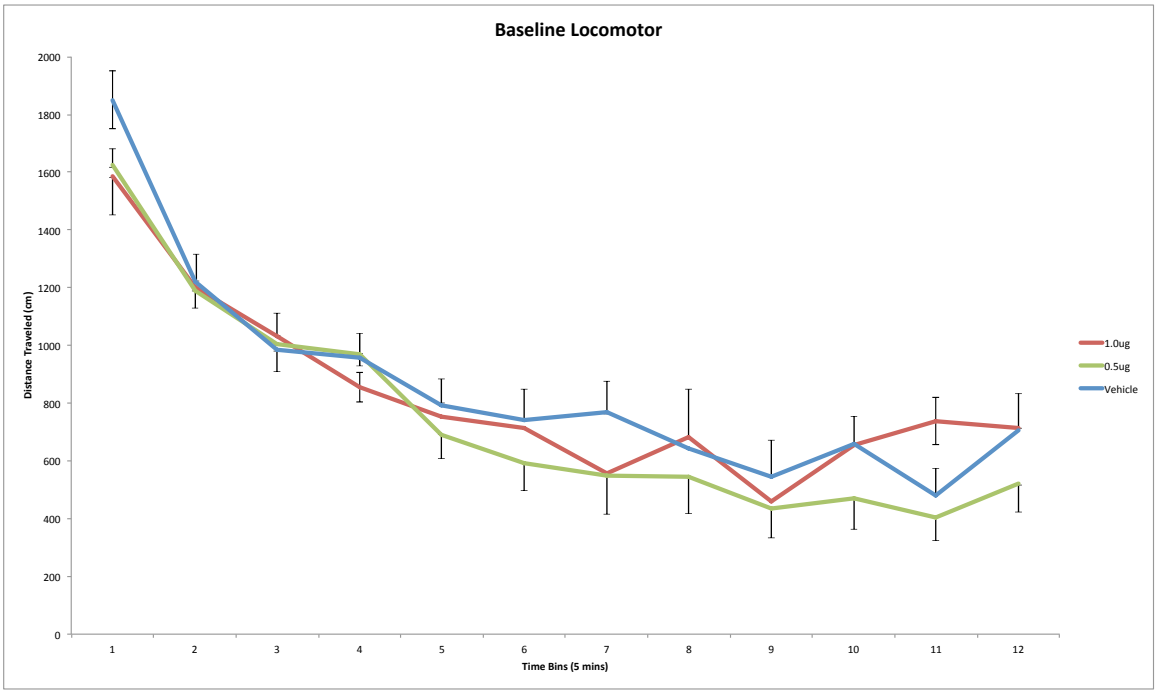


Fig 4. Group Means (±SEM) of Total Run Time data obtained in the runway. Total run time was calculated as Latency to break photobeam 13 (i.e., enter the goal box) minus the latency to break photobeam 2 (i.e., the start latency). A total of 33 animals were included in the analysis (n=11 per treatment group).

5-HT1B ACTIVATION IN THE BSNT ATTENUATES THE NEGATIVE EFFECTS OF COCAINE

Figure 5. Locomotor Data

A.



B.

