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Cocaine conditioning: Reversal by autoreceptor dose levels of 8-OHDPAT

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ABSTRACT

In order to investigate the contribution of serotonergic effects of cocaine to Pavlovian conditioning of cocaine locomotor stimulant effects, two experiments were conducted in which groups of rats ($N=10$) received cocaine treatments (10 mg/kg) paired or unpaired to placement in an open-field environment. Initially, a cocaine conditioned locomotion stimulant effect was established. Next, additional Coc-P and Coc-UP pairings were carried out in conjunction with pretreatment injections of the 5-HT_{1A} agonist, 8-OHDPAT (0.01, 0.025 and 0.05 mg/kg) or saline. In experiment 1, the Coc-P group which received the saline pretreatment again exhibited conditioning but in the 8-OHDPAT pretreatment Coc-P group conditioning was eliminated. In the second experiment, the protocol of the first experiment was repeated but expanded in the post-conditioning phase to include an 8-OHDPAT plus the 5-HT_{1A} antagonist pretreatment Coc-P group. As in the first experiment, the 8-OHDPAT pretreatment Coc-P group did not exhibit a cocaine conditioned locomotion stimulant effect; whereas, the saline pretreatment Coc-P and the 8-OHDPAT plus WAY-100635 pretreatment Coc-P groups did exhibit the cocaine conditioned locomotion stimulant effect. These findings are consistent with an important role for serotonin in the maintenance of cocaine Pavlovian conditioned effects.

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1. Introduction

Cocaine is a highly addictive drug. The acute neurochemical effects of cocaine have been extensively investigated and it is now well-established that cocaine binds to transporters for dopamine (DAT), norepinephrine (NET) and serotonin (SERT). The occupancy of these transporter sites by cocaine prevents the re-uptake of the released transmitters; and, thereby, prolongs their activity on pre- and post-synaptic receptors. This potentiation of neurotransmitter activity in brain systems, critical for movement, reward, memory, attention and stimulus salience, can lead to persistent neurobiological (Franklin et al., 2002; Goldstein and Volkow, 2002; Goldstein et al., 2004; Le Foll et al., 2005; Lee et al., 2003; Leshner and Koob, 1999; Gignaschi et al., 2004) and behavioral effects (Lee et al., 2006). One way in which cocaine can produce lasting effects upon behavior is by Pavlovian conditioning processes. That is, the motoric and hedonic states evoked by cocaine occur in an environmental context which by Pavlovian temporal/spatial contiguity transforms these context cues into cocaine conditioned stimuli. This association between contextual cues and cocaine effects forges a cocaine memory traces which can persist long after the acute effects of cocaine have worn-off (O'Brien et al., 1992a,b). The increase in dopamine evoked by cocaine would appear to be a critical contributor to the conditioning process in that the increase in dopamine would facilitate locomotor activation and, at the same time, elicit hedonic/reward effects to reinforce and strengthen the behavioral activation (Koob et al., 1998).

The neurochemical effects of cocaine have been extensively documented for DA, particularly, in subcortical areas critical for movement and reward such as the neostriatum and nucleus accumbens. Recent reports have shown that cocaine increases 5-HT levels widely in cortical areas including the medial prefrontal cortex (mPFC) primary and secondary sensory cortices (Pum et al., 2007) and rhinal cortices (Müller et al., 2007). Cocaine, therefore, can modulate sensory processing systems (Devonshire et al., 2007) as well as brain structures that are important for memory processes. In this way, the 5-HT increases generated by cocaine contribute substantially to the transformation of cocaine associated stimuli into cocaine conditioned stimuli, critically important to the maintenance and persistence of addictive behavior (Di Ciano and Everitt, 2004).

The possible contribution of serotonin to cocaine Pavlovian conditioning has received relatively little experimental attention (Carey and Damianopoulos, 1994). In this report, we present the results of two studies designed to assess the possible contribution of cocaine induced serotonin effects to cocaine Pavlovian conditioned behavior. The strategy underlying the present experiments was to first establish cocaine conditioned stimuli and then expose animals to these conditioned stimuli to activate the cocaine memory trace in a deactivated 5-HT state so as to diminish and degrade the salience and significance of the conditioned stimuli. Accordingly, we first established cocaine

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conditioned behavior. After the cocaine conditioning was validated by a post-treatment saline conditioning test, the animals were subjected to pharmacological manipulations designed to attenuate the availability of serotonin using low dose 8-OHDPAT pretreatments (Carey et al., 2008b). The 8-OHDPAT pretreatments were administered during cocaine reconditioning. Under these conditions, the low dose centrally acting 8-OHDPAT was expected to reduce the cocaine induced serotonin response in the presence of the cocaine conditioned stimuli. The possible effect of a lowered cocaine 5-HT response upon the cocaine conditioned stimuli were then assessed in a second saline conditioning test.

