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The effects of medial prefrontal cortex infusions of cocaine in a runway model of drug self-administration: evidence of reinforcing but not anxiogenic actions

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Abstract

In previous work we have shown that rats running a straight alley for intravenous (i.v.) or intracerebroventricular (i.c.v.) injections of cocaine develop an ambivalence about entering the goal box that results from cocaine's mixed reinforcing and anxiogenic properties. What remains unclear is whether or not cocaine's opposing properties stem from actions on a common neuronal system or from dual actions on separate systems – one related to reward and another to anxiogenic responses. One way to address this question is to deliver cocaine into discrete brain areas as a means of assessing whether or not the positive and negative effects of the drug can be spatially dissociated. Given the putative role of mesocorticolimbic dopamine pathways in the mediation of cocaine-reinforced behavior, the current study examined the cocaine-seeking behavior of rats permitted to run an alley once each day for bilateral medial prefrontal cortex microinjections of cocaine (0.0, 12.5, 25 or 50µg/0.5 µl per side) delivered upon goal-box entry. The results demonstrated that undrugged animals are highly motivated to seek medial prefrontal cortex cocaine without any evidence of negative or anxiogenic effects at any dose. These results are therefore consistent with suggestions of a medial prefrontal cortex involvement in the reinforcing actions of cocaine, and indicate that the dual and opposing actions of the drug can be dissociated and hence may be mediated by the drug's actions on separate neuronal systems.

Keywords

intracranial self-administration; drug reward; runway; opponent processes; operant behavior

1. Introduction

In addition to its well documented positive reinforcing properties, cocaine has been shown to have strong negative affective consequences that are unmasked as the initial euphoric actions of the drug subside. For example, while animals develop conditioned preferences for locations paired with the immediate effects of i.v. cocaine, they learn to avoid places associated with the effects of the drug present 15 min post i.v. injection (Ettenberg and Bernardi, 2007; Ettenberg et al., 1999; Knackstedt et al., 2002). Humans also report that the initial “high” from cocaine

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is often followed by a strong aversive “crash” that is characterized by feelings of anxiety, agitation and strong drug craving (Anthony et al., 1989; Resnick et al. 1977; Rohsenow et al., 2007; Spotts and Shontz, 1984; Williamson et al., 1997).

As a means of investigating the dual nature of cocaine’s actions, our laboratory developed a runway model of drug self-administration that is sensitive to both the positive and negative properties of cocaine in the same animals during the same test session (e.g., Ben Shahr et al., 2008; Ettenberg and Bernardi, 2006; Ettenberg and Geist, 1991, 1993; Knackstedt and Ettenberg, 2005; Raven et al., 2000). Animals trained to run a long straight alley once a day for goal box administration of i.v. cocaine develop a unique behavioral profile characterized by a growing ambivalence about entering the goal box as testing proceeds (e.g., see review by Ettenberg, 2004). This is operationally defined by the occurrence of “retreat behaviors” where animals rapidly traverse the alley and approach the goal, but stop at the threshold of the goal box, hesitate, and then rapidly retreat back to the start box. Retreat behaviors develop over the course of the training, increase in frequency over trials, and have been shown to represent a form of “approach-avoidance conflict” about entering a goal box that has concurrent positive and negative associations (e.g., Ettenberg, 2004; Geist and Ettenberg, 1997; Miller, 1944).

