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ORIGINAL INVESTIGATION

Aberrant approach-avoidance conflict resolution following repeated cocaine pre-exposure

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Abstract
Rationale Addiction is characterized by persistence to seek drug reinforcement despite negative consequences. Drug-induced aberrations in approach and avoidance processing likely facilitate the sustenance of addiction pathology. Currently, the effects of repeated drug exposure on the resolution of conflicting approach and avoidance motivational signals have yet to be thoroughly investigated.
Objective The present study sought to investigate the effects of cocaine pre-exposure on conflict resolution using novel approach-avoidance paradigms.
Methods We used a novel mixed-valence conditioning paradigm to condition cocaine-pre-exposed rats to associate visuotactile cues with either the delivery of sucrose reward or shock punishment in the arms in which the cues were presented. Following training, exploration of an arm containing a superimposition of the cues was assessed as a measure of conflict resolution behavior. We also used a mixed-valence runway paradigm wherein cocaine-pre-exposed rats traversed an alleyway toward a goal compartment to receive a pairing of sucrose reward and shock punishment. Latency to enter the goal compartment across trials was taken as a measure of motivational conflict.
Results Our results reveal that cocaine pre-exposure attenuated learning for the aversive cue association in our conditioning paradigm and enhanced preference for mixed-valence stimuli in both paradigms.
Conclusions Repeated cocaine pre-exposure allows appetitive approach motivations to gain greater influence over behavioral output in the context of motivational conflict, due to aberrant positive and negative incentive motivational processing.

Keywords Cocaine · Addiction · Associative learning · Motivation · Animal model

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Introduction

Drug addiction is a disorder characterized by compulsive drug-seeking and taking behaviors that persist despite the recurrent experience of withdrawal and other negative consequences (American Psychiatric Association 2013). From this perspective, it is likely that addicts' compulsive behaviors are influenced by aberrant processing of competing motivational signals: those that compel the individual to simultaneously seek and avoid the drug. In a seminal study, Ettenberg and Geist (1991) demonstrated these opponent properties of cocaine utilizing a runway paradigm, in which rats were trained to traverse an alleyway for intravenous injections of cocaine in a goal compartment. Rats displayed shorter latency values in leaving the start compartment over subsequent trials; however, these shorter latency values to initiate trials corresponded to longer latency values to enter the goal compartment. This observation led to the interpretation that the goal compartment became imbued with both appetitive and aversive qualities, thereby presenting an approach-avoidance conflict for the animal as they pursued cocaine administration (Ettenberg 2004).

The resolution of this motivational conflict is likely to involve a decision-making process, whereby both positive

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and negative outcomes are weighted before the final behavioral response of entering the goal compartment is determined. While the motivational value of cocaine's positive effects may naturally outweigh that of its aversive effects, it is also plausible that cocaine exposure leads to fundamental disruptions in the processing of information with competing positive and negative incentive valences; that is, cocaine exposure may shift the balance of behavioral control presented by opposing incentive systems in favor of approach, thereby facilitating compulsive cocaine seeking. Indeed, drug-induced long-term perturbations occur in mesolimbic systems implicated in the processing of both appetitive and aversive stimuli (Koob and Volkow 2010; Wheeler and Carelli 2009). For example, rodents with a history of cocaine exposure show neurochemical evidence of enhanced excitability of dopaminergic neurons originating in the ventral tegmental area (VTA) and enhanced cue-induced dopamine (DA) signaling in the nucleus accumbens (NAc) (Bellone and Luscher 2006; Borgland, Malenka, and Bonci 2004; Chen et al. 2008; Gratton and Wise 1994; Ungless et al. 2001). Moreover, there is much behavioral evidence that prior repeated stimulant drug exposure enhances positive incentive motivation. For example, rodents with a history of cocaine exposure show enhanced acquisition of conditioned place preference for cocaine-reinforced locations (Lett 1989; Seymour and Wagner 2008) and decreased latency values to obtain cocaine reward in a runway task (Deroche et al. 1999). Similarly, rodents with a history of amphetamine exposure show enhanced Pavlovian conditioning to instrumental transfer for sucrose reinforcement (Wyvell and Berridge 2001), enhanced conditioning for cues predictive of sucrose reinforcement (Harmer and Phillips 1998), and enhanced sexual pursuit behaviors (Fiorino and Philips 1999). Taken together, these findings suggest a psychostimulant-induced effect facilitating appetitive incentive motivation. What is less clear is whether repeated drug exposure influences aversive incentive motivations and, if so, in what direction.

