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ScienceDirect
Pharmacology, Biochemistry and Behavior 87 (2007) 171 – 178
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Effects of buspirone on the immediate positive and delayed negative properties of intravenous cocaine as measured in the conditioned place preference test

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Received 30 April 2006; received in revised form 16 April 2007; accepted 19 April 2007
Available online 4 May 2007

Abstract

In prior work, we have demonstrated that the behavioral effects of cocaine adhere to the predictions of the opponent-process theory of drug action. Animals develop conditioned place preferences for distinct locations paired with the immediate effects of IV cocaine, but learn to avoid places paired with the effects present 15-min post-injection. It was of interest to assess the putative role of 5-HT in producing the negative properties of cocaine since cocaine acts to inhibit the reuptake of serotonin (5-HT) and since such actions have been associated with anxiogenic consequences. Male rats were administered a reinforcing dose of cocaine (1.0 mg/kg IV) and then placed – either immediately or after a 15-min delay – into one side of a two-compartment (black–white) conditioned place preference (CPP) box for 5-min. On alternate days, the animals received IV saline injections and were placed in the opposite side of the CPP box. This continued for eight days after which animals had experienced 4 pairings of cocaine with one side (black or white) of the CPP apparatus, and 4 saline pairings with the opposite side. Other groups of rats were treated identically except that 30-min prior to placement into the apparatus, these animals received an IP injection of saline or buspirone (a partial 5-HT_{1A} agonist) at a dose that we have shown to be anxiolytic (2.5 mg/kg IP). Control animals experienced either buspirone or saline pretreatments without cocaine. Our results confirm that animals increase the time spent on the side paired with the immediate effects of cocaine (compared to baseline), but tend to avoid the side paired with effects present 15-min post-injection. Buspirone had no effect on the immediate rewarding properties of cocaine, but completely reversed the negative properties present 15-min post-cocaine. These results are consistent with the view that attenuation of 5-HT neurotransmission (via the autoreceptor agonist properties of buspirone) can reverse the negative impact of IV cocaine.

Keywords: Drug reward; Serotonin; 5-HT; CPP; Cocaine-induced anxiety; Partial 5-HT_{1A} agonist

1. Introduction

The opponent-process theory of motivated behavior postulates that the presentation of an affective stimulus has two opposing consequences: an initial subjective experience (either positive or negative) that is subsequently replaced by a second experience whose nature is diametrically opposite to that of the original affective state (Solomon and Corbit, 1974; see also more recent variations by Baker et al., 1986; Koob et al., 1989, 1997). The consequences of cocaine administration appear to adhere to this notion of dual opposing processes. For example, human cocaine users report that the initial euphoric or rewarding state produced by the drug is typically followed in time by a state characterized by anxiety, fatigue, agitation, anhedonia and often cravings for more cocaine (Anthony et al., 1989; Spotts and Shontz, 1984; Washton and Gold, 1984; Williamson et al., 1997). Animal studies have similarly confirmed that although cocaine is self-administered (e.g., see reviews by Fibiger et al., 1992; Folton and Fischman, 1994; Morgan and Roberts, 2004; Porrino et al., 2004; Shalev et al., 2002; Wolverton, 1992), produces preferences for distinct environments in which it is administered (e.g., Bardo et al., 1995; Calcagnetti et al., 1995; Carr et al., 1989; McBride et al., 1999), and lowers thresholds for rewarding brain-stimulation (Gill et al., 2004; Gilliss et al., 2002;

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doi:10.1016/j.pbb.2007.04.014

Kenny et al., 2003), the drug also has distinct aversive/negative properties. Cocaine increases thigmotaxic behavior (Simon et al., 1994), exacerbates the behavioral effects of aversive stimuli or punishment (Dworkin et al., 1989; Fontana and Commissaris, 1989), heightens the anxiogenic response of animals in an elevated plus maze (Hayase et al., 2005; Paine et al., 2002; Rogerio and Takahashi, 1992), and potentiates the avoidance of inherently aversive environments (Costall et al., 1989). In our own laboratory, animals running a straight alley for IV cocaine demonstrate concurrent positive and negative associations with the goal box that are manifested by the development of a unique approach-avoidance conflict about entering the goal area (e.g., Ettenberg and Geist 1991, 1993; Guzman and Ettenberg, 2004; Knackstedt and Ettenberg, 2005; Raven et al., 2000). Together, the data from these and other human and animal reports clearly suggest that cocaine produces both the positive and negative actions predicted by opponent-process theory.