2. Materials and methods

2.1. Animals

80 naïve male Sprague–Dawley rats from Taconic Farms (Germantown, NY), 4 months old and weighing approximately 400 g at the start of the experiments were used. Upon arrival, the animals were housed in individual 48×27×20 cm clear polycarbonate cages in a climate-controlled room at 22–24 °C with a 12-h dark and 12 h light cycle. During the 1st week after arrival, all animals were handled and weighed daily for 7 days. During the second week the animals received three injections (i.p.) of 0.9% saline (1.0 ml/kg) in order to acclimate the animals to the injection procedure. All experiments occurred during the 12-h light cycle (6 AM–6 PM). This protocol (IACUC 4-E) was approved by the Veterans Administration Medical Center's Subcommittee for Animal Studies.

2.2. Drugs

Cocaine hydrochloride (Sigma Chemical, St. Louis MO) was dissolved in sterile distilled H_20 to a concentration of 10.0 mg/ml. Cocaine injections were administered i.p. in a volume of 1.0 ml/kg. Saline injections (sterile 0.9% sodium chloride) were administered in a volume of 1.0 ml/kg (i.p.). ±8-OH-DPAT (Sigma Chemical, St. Louis MO) and WAY-100635 (Sigma Chemical, St. Louis MO) were dissolved in sterile H_2O to concentrations of 0.25, 0.5 and.1 mg/ml and injected s.c. in a volume of 0.5 ml/kg.

2.3. Apparatus

Behavioral tests were conducted in two identical 60 cm square by 40 cm high open-field arenas. To reduce noise and control ambient light each arena was placed in one of two smaller rooms within the main laboratory. Test room exposure was equated across groups and treatments for each experiment to control for any potential effects on behavior. In the present experiments, test room was not a statistically significant variable on any behavioral measure ($P>0.05$).The interior walls and floor of each arena were white. Two overhead 12 V projection lamps provided illumination. These were placed 50 cm above the arena floor adjacent to the video camera. Each lamp was fitted with a red filter since testing under red light conditions is less stressful, thus favoring locomotor activation as the rats are transferred from the ambient light of the vivarium to the red light of the testing room (Nasselo et al., 1998). A white noise generator (San Diego Instruments, San Diego, CA), also placed 50 cm above the arena floor, provided masking sound (75 dB). It was turned on immediately prior to placement of the animal into the test arena and turned off upon removal from the arena.

Each arena was monitored by a closed-circuit video camera (Sanyo VCB-5123B) mounted 50 cm above the arena floor. Analog video signals were digitized and analyzed by an automated video tracking system (Ethovision, Noldus Information Technology, Inc, Leesburg, VA). The accuracy of the system for distance measurements was corroborated by moving objects a measured distance and confirming that the tracking system generated the same distance. To provide contrast, the animal's head was blackened with a non-toxic marker, the camera tracked only this feature of the rat's body. data were captured at a rate of six samples per second and the input filters were set to a minimum distance of 2 cm per sample. In addition to distance measurements, the locomotion paths in the arena were recorded and these were similar to those reported previously (Dai et al., 1995). The paths recorded by the software were used to identify small repetitive movements. Such movements occurred infrequently and idiosyncratically. After each rat was marked and placed in the arena and the behavior capture session was initiated the test proceeded without the experimenters in the room. A small TV screen connected to the video system was located outside of each room. It enabled experimenters to monitor the rats throughout each test trial. At the completion of each trial, the arenas were cleaned and dried. A VHS VCR was connected to each camera to provide a complete record of an animal's behavior during a test trial. The videotapes were reviewed following completion of experimental treatments at the end of each to validate or take into account any abnormalities shown by the automated records.