In the present study, we sought to investigate the effects of prior repeated cocaine exposure upon opposing incentive motivational processes. To this end, we used two novel approach-avoidance paradigms. The first was a mixed-valence conditioning paradigm designed to assess incentive cue learning and to measure the behavioral resolution of conflicting motivational signals. The second paradigm was a modified runway task designed to assess trajectory to a goal compartment conditioned to both rewarding and aversive outcomes. Here, we report that rats with a prior history of cocaine exposure, relative to drug-naïve rats, were attenuated in the acquisition of aversive cue associations and exhibited enhanced preference for mixed-valence stimuli.

Materials and methods

Subjects

The studies were carried out with 47 male Long Evans rats (Charles River, QC, Canada) weighing 300–400 g at the start of the experiment. All rats were pair-housed and maintained on a 12-h-light/dark cycle (lights on at 700 hours) at a constant room temperature of 22 °C. Up until the time of training on the approach-avoidance procedures, rats were fed ad libitum. Subsequently, they were shifted to a food-restricted diet (laboratory chow, Purina) to maintain their weights at 85 % of their free-feeding weight. Water was available ad libitum throughout the experiments. All behavioral testing took place during the light cycle and was in accordance to the ethical and legal requirements under Ontario's Animals for Research Act and the federal Canadian Council on Animal Care.

Apparatus

Elevated plus maze

The apparatus consisted of four arms arranged in the shape of a plus (43.2-cm L×10.2-cm W). Two of the arms were enclosed by walls (24.8-cm H). The entrances to these arms were positioned directly across from each other and were accessible from the maze center. The other two arms were not enclosed by walls and were also accessible from the maze center. The apparatus was elevated 43.2 cm from the ground by a pedestal.

Radial maze

The apparatus (Med Associates, VT, USA) was fully automated and consisted of six enclosed arms (45.7-cm L×9-cm W×16.5-cm H) radiating from a central hub compartment (19.5-cm L×19.5-cm W). Three of the six arms were kept closed throughout the experiment; the remaining three arms, each positioned 120° relative to the other, were used for the various conditioning trials. Arm walls and removable lids were made of Plexiglas and covered with red cellophane to minimize the visibility of extra-maze cues. The entrance to each arm was blocked by an automated stainless steel door. The arm floors contained grids that were connected to a shock scrambler (Med Associates, VT, USA) for delivery of mild foot shocks. A stainless steel well was located at the end of each arm for sucrose delivery. A camera positioned above the apparatus was used to record behavior during each trial.

Runway

The apparatus (Lafayette Instrument Co., IN, USA) was divided into three compartments separated by steel guillotine

doors. Identically sized start and goal compartments (24.5-cm L, 11.4-cm W, 21.1-cm H) were located at opposite ends of an alley (98.8-cm L, 11.4-cm W, 21.1-cm H). The floor of the apparatus consisted of steel rods laid parallel across the width of the entire apparatus 1.3 cm apart. The steel rods located inside of the goal compartment were connected to a shock generator (Lafayette Instrument Co.). The steel doors separating the three compartments were manually controlled to allow access to the alley and goal compartments. The entire apparatus was covered with red cellophane to minimize distraction by extra-maze stimuli. A ceiling-mounted infrared camera was positioned above the apparatus to record each trial.

Behavioral procedures

In each of two experiments, rats were first pre-exposed to cocaine (or saline) injections and then tested for anxiety in the elevated plus maze. Subsequently, they were trained and tested in one of the two novel approach-avoidance procedures: one utilizing the radial arm maze (experiment 1) and the other utilizing the runway apparatus (experiment 2). Finally, at the end of each experiment, all rats were given a test for locomotor sensitization.