The approach-avoidance conflict observed in animals running an alley for i.v. cocaine, has also been shown to develop in rats reinforced by intracerebroventricular infusions of cocaine (Guzman and Ettenberg, 2007), suggesting that both the positive and negative properties of cocaine may involve central mechanisms of action. Several important questions stem from these findings. First and foremost, it is of interest to determine whether or not the same or separate neuronal systems mediate the reinforcing and anxiogenic properties of cocaine. One way to address this issue is to examine the impact of intracranial cocaine delivered to discrete brain areas as a means of assessing whether or not the positive and negative effects of the drug can be spatially dissociated. Given the putative role of the mesocorticolimbic dopamine system in the mediation of drug-reinforced behavior (e.g., Ikegami and Duvauchelle, 2004b; Thomas et al., 2008; Volkow et al., 1999; Wise, 2004), the current study examined the motivational impact of intracranial cocaine delivered directly into one of the terminal regions of this system – i.e., the medial prefrontal cortex (mPFC). This brain region was selected as the target of our initial investigation since the mPFC has been shown to modulate the positive reinforcing properties of i.v. cocaine (e.g., Di Pietro et al., 2008; McGregor et al., 1996; Olsen and Duvauchelle, 2006) and to readily support intracranial self-administration of the drug (e.g. Goeders and Smith, 1983, 1984, 1986, 1993; Goeders et al., 1986; McBride et al., 1999; see review by Tzschentke, 2000). Additionally, intra-PFC cocaine reportedly does not activate the hypothalamic-pituitary-adrenal (HPA) axis (Ikemoto and Goeders, 1998) and lesions of the mPFC that disrupt cocaine-induced conditioned place preferences, have no effects on conditioned taste aversions (Isacc et al., 1989) – studies that suggest an involvement of mPFC in the positive but not the aversive effects of cocaine. In the current work, we report that undrugged animals will readily seek cocaine each day by quickly traversing a runway and entering a goal box where bilateral intracranial infusions of the drug are delivered directly into the mPFC. Additionally, and perhaps most importantly, our results showed that, unlike the behavioral impact of i.c.v. or i.v. cocaine, animals running for intra-mPFC cocaine exhibited no evidence of retreat behaviors at any of the doses tested. These data therefore suggest that the anxiogenic and reinforcing actions of cocaine can be dissociated and hence support the view that the drug’s dual actions are mediated by separate neuronal and/or neurochemical systems

2. Materials and Methods

2.1 Animals

Forty-two male albino Sprague Dawley rats (weighing 340–470 g at time of surgery) were obtained from Charles River Laboratories (Wilmington, Massachusetts, USA) to serve as subjects. Each animal was individually housed within a temperature-controlled (23 °C), 12 hour light-dark vivarium environment (lights on at 0700 hours). Animals were provided *ad libitum* access to food and water throughout the experiment. The animals' care and all experimental procedures were reviewed and approved by the University of California at Santa Barbara's Institutional Animal Care and Use Committee and are in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

2.2 Surgery

Each rat was stereotaxically implanted with bilateral intracranial guide cannulae (Plastics One, Roanoke, Virginia, USA, 22 gauge, 8-mm long) during deep anesthesia induced by continuous inhalation of isoflurane gas (4% for induction and 1.5%–2.5% for maintenance). Each animal was also administered single injections of 0.04mg/kg/im atropine to prevent respiratory congestion and the non-opiate analgesic flunixin meglumine (FluMeglumine; Phoenix Pharmaceuticals, Belmont, California, USA) (2.0 mg/kg/sc) to control post-surgical pain. The intracranial guide cannulae were stereotaxically aimed 1.0 mm above the mPFC using the following coordinates relative to bregma (with the tooth bar set at 3.2mm: AP = + 2.7, ML = +/- 0.5, DV = - 3.0 from skull surface; coordinates from Paxinos and Watson, 1986). The cannulae were secured to the skull with the use of dental cement and four stainless steel screws. Once the dental cement hardened, an obturator was inserted into each guide cannula to seal the opening and thereby maintain cannula patency and reduce the risk of infection. Finally, before returning animals to their home cages for recovery, each subject was injected s.c. with 3.0 ml of 0.9% physiological saline to prevent dehydration. Animals were permitted to recover from surgery for at least 7 days prior to the initiation of runway testing. During this period, a topical antibiotic cream (Neosporin; Pfizer, New York, NY, USA) was applied to each rat's wound to aid the healing and to prevent infection.

2.3 Runway Apparatus

All trials were conducted in two identical wooden straight-arm runways (155 L × 15 W × 40 cm H). At opposite ends of each runway were equal-sized start and goal boxes (24 L × 25 W × 40 cm H). Suspended above and along the entire length of each runway apparatus were two magnetic rails spaced 3 cm apart and between which a flow-through swivel rested. A disc-shaped Plexiglas collar was affixed to the swivel to prevent it from falling between the rails. Attached to the bottom of the collar was a pot magnet whose polarity was arranged to repel that of the magnetic rails thereby creating a low-resistance system in which the swivel literally floated a few centimeters above the rail surface. As an animal ran down the runway it pulled along and behind it the swivel assembly one end of which was connected by PE 50 tubing to two 25µl Hamilton syringes (each seated within a Razel Co. syringe pump) and the other end to two internal cannulae that were inserted into (and extended 1 mm beyond) the animal's implanted bilateral guide cannula. This arrangement permitted the subjects complete freedom of movement within the alley while maintaining their connection to the drug delivery system (for a more detailed description of the runway apparatus see Geist and Ettenberg, 1990). Imbedded in the walls along the length of the runway were 13 infrared photodetector-emitter pairs whose output was sent to a Windows-based personal computer running custom software that identified the animals' position in the apparatus in real time throughout each trial. The first photodetector-emitter pair was located within the start box and the final pair was located within the goal box. The remaining 11 photodetector-emitters pairs were set equally spaced along the walls of the alley. To ensure common environmental/olfactory cues on each trial, the animals