2.4. Design and procedure

2.4.1. Experiment I

Four groups (N=10) were used. All animals received six 20 min saline tests to establish stable baseline locomotor levels prior to conditioning protocol treatment. The groups were then equated for baseline locomotion on the basis of the final saline test before initiation of the conditioning procedures.

Phase 1. Conditioning A. Two groups received four cocaine paired treatments (Coc P) in which cocaine (10 mg/kg) was administered immediately prior to placement in the test environment. The other two groups were cocaine unpaired groups (Coc-UP) which received saline immediately prior to placement in the test environment and cocaine (10 mg/kg) in the homecage 2 h after testing. The day after completion of this treatment protocol, all groups were given a saline conditioning test.

Phase 2. Conditioning B. On the day following the conditioning test, the groups received three additional Coc-P or Coc-UP conditioning treatment sessions to assess the effect of reduced 5-HT availability on the maintenance of conditioning. One Coc-P and Coc-UP pair was given three additional Coc-P and Coc-UP treatment sessions. The other pair also received three Coc-P and Coc-UP treatments; but, in addition, received 8-OHDPAT pretreatments 10 min before saline (Coc-UP) or cocaine (Coc-P). The three 8-OHDPAT pretreatments were: 0.01, 0.025 and 0.05 mg/kg, respectively. These treatments were designed to induce a progressive decrease in serotonin availability prior to testing. Previously, we reported (Carey et al., 2004a,b, 2008a,b) that an ascending low dose regimen of 8-OHDPAT such as that described above did not reduce the locomotion stimulant effect of cocaine. Maintenance of the unconditional response is critical in that the locomotion stimulant effect of cocaine was the unconditioned response to be conditioned. After completion of the Coc-P and Coc-UP treatments for both pairs, each group received a second saline conditioning test.

2.4.2. Experiment 2

In the second experiment, four groups ($N=10$) were used. As in the first experiment, all groups received six 20 min saline test sessions to establish stable baseline levels prior to the start of the conditioning protocol.

Phase 1. Conditioning A. The four groups were: 3 Coc-P groups and 1 Coc-UP group. In phase 1, the 3 paired groups Coc-P1, Coc-P2, Coc-P3 groups received four Coc-P treatments (10 mg/kg cocaine) and the Coc-UP group received four Coc-UP (10 mg/kg cocaine) treatments. One day after completion of this conditioning protocol, all groups received a saline conditioning test.

Phase 2. Conditioning B. The Coc-P and Coc-UP protocols were repeated with 4 additional conditioning sessions. One Coc-P group (Coc-P1) received a saline pretreatment 10 min before the same Coc-P treatment as in phase 1. Similarly, the Coc-UP group also received a saline pretreatment 10 min before the same Coc-UP treatment as in phase 1. The remaining two Coc-P groups were given drug pretreatments 10 min prior to the Coc-P cocaine (10 mg/kg) conditioning protocol. One group (Coc-P2) received 8-OHDPAT pretreatments 10 min prior to the cocaine (10 mg/kg) conditioning protocol and the other paired group (Coc-P3) received 8-OHDPAT plus WAY-100635 10 min prior to cocaine (10 mg/kg). The four 8-OHDPAT pretreatments and sequence were: 0.025, 0.05, 0.01 and 0.075 mg/kg and for the combined 8-OHDPAT plus WAY-100635, the matched dose pretreatments and sequence were: 0.025, 0.05, 0.01 and 0.075 mg/kg of 8-OHDPAT and WAY-100635 (0.025, 0.05, 0.01 and 0.075 mg/kg. Previously, (Carey et al., 2004a,b) we have shown that the WAY-100635 reverses the behavioral inhibitory effect of 8-OHDPAT when both drugs are given at the same dose level. Thus, if the 8-OHDPAT treatments were effective in blocking conditioning, then, the combined treatment with WAY-100635 should prevent this effect and be equivalent to the saline treatment. One day after completion of the additional four cocaine conditioning reacquisition treatments, all groups received a second saline conditioning test.