Cocaine pre-exposure

Habituation Rats were individually placed in separate opaque plastic chambers and locomotor activity was monitored for 60 min by an overhead CCD camera and infrared sensor connected to an Ethovision tracking system (Noldus Information Technology). Rats were then given an intraperitoneal (i.p.) injection of saline (0.9 %) and replaced in the activity chambers for an additional 60-min monitoring period.

Pre-exposure Rats were administered an i.p. injection of cocaine (n=24) or saline (n=23) once daily for seven consecutive days, beginning 1 day after habituation. The first and last daily injections (15 mg/kg, i.p.) were given in the activity chambers, immediately following a 60-min pre-injection period. Activity was monitored during the 60-min pre- and post-injection periods. Injections 2–6 (30 mg/kg, i.p.) were administered in the home cages but in a room other than the housing or behavioral testing rooms. Activity was not recorded on these days.

Elevated plus maze testing

Ten days after the 7-day cocaine pre-exposure, all rats were tested for anxiety in the elevated plus maze. Each rat was tested in a single trial, which began by placement in the center compartment of the apparatus, which was located in a well-lit room. The rat was allowed to freely explore the apparatus for 10 min. Time spent exploring the open arms was measured in comparison to time exploring the closed arms.

Experiment 1: effect of cocaine pre-exposure on mixed-valence conditioning

Cocaine (n=15) and saline (n=13) pre-exposed rats were trained to associate different visuo-tactile cues in separate arms with either rewarded or aversive or neutral outcomes. The three cues were distinctly textured "whisker bars" (wood, duct tape, and cloth) that ran the entire length of a single maze arm. The experiment was comprised of a total of three habituation, nine training, and three preference/avoidance testing sessions, followed by a final conflict test in which the positive and aversive cues were superimposed in one arm.

Habituation

Rats were given three habituation sessions. In the first session, the rat was placed in the central hub compartment for 1 min, after which all three guillotine doors were raised to allow free access to all three arms. The rat was given 5 min to freely explore the apparatus. In the second session, the rat was exposed, for the first time, to the three sets of discrete whisker bar cues. Each set of cues was placed in one of three maze arms, and the session followed the same procedures as described for the first habituation session. The assignment of affective valence to the whisker bar cues (for subsequent training sessions) was assigned according to the amount of time that the rat spent exploring each arm, which was taken as an indication of how much they preferred each set of stimuli. The most preferred stimulus was assigned as the negative cue, the least preferred stimulus as the positive cue, and the remaining stimulus as the neutral cue. For the third habituation session, the rat was given access to two arms, which contained either the neutral cue or a superimposition of the appetitive and aversive cues to mirror the conditions of the final conflict test. This session followed the same procedures described for the first and second habituation sessions, except that rats were given access to only the two rather than all three arms.

Training

The valence conditioning consisted of nine identical daily training sessions. In each session, the rat was placed in the central hub compartment for 30 s. One of three guillotine doors was then raised, allowing access to that arm, which contained one of the three whisker bars. Upon entry, the door was lowered, confining the rat in the arm for 2 min, during which the outcome assigned to the cue was administered (appetitive outcome, 0.4 ml of 20 % sucrose solution, administered once at the onset of each 30-s interval; aversive outcome, 0.5 s, 0.25–0.30-mA shock administered once at a random

time point within each 30-s interval; neutral, no outcome). Importantly, in order to mitigate the potentially confounding effect of treatment-induced changes in pain thresholds on the degree of aversive cue conditioning, the shock level was calibrated for each rat in the first conditioning session and fixed at a value that elicited a mild startle and defensive treading behavior for the rest of the conditioning sessions. For this experiment, it was found that a shock level of 0.27 mA was sufficient to elicit the desired reaction in all animals, except for one rat (0.3 mA). After the completion of the 2-min period, the guillotine door was raised again, allowing access to the central hub. After 30 s in the hub, the same procedure was repeated until training in all three arms was completed. The rat was then removed from the central hub and returned to its home cage.