were tested in the same order and in the same apparatus each day. Upon completion of testing, the runway walls and floors were wiped clean with a weak ethanol solution each day, and clean trays of fresh bedding (to catch the animals' droppings) were placed beneath the entire length of the runway.

2.4 Drug reinforcer

Cocaine hydrochloride (Sigma Co., St. Louis MO) was dissolved in a vehicle solution of cerebrospinal fluid and injected intracranially by Razel infusion pump in a volume of 0.5 μ L/side over 74 s. The cerebrospinal fluid was prepared by combining 2.15 g NaCl, 42.5 mg CaCl₂, 50 mg KCl, 50 mg MgCl₂ in a volume of 250 mL of nanopure water resulting in a pH of 4.7. Each animal received the same dose of cocaine (delivered bilaterally into the mPFC) on each trial: 50 μ g (n = 11), 25 μ g (n = 10), 12.5 μ g (n = 10), or 0.0 μ g cocaine (n = 10). These doses were selected both on the basis of previous work demonstrating conditioned place preferences with i.c.v. cocaine (e.g., Hemby et al., 1994; Morency and Beninger, 1986), and from our own cocaine studies where doses of 25 μ g i.c.v. or greater generated evidence of mixed positive+ negative approach-avoidance retreat behaviors in the runway (Guzman and Ettenberg, 2007).

2.5 Procedure

Prior to testing, all rats were placed individually into one of the two runways and allowed to acclimate to the start box and alley portion of the apparatus during a single 20 min session. A closed goal door prevented the animals from accessing the goal box during acclimation. Once assigned in this way, each animal was tested in the same runway throughout the duration of the experiment. Runway testing was initiated on the day following the acclimation session and consisted of 15 single daily trials. On each trial, the subject was connected to the drug-delivery system by threading the bilateral internal infusion cannulae into the implanted intracranial guide cannulae mounted on the animal's head. The subject was then placed into the start box of the runway and, after 5 s, the start door was opened and the trial thereby initiated. Animals were permitted to freely travel the length of the alley. Upon goal box entry, the goal door was automatically raised from below the floor (thereby restricting the subject to the goal box), and the bilateral intracranial cocaine injections were administered. Five minutes post-injection, each animal was removed from the goal box, disconnected from the drug delivery system, and returned to its home cage.

2.6 Dependent measures

There were two dependent measures collected for each animal on every trial: run times and retreat behaviors. Run times (defined as the time required for the animal to enter the goal box after it has left the start box) have long been used to assess the motivation of animals to seek positive incentive goal box stimuli (e.g., Beach and Jordan, 1956; Capaldi and Robinson, 1960; Kintsch, 1962; Olds, 1956; Stellar and Gallistel, 1975), while "retreat behaviors" provide an index of the approach-avoidance conflict that develops when animals approach a goal-box having mixed positive and negative properties, such as food and shock (e.g., Geist and Ettenberg, 1997; Miller, 1944). As reviewed in the Introduction of this paper, animals running for i.v. or i.c.v. cocaine behave comparably to animals working for food+shock suggesting that the drug has mixed positive and negative qualities. A major goal of the current study was to assess whether or not cocaine delivered into the mPFC produces a similar behavioral profile to that observed with other routes of drug administration. Retreat behaviors were therefore assessed on every trial and defined as a stop in a subject's forward motion followed by a retreat back towards the start box by a length of at least two infrared photodetector-emitter sensors (i.e., approximately 35 cm). Both run times and retreat frequency were automatically calculated by computer from the raw data provided via the infrared photobeam system.