In a more direct test of the opponent-process model, we employed a conditioned place preference (CPP) procedure in which rats readily learn to prefer or avoid distinctive environments associated with the positive or negative properties of drug administration (e.g., Bardo and Bevins, 2000; Carr et al., 1989; Schechter and Calcagnetti, 1993; Tzschentke, 1998). We demonstrated that while animals came to prefer an environment associated with the immediate "positive" effects of cocaine, they learned to avoid the environment associated with the effects of the same dose of the drug present 15-min post-injection (Ettenberg et al., 1999; Knackstedt et al., 2002). Such results are consistent with the view that cocaine produces two opposing affective consequences — an immediate positive/rewarding state, followed temporally by a negative/aversive state (see review by Ettenberg, 2004).

Cocaine's reinforcing/rewarding properties are generally attributed to the drug's capacity to prevent the reuptake of dopamine in the terminal regions of the mesocorticolimbic system (e.g., Anderson and Pierce, 2005; Dutta et al., 2003; Zahniser and Sorkin, 2004). However, the precise nature underlying its negative properties remains unclear. For example, cocaine increases heart rate, blood pressure and respiration (e.g., Pitts et al., 1987; Schmidt et al., in press), all of which could conceivably produce or contribute to the negative subjective experience of anxiety or agitation that the drug has been reported to produce. Cocaine has also been reported to alter benzodiazepine binding in rat brain (Goeders et al., 1997; Keys and Ellison, 1999; Suzuki et al., 2000) and to activate the hypothalamic-pituitary-adrenal axis by stimulating the release of corticotropin-releasing factor (CRF), which thereby produces elevations in corticosterone and ACTH, both of which are naturally released during periods of stress and anxiety (Borowski and Kuhn, 1991; Goeders, 2002a,b; Rivier and Vale, 1987; Sarnyai et al., 1995, 2001; Sholar et al., 1998). Another mechanism through which cocaine might produce its negative/aversive effects is the serotonergic (5-hydroxytryptamine; 5-HT) system. Cocaine has potent reuptake inhibiting actions at 5-HT synapses (Filip et al., 2005; Koe, 1976; Ritz et al., 1990), and there is a growing literature implicating the activation of 5-HT pathways to the production of aversive/anxiogenic states (e.g., Abrams et al., 2004; 2005; Eison and Eison, 1994; Graeff, 2002; Griebel, 1995; Matsuo et al., 1996; Reuter and Jacobs, 1996; Rex et al., 2005; Sena et al., 2003).

The current study was therefore devised as a means of investigating the nature of the relationship between cocaine's negative/aversive actions and the drug's actions at 5-HT synapses. The tendency of animals to develop preferences for places associated with the immediate effects of cocaine and avoid places associated with the drug's delayed effects (e.g., Ettenberg et al., 1999; Knackstedt et al., 2002), was examined during challenge with the partial 5-HT_{1A} agonist buspirone. It was hypothesized that the reduction in 5-HT release resulting from buspirone's agonist actions at the 5-HT autoreceptor (Pecknold, 1994) would have no effect on the immediate "rewarding" properties of cocaine (since the onset of the negative process is presumed to be temporally delayed), but would attenuate the delayed negative properties of cocaine as measured in the conditioned place test.

2. Methods

2.1. Subjects

The subjects were 53 male Sprague Dawley rats (300–325 g at the time of surgery) obtained from Charles River Laboratories (Wilmington, MA). The animals were housed individually in metal wire hanging cages located within a secure and temperature-controlled 23 °C vivarium. The animals were gentled for a period of 7 days prior to catheterization and were provided ad libitum access to food and water throughout the study. The care and use of the animals including all aspects of the experimental protocol were reviewed and approved by the campus IACUC (Institutional Animal Care and Use Committee) for compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