Several measures were taken to ensure that the animals did not associate the outcomes with the sequence of arm presentation, spatial location of the arms, or other intramaze cues. Firstly, each of the three cues was placed in a different arm for each session; a cue was never located in the same arm for consecutive sessions. Secondly, the order in which each cue and its corresponding arm were presented was changed for each training session. Lastly, the relative orientation of the arms was held constant (i.e., Y-maze configuration); however, the maze was rotated either clockwise or counterclockwise by 60° between training sessions.

Tests 1–3: conditioned cue preference/avoidance

To assess the acquisition of learning, each animal underwent a conditioned cue preference/avoidance test after the third, sixth, and eighth conditioning sessions. The procedures utilized for each test were identical to those described for the second habituation session, such that rats were allowed to freely explore all three cued arms under extinction conditions. Each test was immediately followed by a training session to minimize the effect of extinction.

The total time spent exploring each of the cued arms was recorded for each test. Successful acquisition of conditioned cue preference and avoidance was indicated by (1) time spent exploring the appetitive cued arm being greater than time spent exploring either the neutral or aversive cued arms (conditioned cue preference) and (2) the time spent exploring the aversive cued arm being less than time spent exploring either the appetitive or neutral cued arms (conditioned cue avoidance).

Test 4: conflict test

This final test was conducted on the day following the last training session. The procedure carried out for this test was identical to the procedure described for the third habituation session, such that rats were allowed to freely explore one arm containing a superimposition of the appetitive and the aversive cues and another arm containing the neutral cue, under extinction conditions. The total time spent exploring the arms was recorded (see Fig. 1a for schematic).

Experiment 2: effect of cocaine pre-exposure on mixed-valence conditioning using a modified runway procedure

Cocaine (n=9) and saline (n=10) pre-exposed rats were trained in a runway apparatus to traverse an alleyway to receive sucrose reward within the goal compartment. This initial acquisition phase was then followed by a conflict phase in which foot shocks were delivered in the goal compartment, in addition to it still containing sucrose reward. The latency to enter the mixed-valence goal compartment was compared with the latency values recorded during the initial acquisition phase. The amount of sucrose consumed in the goal compartment was also recorded for all training trials.

Habituation

Rats were given two daily habituation sessions on consecutive days. At the start of each habituation session, rats were confined to the start compartment of the apparatus for 1 min.

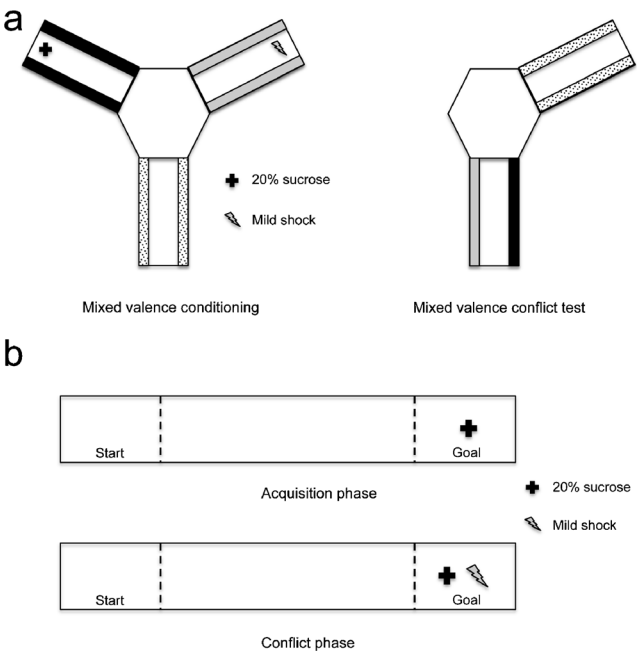

Fig. 1 This schematic shows the apparatus used for experiments 1 and 2. **a** The radial maze consisted of a central hub with three radiating arms in a Y formation. The inside walls of each arm were lined with visuo-tactile whisker bar cues conditioned to appetitive, aversive, or neutral outcomes. During a mixed-valence conflict test, rats are presented with an arm containing a superimposition of the appetitive and the aversive cues and an arm containing the neutral cues. **b** The runway consisted of a start compartment, alleyway, and goal compartment. Sucrose reinforcement and mild shock administrations were initiated upon entry of the goal compartment

Subsequently, the steel guillotine door was raised, allowing access to the alley. Rats were allowed to freely explore the alley and the start compartment for 3 min. On the first day, rats were removed from the apparatus upon completion of the 3-min period. On the second day, upon completion of the 3-min exploration period, the guillotine door blocking the goal compartment was lifted, and the goal compartment, containing a well filled with a 20 % sucrose solution, was available for an additional 1 min during which rats had unlimited access to the sucrose solution.