2.5. Statistical analysis

In experiment 1, a two-way, mixed factor, analyses of variance (ANOVA) with repeated measures was used to assess the cocaine drug treatment effects for each Coc-P/Coc-UP pair. Separate two-way ANOVAs were performed for phase 1 and phase 2 of the repeated treatment phases of the experiment. In order to compare groups in the conditioning tests, independent and paired *t*-tests were used. $P<.05$ was used as the statistical criterion for rejection of the null hypothesis. In experiment 2, two-way ANOVAs with repeated measures were performed in the cocaine treatment phases 1 and 2 of the experiment. In this experiment, the four treatment groups (3 Coc-P and 1 Coc-UP) were compared. In order to make specific group comparisons, post-hoc, Duncan multiple range tests were performed. For the saline/conditioning test results, paired *t*-tests were performed in which the locomotion scores for each group were compared to their respective baseline scores. In this way, a change from baseline in each treatment group could be assessed independently from each of the other groups. This provided a statistical assessment of the cocaine treatment effects which were not directly linked to control group performance. That is, did a particular treatment protocol change the behavior of a specific group in terms of its pretreatment baseline? This within-group analysis supplements the between-group analysis used for the conditioning results in Experiment 1.

3. Results

3.1. Experiment 1

Fig. 1A presents the phase 1 results for the saline/cocaine paired/unpaired groups ($N=10$). In phase 1 of the experiment, there were four paired/unpaired treatments followed by a saline conditioning test. As can be seen in Fig. 1A the cocaine paired treatment generated the expected locomotion stimulant effect ($F_{1,18}=19.2$, $P<.001$). The group differences were across the four treatment days and there were no day or interaction effects ($P>.05$). The first conditioning test results in Fig. 1B show that the paired/unpaired protocol induced a cocaine conditioned locomotion response ($t_{18df}=2.6$, $P<.02$). Fig. 1A, phase 2, shows the second conditioning procedure in which there were three paired/unpaired treatments. Again cocaine generated a locomotion stimulant effect ($F_{1,18}=20.4$, $P<.001$). As shown in Fig. 1B, a cocaine conditioned locomotion response persisted in phase 2 ($t_{18df}=2.8$, $P<.02$).

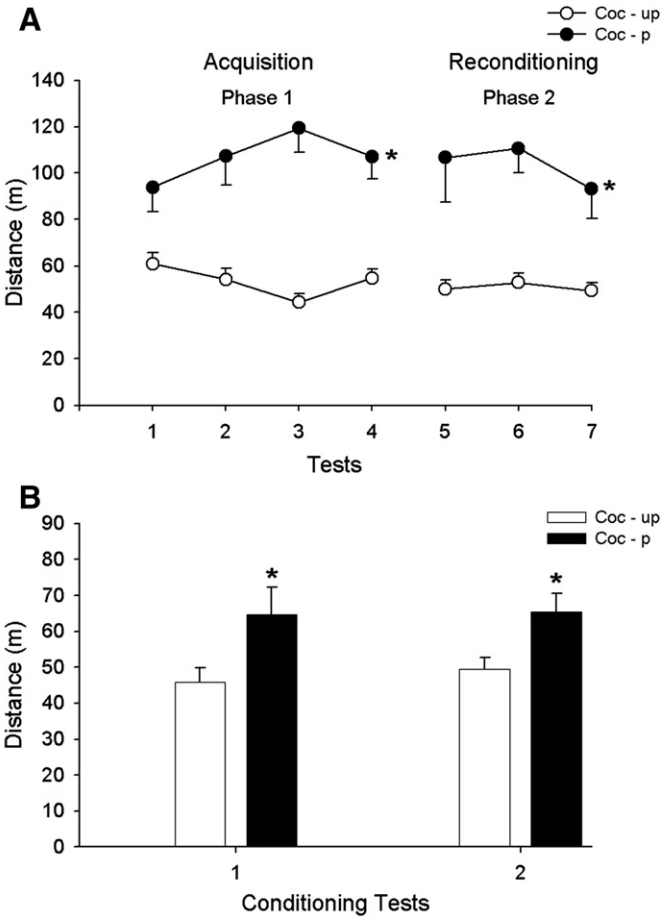

Fig. 1. A. Means and SEMs of scores for locomotion distance in acquisition and reconditioning phases of experiment 1. The Coc-P group received cocaine (10.0 mg/kg) immediately before testing; and, the Coc-UP group received saline immediately before testing and cocaine in the home cage. B. Saline conditioning tests following acquisition (Conditioning Test 1) and following reconditioning (Conditioning Test 2). The Coc P and Coc UP groups received saline immediately befoe testing. *denotes $P<.05$.