2.2. Surgery

A chronic silastic jugular catheter was implanted into the jugular vein of each rat under deep isoflurane-induced anesthesia (4% for induction and 1.5–2.5% for maintenance) administered continuously via inhalation. At the time of surgery, atropine sulfate (.04 mg/kg IM) was applied to prevent respiratory congestion, and the non-opiate analgesic flunixin meglumine (FluMe-glumine; 2.0 mg/kg SC) was administered to control post-surgical pain. Catheter implantation involved making a small incision in the animal's neck to expose the jugular vein. One end of the catheter was then inserted into the vein and sutured in place. The other end was passed subdermally to the animal's back where it was fused to a threaded guide cannula (Item 313G, Plastics One) that protruded through a small 3 mm diameter opening. The guide cannula was commented to a 2-cm square piece of surgical Mersilene mesh (Ethicon) that was laid flat subdermally on the animal's back and sutured in place. Between test sessions, the open end of the guide cannula was sealed by insertion of a dummy cannula (Item 313DC, Plastics One) that screwed down securely onto the guide. The administration of intravenous

heparin or drug was accomplished with an internal cannula (Item 313I, Plastics One) that was inserted into the open end of the guide cannula (in place of the dummy cannula) and was connected by PE 20 tubing to a fluid-filled syringe containing the drug. Animals were permitted 10 days of recovery from surgery before behavioral testing began.

Immediately following the surgery, each animal was administered 50 mg IV (in .25 ml) of the antibiotic ticarcillin disodium/clavulanate potassium (Timentin) through the implanted catheter. To ensure catheter patency and reduce the risk of infection, each subject was injected daily (beginning the day after surgery) with Timentin (20 mg/.1 ml IV) followed by IV heparin (1000 IU/.1 ml prepared in .9% physiological saline) approximately 60 min after each conditioning trial. At the end of the experiment, a low dose of the fast acting barbiturate Brevital (methohexital sodium, .1 mg/kg in .1 ml) was injected through the IV catheter to confirm catheter patency.

2.3. Place preference apparatus

The conditioned place preference (CPP) box consisted of a large rectangular wooden box measuring 94 cm long × 43 cm wide × 61 cm high. Two removable walls could be put in place to create three separate compartments: on opposite ends of the apparatus were equally-sized black and white compartments (42 × 43 × 61 cm) separated by a central neutral gray compartment (10 × 43 × 61 cm). The floor of the black side of the apparatus was lined with smooth Plexiglas and the top of each wall was wiped prior to each trial with a 1.0 ml of a dilute 2% acetic acid solution (to provide a novel olfactory cue). No acetic acid odor was applied to the white compartment of the apparatus and the floor on this side was covered with wood shavings. The central gray area had a painted gray wooden floor and walls. This arrangement served to provide the animal with three distinct environments within the CPP box each differing in color, odor and floor texture. The location of the animal within the apparatus was determined in real time through the use of 15 pairs of evenly spaced infrared emitter-detectors that lined the long axis of the apparatus approximately 1.0 cm above the floor. Input from these infrared sensors was recorded by a desktop personal computer equipped with an I/O interface and running custom software. The apparatus and computer were located within a sound-attenuated room and all conditioning and testing took place under low light conditions (i.e., a single 40 W lamp located on the floor in one corner of the test room served as the sole source of illumination).

2.4. Drugs

Cocaine hydrochloride was prepared in a vehicle solution of .9% physiological saline and infused intravenously in a volume of .1 ml over a period of 4.6 s via a 10-ml syringe nested in a motorized syringe pump (Razel). The dose of cocaine employed in this study (1.0 mg/kg IV) was specifically selected on the basis of prior work in our laboratory demonstrating that this dose optimally supported operant responding in a runway model of self-administration and produced reliable conditioned place preferences, yet had demonstrable negative/aversive side effects 15-min post-injection (Ettenberg et al., 1999; Knackstedt et al., 2002; Raven et al., 2000).