2.7 Histology

After completion of the experiment, animals were euthanized by overdose of sodium pentobarbital (50mg/ml IP) and perfused transcardially with 60 ml of 0.9% physiological saline followed by 60 ml of 10% Formalin. Brains were removed, stored in 10% Formalin, and subsequently sliced into 40 μ m frozen sections that were mounted on gelatinized slides and stained with 0.1 % cresyl violet acetate. The slides were then visually inspected under magnification to determine the locations of the guide cannula tips within the brain.

3. Results

The results of the histological analysis confirmed the location of the cannula immediately above the PFC in all but four animals. Those four subjects were therefore removed from the data analysis. Figure 1A depicts the mean run times ($\pm$ S.E.M.) of each group of rats (corresponding to different doses of intra-mPFC cocaine reinforcement) over the course of the two weeks of runway testing. Note that, for clarity, and to reduce some of the inherent inter-trial variability, each data point in the figure represents the average performance of each group over two consecutive days of testing. A two-factor (Group $\times$ Trial) mixed design Analysis of Variance (with repeated measures on one factor) computed on the data from Figure 1A revealed a statistically significant main effect of Group [$F(3, 38) = 3.76, P = 0.019$] with no main effect for Week nor a Group $\times$ Week interaction ($P > .05$). To illustrate the source of the “group” effect, the average run times exhibited over the course of the entire two-week test session were computed for each group (see Figure 1B). Post-Hoc Newman Keuls comparisons confirmed that each of the three cocaine-reinforced groups exhibited faster run times than did the non-reinforced control group ($P < .05$).

Figure 2A shows the mean ($\pm$ S.E.M.) number of retreats produced by each group during each two-day interval over the entire course of the experiment. A two-factor (Group $\times$ Trial) mixed design Analysis of Variance computed on these data identified a statistically significant main effect for Group [$F(3, 38) = 7.65, P < 0.001$], but once again there was no main effect for Trials and no Group $\times$ Trial interaction. The average total number of retreats made by each group during the entire two-week test period is illustrated in Figure 2B. As was the case for the run time measure, all three cocaine-reinforced groups were different than (i.e., made fewer retreats compared to) the non-reinforced control animals ($P < .05$). Two additional pieces of information are worthy of note with respect to the frequency of retreat behaviors: first, the incidence of retreats in the control group was very small – on average, animals in this group made only 8 total retreats over the course of the entire test session (i.e., less than 1 per trial); additionally, the location of those retreats reflected an exploratory pattern of activity and not an ambivalence about entering the goal box – only 28% of the retreats occurred in the immediate vicinity of the goal box entry.

4. Discussion

Rats provided the opportunity to traverse an alley and enter a goal box for the delivery of bilateral mPFC infusions of cocaine, exhibited faster running over trials in much the same way that animals do when running an alley for natural reinforcers (e.g., Beach and Jordan, 1956; Ettenberg and Camp, 1986; Ettenberg and Horvitz, 1990; Lopez et al., 1999; Nofrey et al., 2008). These results therefore compliment and extend the findings of previous investigators who have reported that rats will readily learn to lever-press for self-administration of cocaine directly into the mPFC (e.g., Goeders and Smith, 1983, 1984; 1993; Goeders et al., 1986; McBride et al., 1999; see review by Tzschentke, 2000). However, unlike the lever-press procedure where animals work to maintain their drugged state, the runway protocol assesses the motivation of *undrugged* animals to return to a location (the goal box) where on previous trials cocaine had been delivered. Thus, by testing animals a single trial per day the procedure

permits the measurement of drug-seeking behavior independent of the potentially confounding direct pharmacologic effects of the drug. So, for example, the increases in operant running observed over trials cannot be easily accounted for by cocaine's psychomotor stimulant properties nor by the potential development of drug-induced sensitization since the animals' behavior was assessed prior to the delivery of the drug reinforcer. Additionally, while conditioned locomotor behavior has been observed in "sensitized" animals injected with a vehicle and placed into environments in which they had previously received injections of cocaine (e.g., Post et al., 1992) in the current study there was no vehicle injection to induce the conditioned hyperactivity (i.e., no conditioned stimulus), and the behavioral measures were assessed in the runway which, unlike the goal box, was never directly paired with cocaine infusions. Thus, while the mPFC has been implicated in the development of cocaine-induced behavioral sensitization (e.g., Steketee, 2005), it seems unlikely that this phenomenon can account for the current changes observed in runway behavior observed over trials. The current results are also not likely to be attributable to the well-known anesthetic properties of cocaine (e.g., Fairbanks and Fairbanks, 1983; Verlander and Johns, 1981) since other local anesthetic agents (e.g., lidocaine) are not readily self-administered into the mPFC (Goeders et al., 1986). We conclude, therefore, that the shortened run times that occurred over the course of training reflect an increase in drug-seeking motivation stemming from the animals' association of the goal box with the positive reinforcing actions of mPFC cocaine.