Fig. 2A presents the phase 1 results for the 8-OHDPAT treatment groups. The phase 1 part of the experiment was exactly the same as the preceding Coc-P/Coc-UP pair. As can be seen in Fig. 2A, in phase 1, cocaine enhanced locomotion across the four paired/unpaired sessions ($F_{1,18}=18.7$, $P<.001$). Again, there were no day or interaction effects $P>.05$).The conditioning test following phase 1 (Fig. 2B), generated a cocaine conditioned effect similar to that of the Coc-P/Coc-UP pair shown in Fig. 1 ($t_{18df}=2.7$, $P<.02$). In phase 2, both the paired and unpaired groups had received the ascending 8-OHDPAT treatments (0.01, 0.025, 0.05 mg/kg) 10 min prior to the paired/unpaired cocaine treatments. As can be seen in Fig. 2A for phase 2, the 8-OHDPAT treatments did not modify the locomotion response of the Coc-P group from that of phase 1 but it did substantially affect the locomotion response of the Coc-UP group. As shown in Fig. 2A phase 2, the 8-OHDPAT treatment produced a dose-related decrease in locomotion in the Coc-UP group. There were both group effects and group ×8-OHDPAT dose interactions. The group differences were statistically significant ($F_{1,18}=51.2$, $P<.001$). The group ×8-OHDPAT dose level interaction was significant ($F_{2,36}=4.8$, $P<.05$). This interaction is attributable to the profound decrease of locomotion in the Coc-UP group at the 0.05 mg/kg dose level. On the conditioning test following phase 2, as shown in Fig. 2B, there was no significant difference between groups on locomotion ($t_{18df}=0.4$, $P>.05$). The 8-OHDPAT treatment, therefore, had a marked effect upon the cocaine conditioned locomotion response. The loss of the conditioned locomotion response in the Coc-P group following the 8-OHDPAT

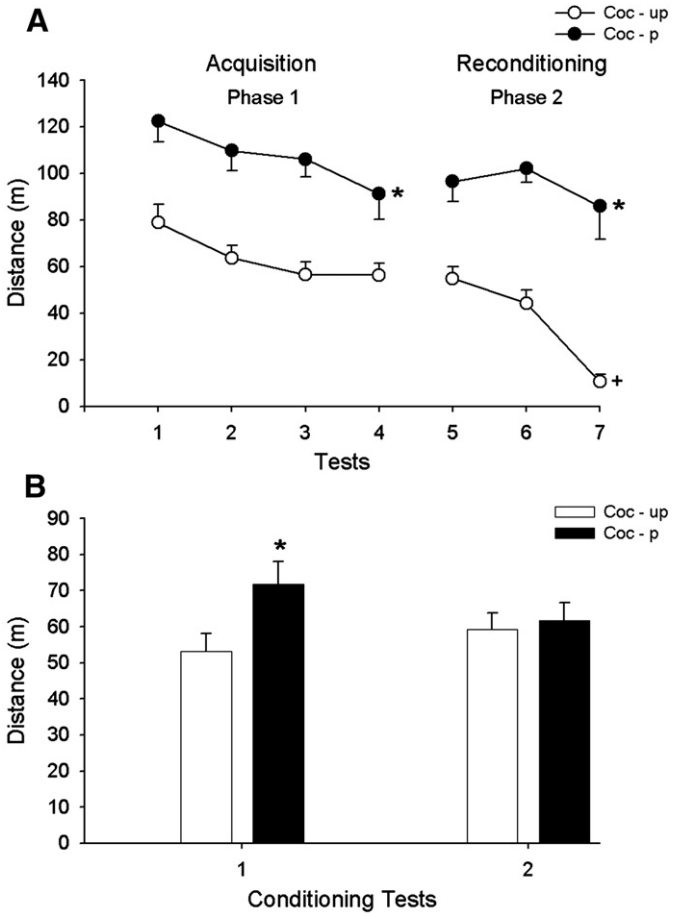

Fig. 2. A. Means and SEMs of scores for locomotion distance in acquisition and reconditioning phases of experiment 1 for the Coc-P and Coc-UP groups which received 8-OHDPAT. In acquisition, the Coc-UP group received saline immediately before testing and the Coc-P group receive cocaine (10.0 mg/kg). In reacquisition, the Coc-P group received 8-OHDPAT 10 min before cocaine (10.0 mg/kg) and the Coc-UP group received 8-OHDPAT 10 min before saline. *denotes $P<.05$. B. Means and SEMs of scores for locomotion distance following acquisition (ConditioningTest 1) and following reconditioning (Conditioning Test 2). *denotes $P<.05$.