The presynaptic 5-HT_{1A} receptor has been implicated as the mechanism through which several new agents produce their anxiolytic effects (e.g., Dekeyne et al., 2000; Koek et al., 1998; Millan et al., 1997; Schreiber et al., 1995). Buspirone was therefore employed in the current study both because it acts as a partial 5-HT_{1A} agonist (Pecknold, 1994) and because it has been shown to have anxiolytic actions in both human (e.g., Apter and Allen, 1999; Argyropoulos et al., 2000; Bond et al., 2003; Gale, 2002; Hellewell et al., 1999; Rakel 1990, Bohm et al., 1990; Fulton and Brogden, 1997) and animal studies (e.g., Angrini et al., 1998; Chang and Liao, 2005; Costall et al., 1988; Jelen et al., 2003; Leveleki et al., 2006; Risbrough et al., 2003; Simon et al., 1994). Buspirone was prepared in a .9% physiological saline vehicle solution and injected IP in a dose of 2.5 mg/kg (injection volume 2.0 ml/kg). This dose was carefully selected on the basis of our prior dose-response analysis where it was found to reliably decrease the approach-avoidance conflict observed in animals running a straight-arm runway for IV cocaine (e.g., Ettenberg and Bernardi, 2006).

2.5. Procedure

Approximately 10 days after catheterization, each animal was placed in the CPP apparatus for 15-min with the internal walls removed. The amount of time that each subject spent in each of the three compartments was recorded and served as an initial baseline score. After each trial, the entire apparatus was thoroughly cleaned with a dilute solution of ethanol, the wood shavings were replaced in the white compartment, and fresh acetic acid was laid down in the black compartment. Baseline data were used to assign animals to one of five groups in a manner that ensured that there were no between-group differences in preconditioning baseline performance. Conditioning then began on the day following baseline. Each conditioning trial consisted of an IP injection of .0 or 2.5 mg/kg buspirone, followed either 15 or 30 min later by an IV injection of .0 or 1.0 mg/kg cocaine, and then placement into the CPP apparatus either immediately or 15-min post-injection. It is important to note that buspirone (or saline control) injections were administered with respect to the putative onset of cocaine's subjective rewarding and aversive effects (0 and 15 min post-injection, respectively). Thus, Ss placed immediately into the apparatus after IV cocaine (or vehicle) were administered buspirone (or vehicle) 30 min prior to the IV injections, while Ss in the 15-min delay conditions were administered buspirone (or vehicle) 15 min prior to cocaine injections, but still 30-min prior to placement into the apparatus. The delay condition involved placing animals in a plastic holding cage during the 15-min period between the IV injection and placement into the CPP apparatus.

Conditioning trials consisted of four drug-place trials alternating daily with four saline-place trials. On a given trial, half the animals were administered drug and half were administered saline before being placed into either the white or black

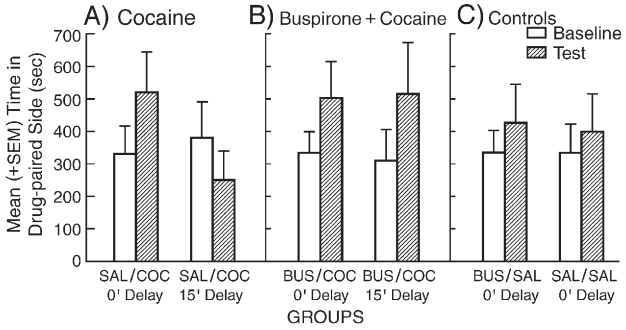

Fig. 1. Mean (+SEM) time spent (s) in the drug-paired side of the place preference apparatus for each group during a pretreatment baseline trial and an identical test trial conducted after drug-place conditioning. Panel A shows the effects of pairing an environment with the immediate effects of cocaine (SAL/COC 0′ Delay) or with the effects present 15-min post-injection (SAL/COC 15′ Delay). Panel B depicts these same two conditions in animals pretreated with buspirone. Panel C depicts the behavior of control animals that received no place-cocaine conditioning (a BUS/SAL 0′ Delay group and a SAL/SAL 0′ Delay group).