5-HT1B ACTIVATION IN THE BSNT ATTENUATES THE NEGATIVE EFFECTS OF COCAINE

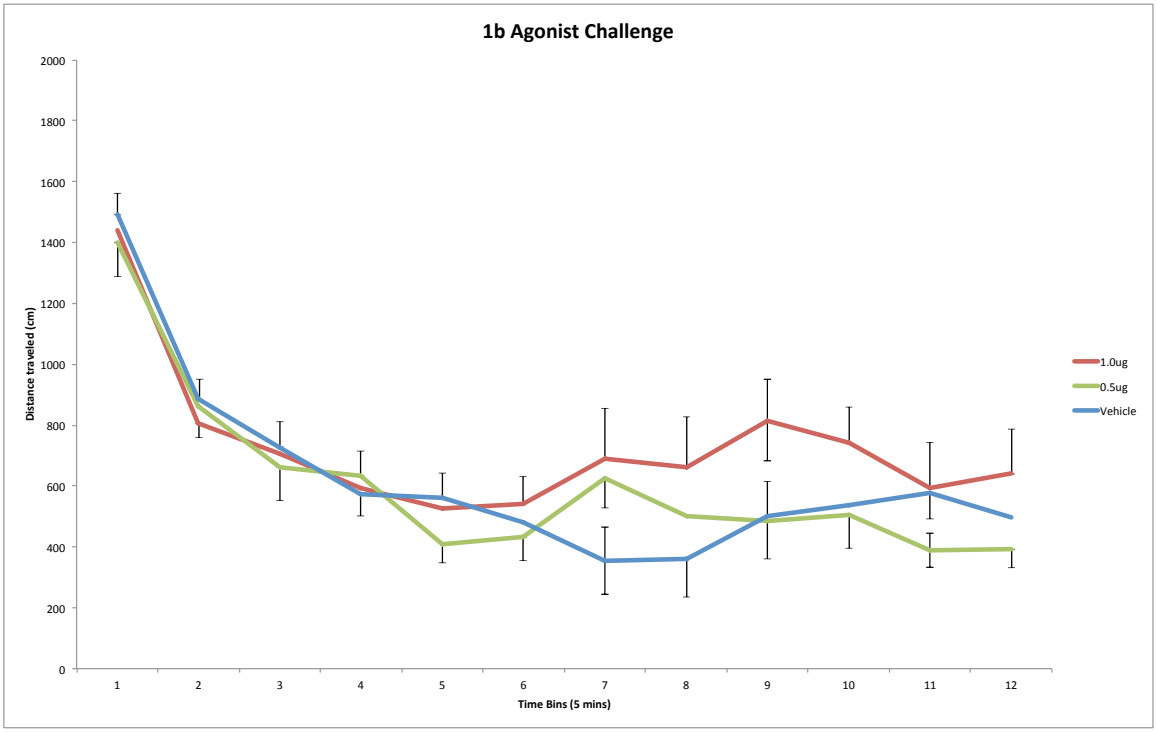


Fig 5. Group Means (±SEM) of spontaneous locomotor activity acquired during habituation (A) and after treatment with CP94,253 (B). Total distance traveled over each one-hour test was averaged into 5 min bins. All groups showed normal habituation to the novel environment (activity monitors) and no significant differences were observed after challenge with CP94,253.

Figure 6. Histology

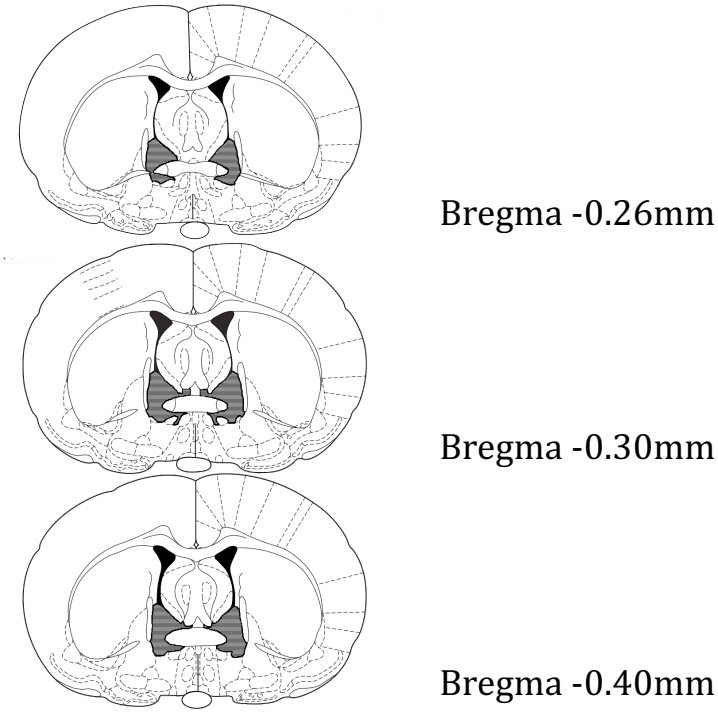


Fig 6. Histological confirmation of cannula placements. Shaded areas indicate regions where successful cannula placements were identified. Animals which could not be verified to have both cannulae located in the target region were excluded from analysis. Figure adapted from Paxinos and Watson (2005).