pretreatment on reconditioning in phase 2 was not due to a generalized inhibitory effect of the prior 8-OHDPAT treatment on conditioning test performance. Specifically the locomotion response of the Coc-UP group on conditioning test 1 remained essentially unchanged on conditioning test 2 (conditioning test 1 vs. conditioning test 2 paired *t*-test: $t_{9df}=0.5$, $P>.05$) with the Coc-UP group exhibiting a slight but statistically non-significant increase in locomotion (+3.1 m) on conditioning test 2. In contrast, the Coc-P group locomotion decreased on conditioning test 2 (−11.4 m) from conditioning test 1. This decrease was statistically significant (conditioning test 1 vs. conditioning test 2 paired *t*-test: $t_{9df}=2.7$, $P<.02$). These findings indicate that the 8-OHDPAT treatment reversed the previously established cocaine locomotion stimulant conditioning.

3.2. Experiment 2

In order to further validate the 8-OHDPAT effect observed in experiment 1, a second experiment was carried out. In this study, three cocaine paired groups (Coc-P1, Coc-P2, Coc-P3) and one cocaine unpaired group (Coc-UP) were used. Initially (phase 1), all groups ($N=10$) received 4 cocaine (10.0 mg/kg) P or UP trials followed by a saline conditioning test. Subsequently (phase 2), one Coc-P group (Coc-P1) received another 4 cocaine paired trials, a second Coc-P group (Coc-P2) received 8-OHDPAT sequentially in the dose level order of 0.025, 0.05. 0.01 and 0.075 mg/kg 10 min prior to each of the 4 cocaine paired trials. A third Coc-P group (Coc-P3) received the 8-OHDPAT treatment plus the 5-HT_{1A} antagonist WAY-100635 at the same doses as were administered for 8-OHDPAT prior to the Coc-P treatment. The Coc-UP group again received the cocaine UP treatment. The expectation was that the addition of WAY-100635 to the 8-OHDPAT pretreatment would reverse the possible inhibitory effect of 8-OHDPAT upon the cocaine conditioned response. In phase 1, of this experiment, in which the 3 Coc-P groups received cocaine (10.0 mg/kg) immediately prior to testing, the results were similar both statistically and in absolute scores to those in Experiment 1. The cocaine treatments increased locomotion activity ($F_{3,38}=14.3$, $P<.001$) and all three Coc-P groups had higher locomotion scores than the Coc-UP groups ($P<.01$) but did not differ from each other ($P>.05$, Duncan multiple range test). The saline pretest and the conditioning results are shown in Fig. 3A–C. As can be seen in Fig. 3A, all groups were virtually equivalent in their locomotion scores in the saline test prior to the start of the phase 1 Coc-P, Coc-UP protocols ($F_{3,36}=0.07$, $P>.05$). In view of this equivalence in pre-conditioning locomotor behavior, we compared the conditioning test scores for each group with its pre-conditioning baseline. This within-group comparison would provide a