compartments (with the walls inserted to restrict the Ss to that conditioning environment) for 5 min. On alternating days, Ss that received drug on the previous day were now administered saline and placed into the opposite side of the apparatus than on the previous day's trial (and vice versa). The order of conditioning trials (drug or saline) and the compartment-type (black or white) were counterbalanced both within and between groups. On "saline" trials, animals received an IP injection of saline followed 15 or 30 min later by an IV infusion of saline; the subjects were then placed into the CPP apparatus. On "drug" trials animals experienced either a saline or buspirone IP injection followed by a saline or cocaine IV injection, and then were placed into the CPP apparatus. Additionally, on "drug" trials, each of the six groups experienced a different drug treatment according to the following protocol: one group ($n=10$) was administered saline IP followed 30 min later by an IV injection of cocaine and then placed immediately into the conditioning apparatus (a SAL/COC 0′ Delay group); the second group ($n=10$) received IP saline, followed 15 min later by IV cocaine, followed 15-min later by placement into the CPP box (a SAL/COC 15′ Delay group); the third group ($n=9$) received buspirone 30 min prior to cocaine and was then immediately placed in the conditioning apparatus (BUS/COC 0′ Delay); the fourth group ($n=8$) received buspirone, then cocaine 15 min later, and then was placed into the CPP box after a 15 min delay (BUS/COC 15′ Delay); and the fifth group received buspirone ($n=8$) followed 30 min later by IV saline followed by immediate placement into the apparatus (BUS/SAL 0′ Delay). A sixth group was later added ($n=8$) to compare the effects of the various treatment groups against a non-drug control. These animals were administered IP saline, followed 15 min later by IV saline, and then followed immediately by placement in the CPP apparatus (SAL/SAL 0′ Delay). For the data analysis in this last group, one of the two sides of the chamber was randomly selected for each animal to serve as the "conditioned side" (paired with IV saline) against which shifts from preconditioning baseline could be compared.

Eight days of place conditioning therefore yielded for each animal four drug experiences (as described immediately above) paired with one of the two distinct compartments, and four saline experiences paired with the other compartment. On the last (10th) day of the experiment, a final 15-min drug-free preference test was conducted in all animals precisely as described for the initial baseline trial. Conditioned place preferences or aversions were subsequently identified as reliable shifts toward or away from the drug-paired environment on test day relative to preconditioning baseline.

By way of summary, the first two groups served as a replication of prior work and were expected to demonstrate learned preferences for the side associated with the immediate effects of cocaine (the SAL/COC 0′ Delay group) or learned aversions of the side associated with the effects of cocaine present 15-min post-injection (the SAL/COC 15′ Delay group). The next two groups were intended to determine the effects of buspirone pretreatment on the immediate rewarding or delayed aversive effects of IV cocaine (the BUS/COC 0′ Delay group and the BUS/COC 15′ Delay group, respectively). The fifth and sixth groups were control conditions intended to assess whether or not buspirone pretreatment was capable of producing learned place preferences or aversions in and of itself (the BUS/SAL 0′ Delay group), and whether or not there were shifts in place preference that could occur as a function of the handling and testing procedures independent of drug treatments (the SAL/SAL 0′ Delay group).

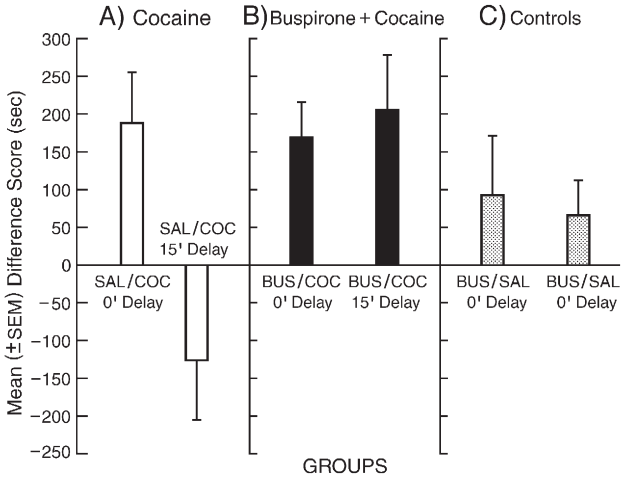

Fig. 2. Mean (+SEM) Difference Scores (Test Trial — Baseline trial) of each of six groups of rats. Bars above the zero-line represent conditioned shifts in preference toward the drug-paired side of the apparatus, while the single bar below the line represents a conditioned avoidance of the drug-paired environment. As shown in Panel A, animals exhibited conditioned place preferences (CPP) for the side of the apparatus paired with the immediate effects of IV cocaine (SAL/COC 0′ Delay), but tended to avoid the side paired with the effects of the drug present 15-min post-injection (SAL/COC 15′ Delay). In Panel B, buspirone pretreatment produced no evidence of an enhancement of cocaine's rewarding effects (BUS/SAL 0′ Delay vs SAL/COC 0′ Delay), but did reverse the delayed negative properties of cocaine (BUS/COC 15′ Delay vs SAL/COC 0′ Delay). Control groups (Panel C) produced no reliable shifts in preference or aversion from baseline to test.