Figure 7. Start Latency- Experiment Two

5-HT1B ACTIVATION IN THE BSNT ATTENUATES THE NEGATIVE EFFECTS OF COCAINE

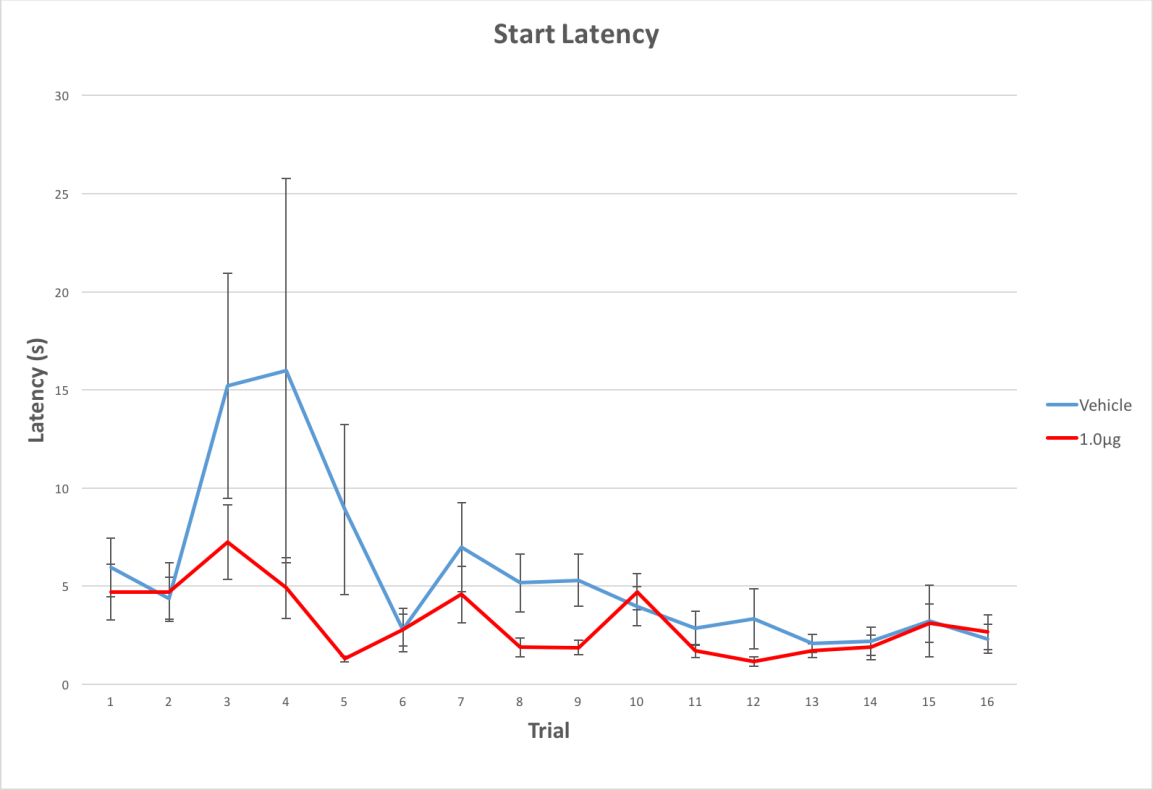


Fig 7. Group Mean (±SEM) start latencies of animals running a straight alley for single daily infusions of 1.0mg/kg cocaine. Animals were treated 5 mins after each trial with CP94,253 or Vehicle. Both groups showed faster start latencies over the course of the experiment.