An additional finding in the current study is that mPFC cocaine had no aversive, negative or anxiogenic actions. In previous work we have shown that rats trained to run a straight alley once-a-day for i.v. or i.c.v. cocaine develop an ambivalence about entering the goal box (Ettenberg and Geist, 1991; Guzman and Ettenberg, 2004, 2007) that is behaviorally comparable to that observed in hungry rats approaching a goal box associated with food + shock (Geist and Ettenberg, 1997). Subjects approach the goal box, stop at the entry point, and retreat back toward the start box in an "approach-avoidance" conflict pattern (e.g. Miller, 1944) that putatively results from associations of the goal box with cocaine's dual opposing (i.e., rewarding and anxiogenic) properties. Note that such retreats are not related to some inherent aversive or negative properties of the goal box itself since when no reinforcer is applied the incidence of retreat behaviors is very low and distributed throughout the alley and not at the threshold of the goal box entryway. For example, in the current study the non-reinforced control group exhibited on average only 8 total retreats across the entire two weeks of testing (under 0.6 retreats/rat/trial) and distributed those retreats in a manner most reflective of exploratory behavior with only 28% occurring in the direct vicinity of the goal box entrance. In contrast, our prior work with i.v. and i.c.v. cocaine using the same apparatus and a comparable testing protocol, resulted in retreats that were 3 to 4 fold higher in frequency and occurred in closer proximity to the goal box entryway (Guzman and Ettenberg, 2004, 2007). Thus, cocaine-induced retreats reflect a quantitatively and qualitatively different profile to non-reinforced runway behaviors.

While others have demonstrated anxiogenic properties of cocaine (e.g., Rogerio and Takahashi, 1992; Simon et al., 1994; Yang et al., 1992) our self-administration runway results are unique in reflecting both positive and negative actions of cocaine in the same animal on the same trial with the same dose (Ettenberg, 2004). In the current study, goal-box delivery of intra-mPFC cocaine yielded minimal levels of retreat behavior even at doses (e.g., 25 μ g and 50 μ g) that produced a high frequency of retreats when injected i.c.v. (Guzman and Ettenberg, 2007). This suggests that the site at which cocaine acts to produce its anxiogenic/negative properties lies somewhere outside of the mPFC. The current results are consistent with the demonstration that mPFC lesions disrupt the development of cocaine-induced place preferences, an index of drug reward, (Isacc et al., 1989; Tzschentke and Schmidt, 1998), while leaving intact conditioned taste aversions, an index of cocaine's negative properties (Isaac et al., 1989). Additionally, the anxiogenic response to systemically administered cocaine, which is reflected by a drug-induced

activation of the hypothalamic-pituitary-adrenal (HPA) axis (Borowsky and Kuhn, 1991; Goeders, 2002; Rivier and Vale, 1987; Sarnyai et al., 2001), is not observed when the drug is applied directly into the mPFC (Ikemoto and Goeders, 1998). Together these data would seem to indicate that intra-mPFC cocaine produces reinforcing but not aversive/anxiogenic consequences and, as such, suggest that the dual opposing actions of cocaine are likely a result of dissociable actions of the drug on separate neural systems.