3. Results

The mean (+SEM) time spent in the drug-paired side of the apparatus on baseline and test trials is depicted for each group in Fig. 1. A two-factor (Group × Trial) mixed-design Analysis of Variance (ANOVA) was computed on the data from Fig. 1. Although the ANOVA yielded no main effect of Group [$F(5, 470 = .97$, p = n.s.], there was a statistically reliable main effect of Trial [$F(1,47) = 12.53$, $p < .002$] and a significant Group × Trial interaction [$F(5,47) = 3.68$, $p < .008$]. The trial effect signifies a significant difference between baseline and test trial performance when averaged across groups, while the interaction confirmed that the shift in behavior from baseline to test differed in magnitude for the different groups.

To more closely examine this reliable Group × Trial interaction, mean difference scores were computed for each group, with the time in the drug-paired side of the apparatus compared on the final test trial relative to that on the initial baseline (Test — Baseline; see Fig. 2). In Fig. 2, bars above the zero-line represent shifts toward the drug-paired side (place preferences) while bars below the line represent post-conditioning shifts away from the drug-paired side (place aversions). A one-way independent group ANOVA computed on the data depicted in the figure identified a highly significant difference among the groups [$F(5,47) = 3.610$, $p < .009$] and post-hoc Tukey HSD comparisons confirmed that the tendency to avoid the side of the chamber paired with the effects of cocaine present 15 min post-injection was significantly different from the preferences that rats exhibited to the side of the chamber associated with the immediate positive effects of the drug. Thus, the SAL/COC 15′ Delay group behaved differently from the SAL/COC 0′ Delay group ($p < .02$), and from the BUS/COC 0′ Delay group ($p < .03$). Pretreatment with buspirone eliminated the delayed "negative" impact of cocaine; i.e., the BUS/COC 15′ Delay group behaved indistinguishably from the BUS/COC 0′ delay group ($p > .05$) and the SAL/COC 0′ delay group ($p > .05$) but was significantly different from the SAL/COC 15′ Delay group ($p < .02$). Finally, there were no discernable effects of buspirone pretreatment on cocaine reward since the SAL/COC 0′ Delay group and the BUS/COC 0′ delay groups behaved equivalently ($p > .05$).

It was of interest to assess whether or not the mean difference score of each group (as depicted in Fig. 2) was statistically different from the "no change" value of zero — i.e., were the shifts in place preference statistically reliable? One-tailed single-sample t-tests confirmed that the immediate effects of IV cocaine were rewarding — the SAL/COC 0′ Delay group spent reliably more time in the drug-paired environment on test day than they did on baseline (i.e., the difference score for this group was reliably different from zero [$t(9) = 2.80$, $p < .02$]). The group exposed to the delayed effects of cocaine (SAL/COC 15′ Delay) exhibited a marginally reliable aversion to the drug-paired side [$t(9) = 1.63$, $p = .06$]. This place aversion was completely reversed by pretreatment with buspirone; thus the BUS/COC 15′ Delay condition continued to show a reliable preference for the cocaine-paired environment [$t(7) = 2.79$, $p < .03$]. The BUS/COC 0′ delay group also demonstrated a cocaine-induced place preference [$t(8) = 3.61$, $p < .05$]. Finally, the two control conditions (buspirone alone and saline alone) produced no reliable shifts in preference from initial baselines (BUS/SAL 0′ Delay, $t(7) = 1.17$, $p > .05$); SAL/SAL 0′ Delay, $t(7) = 1.45$, $p > .05$).