Figure 8. Retreats- Experiment Two

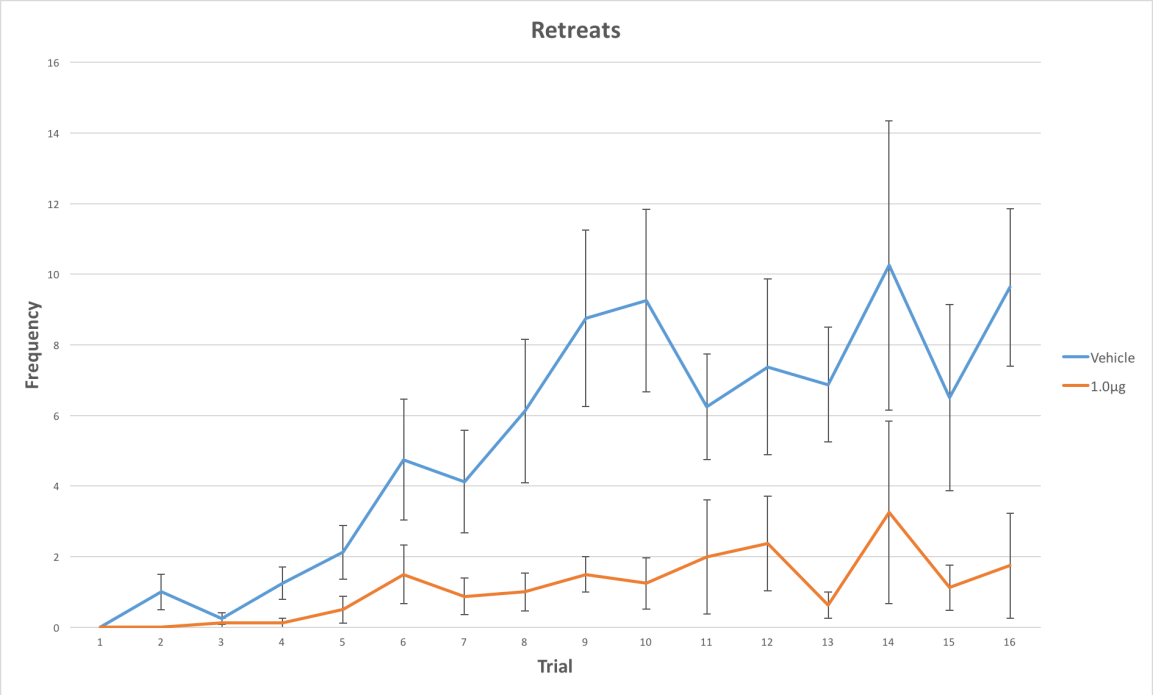


Fig 8. Group Means (±SEM) of retreat frequency data for experiment two. Retreats were defined as crossing 2 photobeams back after moving forward at least 3 photobeams. Treatment with CP94,253 was able to attenuate retreat behavior when delivered 5 mins after each trial.

Figure 9. Total Run Time- Experiment Two

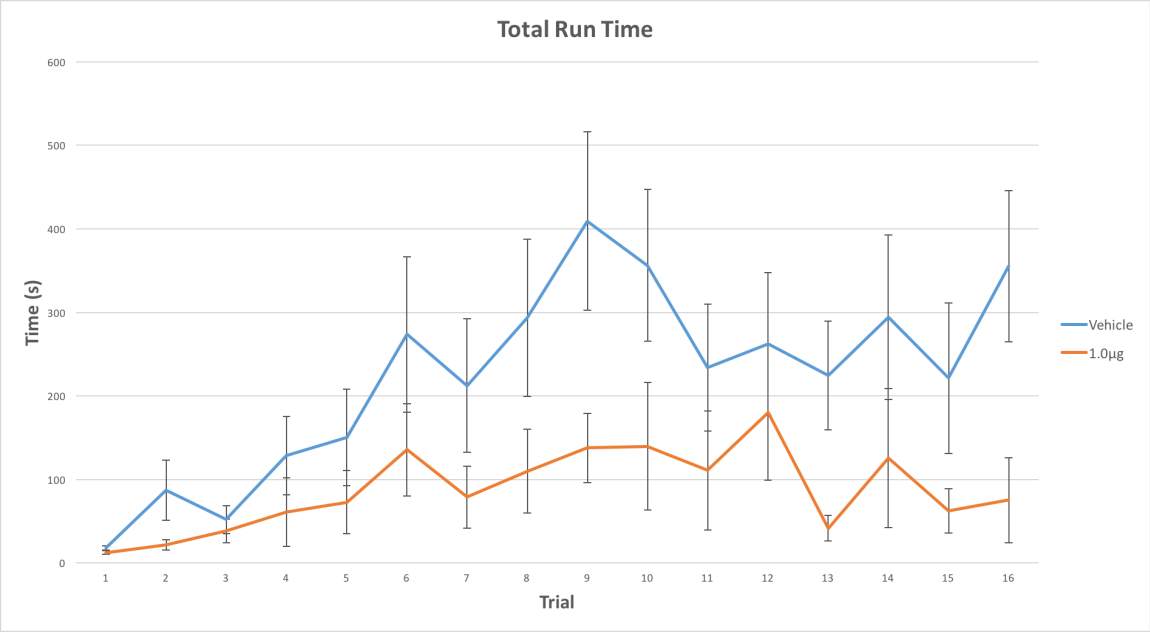


Fig 4. Group Means (±SEM) of Total Run Time data obtained in the runway during experiment two. Total run time was calculated as Latency to break photobeam 13 (i.e., enter the goal box) minus the latency to break photobeam 2 (i.e., the start latency).