The source of the reinforcing actions of intra-mPFC cocaine remains unclear. The mPFC is one of the primary terminal regions of mesocorticolimbic dopamine system emanating from the ventral tegmental area -- a system that many have implicated as a putative final common pathway for reward (e.g., see reviews by Ikegami and Duvauchelle, 2004b; Thomas et al., 2008; Volkow et al., 1999; Wise, 2004). Early on, Goeders and Smith (1986) demonstrated that lever-pressing for intra-mPFC cocaine could be extinguished by selective 6-hydroxydopamine lesions of the dopamine terminals within the region and then reinstated by substituting dopamine for cocaine. Dopamine self-administration into the mPFC could then in turn be attenuated by adding the dopamine D₂ receptor antagonist, sulpiride, to the solution -- all of which suggested that mPFC dopamine receptors were critical for sustaining cocaine intracranial self-administration into this brain region (Goeders and Smith, 1986). There is, however, uncertainty about the precise nature of the role of the mPFC in the reinforcing actions of systemically applied cocaine. For example, disruption of mPFC dopamine function either by selective 6-hydroxydopamine lesions (Martin-Iverson et al., 1986; McGregor et al., 1996) or intracranial administration of dopamine receptor antagonist drugs (Di Pietro et al., 2008; Olsen and Duvauchelle, 2006) reportedly fails to block the self-administration of i.v. cocaine, although such manipulations appear to alter the motivation to sustain reinforced behavior and may therefore suggest a modulatory role for the mPFC. In contrast, selective dopamine lesions within the nucleus accumbens have long been known to produce significant and long-lasting impairments in i.v. cocaine self-administration (e.g; Pettit et al., 1984; Roberts et al., 1980; Zito et al., 1985). Since the mPFC sends projections directly to both the nucleus accumbens and the ventral tegmental area (Gabbott et al., 2005; Sesack et al., 1989, 1992) the latter of which in turn projects to the nucleus accumbens, it may be that the reinforcing actions of intra-mPFC cocaine ultimately depend upon changes in neuronal activity within in the nucleus accumbens (Harden et al., 1998; Ishikawa et al., 2008; Tzschentke, 2001; Tzschentke and Schmidt, 2000). Consistent with this notion are reports that self-administered i.v. cocaine increases dopamine levels both in the nucleus accumbens and mPFC (Ikegami and Duvauchelle, 2004a; Tanda et. al., 1997). However, there is also evidence suggesting that increases in dopamine levels within the nucleus accumbens can be produced by *disruption* of dopamine within the mPFC. For example, depletions of mPFC dopamine produced by intracranial administration of 6-hydroxydopamine have been shown to increase the motivation to self-administer cocaine as measured in a progressive-ratio test (McGregor et al., 1996), and potentiate the nucleus accumbens dopamine response to natural reinforcing stimuli (Mitchell and Gratton, 1992). Hence, while it is clear that the mPFC and nucleus accumbens interact in the production of motivated and reinforced behaviors, the precise nature of that relationship remains to be elucidated.

In summary, the current study has demonstrated that undrugged animals running a straight alley for intra-mPFC infusions of cocaine exhibit progressively stronger drug-seeking behavior (faster running) over trials. Additionally, and unlike i.v. and i.c.v. injections of cocaine, mPFC cocaine produces no evidence of adverse, negative or anxiogenic consequences suggesting that such effects of cocaine are not an inevitable consequence of the drug's delivery, but rather represent an action of the drug on a dissociable mechanism that is likely distinct from the drug's reinforcing actions. In that context, it is relevant to note that systemic cocaine administration is associated with increased levels of norepinephrine and corticotropin-releasing factor within the extended amygdala -- including the bed nucleus of the stria terminalis, shell of the nucleus

accumbens, and central nucleus of the amygdala –systems that have long been implicated in behavioral responses to stress and anxiety (e.g., Koob, 2003; Sarnyai, 1998; Smith and Aston-Jones, 2008). Our laboratory is therefore investigating the hypothesis that cocaine's actions on neurochemical systems within the extended amygdala may be responsible for its anxiogenic properties.

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Figure 1.

Panel A -- Mean ($\pm$ S.E.M.) run times per group expressed per trial during the 15 days of runway testing. For clarity, each data point was plotted from the average of two successive days/trials. The doses represent the bilateral infusions of intra-mPFC cocaine that were delivered once a day upon the animal's entry into the goal box. Panel B -- Mean ($\pm$ S.E.M.) run times per group per trial averaged over the entire two weeks of testing. All three cocaine groups entered the goal box more quickly than the non-reinforced 0 μ g control animals (* P <.05).

Figure 2.

Panel A -- Mean ($\pm$ S.E.M.) retreat frequency per group over each two-day period of the test session (i.e., each data point represents the average 2-day retreat total made by animals in each group). Panel B -- Mean ($\pm$ S.E.M.) total retreat frequency exhibited by each group over the entire two-week test period. The doses represent the bilateral infusions of intra-mPFC cocaine that were delivered once a day upon the animal's entry into the goal box. All three cocaine groups exhibited fewer retreats compared to the non-reinforced 0 μ g control animals (* $P < .05$).