4. Discussion

The results from the current study confirm and extend our laboratory's previous findings on the immediate and delayed actions of IV cocaine. More specifically, animals came to prefer distinct locations paired with the immediate effects of IV cocaine (conditioned place preferences) but came to avoid those locations paired with the effects of IV cocaine present 15 min post-injection (e.g., Ettenberg et al., 1999; Knackstedt et al., 2002). Although the negative effects of cocaine observed in the SAL/COC 15′ Delay group, were only marginally significant ($p < .06$), we note that: a) the current results, when added to those we have reported previously, represent the third demonstration of the negative properties of "delayed" cocaine — hence we believe the phenomenon to be real and replicable; b) the SAL/COC 15′ Delay group was the only one to demonstrate any avoidance of the cocaine-paired side; and c) this group's negative change from baseline to test trial (as revealed by the Difference Score data in Fig. 2) was significantly different from the positive effects of immediate cocaine observed in the Sal/COC 0′ Delay group and the BUS/COC 0′ Delay groups ($p < .05$). Clearly, the preference data are consistent with numerous others reports of cocaine-induced conditioned place preferences (e.g., see reviews by Bardo et al., 1995; Calcagnetti et al., 1995; Carr et al., 1989; McBride at al., 1999), while the place aversion data are likewise consistent with reports of the negative/anxiogenic side effects of cocaine in laboratory animals (e.g., Costall et al., 1989; Dworkin et al., 1989; Ettenberg and Geist 1991, 1993; Fontana and Commissaris, 1989; Hayase et al., 2005; Paine et al., 2002; Simon et al., 1994). What is unique about our work is that the positive and negative features of cocaine were demonstrated in the same apparatus, with the same dose of cocaine, and with the same experimental procedures. Thus, we maintain that the immediate effects of 1.0 mg/kg IV cocaine were rewarding, while the state produced 15 min after an injection of this same dose was aversive. This dual action of cocaine in rats, is comparable to that reported in clinical studies where human cocaine users describe the immediate effects of cocaine as highly euphoric or rewarding, while the affective state present during the subsequent "crash" is described as highly aversive and characterized by anxiety, fatigue, agitation, anhedonia and cravings (Anthony et al., 1989; Spotts and Shontz, 1984; Washton and Gold, 1984; Williamson et al., 1997).

Together, the current data, combined with previous reports from both the human and animal literatures, suggest that cocaine's actions neatly conform to the principles and predictions of the opponent-process theory of drug action (see Ettenberg, 2004). This hypothesis explicitly predicts that the initial euphoric actions of a drug will be counteracted in time by an opposing negative state that serves to return the organism back to some form of

affective equilibrium or homeostasis (e.g., originally formulated by Solomon, 1980; Solomon and Corbit, 1973,1974; see also Baker et al., 1986; Koob et al., 1989, 1997). Central to the original theory was the view that the opposing positive and negative affective states produced by drugs of abuse were mediated by independent neural processes (Solomon and Corbit, 1974). From a neurobiological perspective, cocaine's positive/rewarding actions are widely attributed to its reuptake inhibition of the neurotransmitter dopamine in the terminal regions of the mesocorticolimbic pathways that originate from cells bodies of the Ventral Tegmental Area (e.g., Anderson and Pierce, 2005; Dutta et al., 2003; Zahniser and Sorkin, 2004). However, the neurobiology of cocaine's negative/anxiogenic actions remains less clear. Cocaine has been demonstrated to alter benzodiazepine receptor binding (Goeders et al., 1997; Keys and Ellison, 1999; Suzuki et al., 2000) and to activate the hypothalamic-pituitary-adrenal axis — a system normally responsive to the presentation of stressful stimuli (Borowski and Kuhn, 1991; Goeders, 2002a,b; Rivier and Vale, 1987; Sarnyai et al., 1995, 2001; Sholar et al., 1998). Of particular relevance to the current study is the fact that cocaine is also a potent reuptake inhibiter of 5-HT (Filip et al., 2005; Koe, 1976; Ritz et al., 1990), and numerous studies have demonstrated an anxiogenic consequence of serotonergic activation. For example, animals exposed to anxiety-provoking, stressful, or aversive situations, exhibit increases in the neuronal activation of 5-HT neurons emanating from the raphé nuclei (e.g., Abrams et al., 2004; Jacobs and Azmitia, 1992; Matsuo et al., 1996; Reuter and Jacobs, 1996; Rex et al., 2005). In fact, there is a considerable literature on the role of 5-HT pathways in the neurobiology of anxiety, and the authors of several extensive reviews of this literature have each concluded that drugs acting to stimulate 5-HT neurotransmission produce anxiogenic effects, while drugs that reduce 5-HT neurotransmission tend to be anxiolytic in nature (Abrams et al., 2005; Argyropoulos et al., 2000; Eison and Eison, 1994; Graeff, 2002; Griebel, 1995).