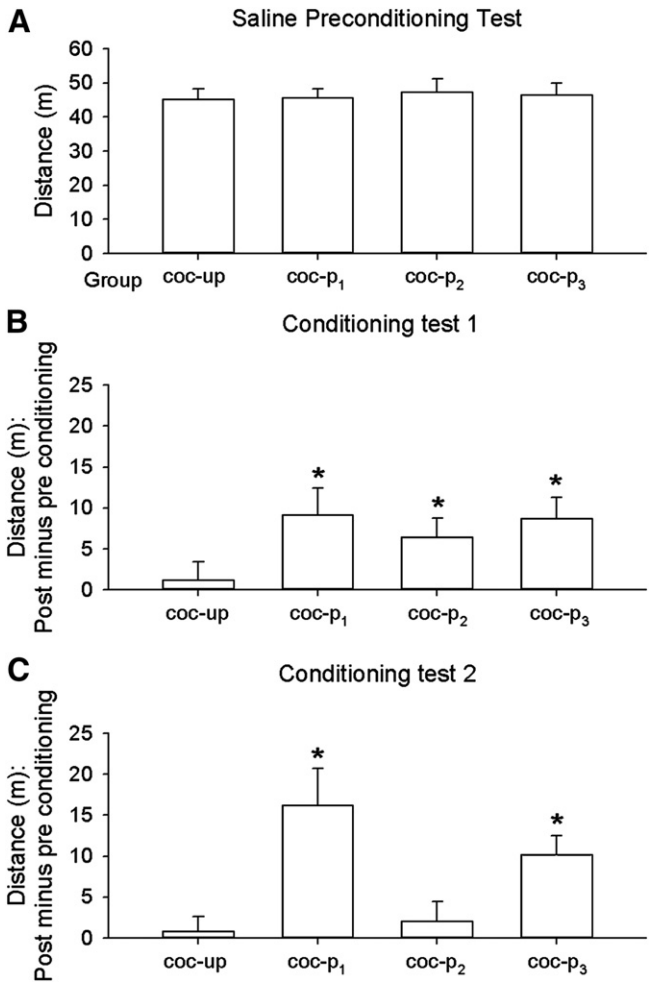

Fig. 3. A. Means and SEMs of scores for locomotion distance on the last saline baseline test prior to initiation of cocaine conditioning treatments. *denotes $P<.05$. B. Means and SEMs for change in locomotion distance scores in the saline conditioning test following cocaine acquisition treatments for the three Coc-P groups (Coc-P1, Coc-P2, Coc-P3) and for the Coc-UP group. *denotes $P<.05$. C. Means and SEMs for change in locomotion distance scores on Conditioning Test 2 following reconditioning phase in which the Coc-P1 group received. Saline (Coc-P1), 8-OHDPAT plus WAY-100635 10 min prior to cocaine (10.0 mg/kg). *denotes $P<.05$.

more direct and more sensitive indicator of whether the conditioning protocol did in fact induce a conditioned behavioral response. Furthermore, the addition of the saline baseline scores plus the difference scores yields absolute scores that are comparable to Experiment 1 and this presentation of the data simply supplements and amplifies the results shown in Experiment 1.

Fig. 3B presents the results in change of locomotion in saline scores before vs. after the Coc-P, Coc-UP protocols. As can be seen in Fig. 3B, the three Coc-P groups showed an increase in locomotion (t_{9df}=2.9, 3.1, 3.5, P<.01, respectively, paired t-tests) while for the Coc-UP group, the difference was not statistically significant (t_{9df}=0.3, P>.05, paired t-test). In the second conditioning phase of the Coc-P, Coc-UP treatments, the results were similar to those of phase 1 ($F_{3,36}$=16.2, P<.001). All Coc-P groups had higher scores over the four conditioning sessions than the Coc-UP group (P<.01) and the three Coc-P groups, saline/cocaine (Coc-P1), 8-OHDPAT/cocaine (Coc-P2) and 8-OHDPAT plus WAY-100635/cocaine (Coc-P3) groups, did not differ from each other (P>.05, Duncan multiple range test). The conditioning test results following this series of cocaine P/UP additional conditioning treatments are presented in Fig. 3C. The paired t-tests revealed that for the Coc-UP group, the pre vs. post treatment differences were not significant (t_{9df}=0.2, P>.05); but were significant for the saline/cocaine group (Coc-P1) (t_{9df}=4.5, P<.05); and for the Coc-P plus 8-OHDPAT and WAY 100635 group (Coc-P3) (t_{9df}=3.1, P<.01). Thus, for these groups, the conditioned response was sustained. In contrast, the Coc-P plus 8-OHDPAT difference scores were not significant from baseline (t_{9df}=0.6, P<.05).

4. Discussion

8-OHDPAT is a 5-HT1A full agonist which at low dose levels acts preferentially and presynaptically at the 5-HT1A autoreceptor site (Blier et al., 1999, 1998; Sprouse and Aghajanian, 1987). As we have previously reported, (Carey et al., 2008b) low dose 8-OHDPAT suppresses spontaneous behavior and reduces cortical 5-HT metabolism. In the present study, we administered the 8-OHDPAT treatment prior to cocaine in order to decrease the availability of serotonin so that cocaine binding to the serotonin re-uptake transporter (SERT) would be less effective in increasing extracellular 5-HT. This is clearly a presumption since we did not measure cocaine evoked 5-HT increases with vs. without 8-OHDPAT pretreatment. In fact the way we administered 8-OHDPAT (i.e., starting with the lower dose levels) was designed to minimize the impact of the 8-OHDPAT pretreatment upon the target conditioned response of locomotion. In this report as well as in our previous studies (Carey et al., 2004a,b, 2008a), the ascending 8-OHDPAT pretreatment protocol did not affect the locomotion response elicited by cocaine. Given as a pretreatment to saline, however, the 8-OHDPAT did have a pronounced dose related inhibitory effect which was similar to our previous finding (Carey et al., 2004a,b). While the 8-OHDPAT pretreatment had a statistically significant inhibitory effect upon locomotion behavior, it did not impact upon the behavioral baseline in that the locomotion response after the 8-OHDPAT regimen did not differ from the locomotion response level prior to the 8-OHDPAT pretreatments. In contrast, the 8-OHDPAT pretreatment given prior to cocaine did not affect the locomotion response elicited by cocaine but did reduce the cocaine conditioned response. A key factor in understanding the differential effect, however, is that for the saline/Coc-UP group, the behavioral response represents baseline spontaneous behavior, whereas, for the Coc-P group, the cocaine conditioned behavior is a learned or a memory based response. The behavioral expression of the cocaine memory trace is, simply, the increase in locomotion above baseline. For the Coc-UP group, the 8-OHDPAT pretreatment decreased behavioral activity, possibly by diminished attention to stimuli and/or suppressing response systems. These inhibitory effects were drug state dependent, so that, when the animals were retested with saline, normative baseline behavior was restored. In the case of the Coc-P groups given the 8-OHDPAT pretreatment, the cocaine memory trace was also activated. Seemingly, this activation occurred in the presence of a reduced serotonin activity state which could interact with and alter the memory trace. Consequently, only the conditioned component of behavioral was lost; and, therefore, behavior returned to baseline. Alternatively, the 8-OHDPAT treatments may have made the reacquisition pairing less effective. In that we have recently found that cocaine conditioned locomotion can be stable and persistent (Carey et al., 2008a), it would appear that the loss of cocaine conditioned behavioral response is not readily explicable by a decrease in efficacy of the additional cocaine paired trials.

It is now well-recognized that learning/memory processes are critical in the acquisition and persistence of cocaine addiction (Everett and Robbins, 2005; Kalivas and Volkow, 2005). It is the capacity of cues associated with drug taking to briefly activate the drug experience which then reinstates craving in the drug abstinent state as well as drug seeking behavior that promotes continued drug taking and enhanced drug addiction (O'Brien et al., 1992a,b). For sometime now, Pavlovian conditioning processes have been recognized as forging associations which enable non-drug stimuli to acquire drug-like properties (Newlin, 1992). The efficacy of Pavlovian conditioned drug stimuli, however, is not easily returned to a non-drug status (i.e., do not readily extinguish). The simplest approach to reducing the potency of a Pavlovian conditioned stimulus is to administer repeated presentations of the stimulus (CS) without the unconditioned stimulus (UCS). Eventually, the CS will no longer evoke the behavior linked to the UCS and it is extinguished. As was thoroughly documented by Pavlov (1927), the CS can spontaneously recover its potency and can be disinhibited as well. In fact, clinical studies of cocaine addiction have shown that while a drug response evoked by drug associated paraphernalia can be extinguished, it can be reinstated by stress (Childress et al., 1994).

In the present study, we undertook to fully reactivate the cocaine memory trace by providing both the contextual cues and cocaine during a post-treatment reconditioning phase. By having the reconditioning occur in the presumed attenuation of widespread cortical serotonergic activation in the 8-OHDPAT pretreatment condition, the memory trace for cocaine could be altered and degraded. The subsequent loss of the cocaine conditioned behavior is consistent with this proposition. Alternatively, it can be argued that the combined 8-OHDPAT and cocaine treatment is non-reinforcing or even aversive. These considerations, however, remain speculative since it requires additional experiments which expand the behavioral analysis beyond locomotion. In the absence of such additional experiments, the present findings are at best suggestive; but yet, do emphasize the potential importance of serotonergic systems in cocaine conditioning, specifically, in the maintenance of the memory traces which underlie addiction.

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