In the current study, the putative role of 5-HT in the anxiogenic state produced by cocaine was assessed by challenge with the partial 5-HT_{1A} agonist, buspirone (Fulton and Brogden, 1997; Goa and Ward, 1986; Griebel, 1995; Pecknold, 1994). As shown in Figs. 1 and 2, the delayed-onset negative properties of IV cocaine were reflected in the rats' tendency to avoid an environment paired with the state present 15-min post-cocaine (the SAL/COC 15′ Delay group). Buspirone pretreatment, at a dose selected for its ability to reverse cocaine-induced approach-avoidance conflict in an operant runway (Ettenberg and Bernardi, 2006), completely reversed the negative properties of cocaine; i.e., the BUS/COC 15′ Delay group exhibited reliable cocaine-induced conditioned place preferences. It would seem that the negative anxiogenic effects of cocaine present 15 min post-injection were prevented by buspirone pretreatment. This result cannot easily be attributed to a simple additive positive effect of buspirone since buspirone alone produced no reliable shifts in place preference (the BUS/SAL 0′ Delay group's difference score was not reliably different from "0", i.e., from baseline). Similarly, buspirone did not simply enhance the positive effects of cocaine; the place preferences exhibited by animals for environments paired with the immediate effects of IV cocaine (SAL/COC 0′ Delay group) were essentially unchanged by the addition of the buspirone pretreatment (compare to the BUS/COC 0′ Delay group). Thus, there is no evidence in our study of buspirone producing either a positive or negative effect on its own, nor of it producing an enhancement of the positive effects of cocaine. Indeed, only buspirone-pretreated animals in the cocaine-delay condition exhibited a change in conditioned performance compared to that of saline-pretreated animals.

Other researchers have similarly shown buspirone to have no effect on place preferences produced by the immediate effects of cocaine in mice (Ali and Kelly, 1997) nor to affect the discriminative stimulus properties of cocaine (Rapoza, 1993). Neisewander et al. (1990) found that at a dose comparable to that employed here, buspirone can produce conditioned place preferences on its own. However, these researchers paired the immediate effects of buspirone with a distinct test environment, while the current study intentionally avoided this potential confound by pretreating animals 30 min prior to placement in the CPP apparatus so that the animals would be less likely to associate distinct places with buspirone injections (as confirmed by the lack of either a place preference or aversion in the BUS/SAL 0′ Delay group). Contrary to the current results, Paine et al. (2002) failed to reverse the cocaine-induced heightened anxiety of rats placed in an elevated plus maze. However, we note that the largest dose employed in their study was 1.0 mg/kg, a dose that we found previously to be ineffective at preventing cocaine-induced approach-avoidance conflict (Ettenberg and Bernardi, 2006) and far lower than that employed effectively in the current study (2.5 mg/kg).

An inherent challenge in interpreting the present results stems from the fact that buspirone is known to have anxiolytic properties in both clinical (Apter and Allen, 1999; Argyropoulos et al., 2000; Gale, 2002; Rakel 1990, Bohm et al., 1990; Fulton and Brogden, 1997) and animal studies (e.g., Angrini et al., 1998; Chang and Liao, 2005; Isogawa et al., 2005; Jelen et al., 2003; Risbrough et al., 2003; Simon et al., 1994). Therefore, one might reasonably argue that the anxiolytic actions of buspirone could have been mediated by an independent and separate neural system than the one(s) responsible for producing the anxiogenic actions of cocaine. The current data cannot refute this possibility. Nevertheless, we note that 5-HT neuronal activation has long been associated with the induction of anxiogenic states, that cocaine potentiates 5-HT neurotransmission by inhibiting the presynaptic transporter, that buspirone has the opposite effect to cocaine by serving as an agonist at the presynaptic receptor, and that buspirone has been widely reported to have anxiolytic actions in both humans and animals. When viewed in this context, the most parsimonious explanation for the current results is that the delayed negative properties of cocaine are reversed by buspirone via opposing actions on a common serotonergic substrate. Additional work is ongoing in our laboratory to further examine this hypothesis.

Acknowledgement

This work was supported by a grant from the National Institute on Drug Abuse (DA05041) awarded to AE.

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