Acquisition phase

Rats were trained to traverse the alley for sucrose reinforcement, during two daily trials over a period of 6 days. At the start of each trial, the guillotine door blocking the goal compartment was raised, and a well containing 20 % sucrose solution was placed inside the goal compartment area. Rats were then placed in the start compartment for 1 min. Subsequently, the guillotine door was raised, allowing free access to the alley and goal compartment. Upon entering the goal compartment, the guillotine door was lowered, separating the goal compartment from the alley. Rats remained inside the goal compartment for 1 min, where they had unlimited access to the 20 % sucrose solution. Rats were then removed from the apparatus and placed in their home cage. The second trial commenced immediately after all rats completed the first trial. The amount of sucrose consumed and latency to enter the goal compartment were measured for each trial.

Conflict phase

In the 16 days following the acquisition phase, the availability of sucrose solution inside the goal compartment was paired with the administration of foot shocks. Rats were given two daily trials under conditions identical to those described for the acquisition phase, with the important exception that in the 1-min period following entry into the goal compartment, a series of foot shocks (0.5 s, 0.25–0.30 mA; once at a random time point within each 15-s interval) was administered. If a rat did not enter the goal compartment within 300 s of the guillotine door being raised, the rat was removed from the apparatus and placed in its home cage (see Fig. 1b for schematic).

Test for locomotor sensitization (experiments 1 and 2)

After completion of behavioral testing in each of experiments 1 and 2, rats remained in their home cages with ad libitum access to food and water for 8–14 days before undergoing a test for locomotor sensitization. Testing took place over 2 days. On the first day, rats were habituated individually in opaque activity chambers for 2 h. On the second day, rats were given another 2-h habituation session, during which locomotor activity was recorded. Subsequently, all rats were given a low dose of cocaine (10 mg/kg, i.p.) and were immediately returned to the activity chambers for an additional 2-h recording session. Rats were then returned to their home cages.

Data analysis

Data were analyzed using SPSS statistical package version 20.0 (IBM, ON, Canada). In experiment 1, two-way repeated-measures analysis of variance (ANOVA) was conducted on the time spent in each of the cued arms (s), with *valence* (appetitive, aversive, and neutral) as the within-subjects factor and *pre-exposure condition* (cocaine and saline) as the between-subjects factor. This analysis was conducted separately for each of the four conditioned preference tests. In experiment 2, two-way repeated-measures ANOVA was conducted on latency to reach the goal compartment (s) and amount of sucrose consumed (ml), with *trial* as the within-subjects factor and *pre-exposure condition* (cocaine and saline) as the between-subjects factor. This analysis was conducted for the acquisition phase only in order to assess acquisition of sucrose reinforcement. In addition, separate two-way repeated-measures ANOVAs were conducted on the latency to reach the goal compartment (s) and on the amount of sucrose consumed (ml), with *phase* (acquisition phase and conflict phase) as the within-subjects factor and *pre-exposure condition* (cocaine and saline) as the between-subjects factor. Analysis of contrasts was used to further examine main effects, and two-way interactions were explored using simple main-effect analyses. Post hoc comparisons for simple effects were subjected to a Bonferoni correction. The test for locomotor sensitization was analyzed using two-way repeated-measures ANOVA. Locomotor activity as a measure of total distance traveled (m) following the cocaine injection was compared with the baseline measure of locomotion recorded prior to the cocaine challenge.

Results

Locomotor activity test during cocaine pre-exposure

ANOVA was conducted to compare locomotor activity following the i.p. injection (cocaine and saline) for days 1 and 7 of pre-exposure. Overall, locomotor activity was higher on day 7 than on day 1 (cocaine, day 1 303.72±17.17, day 7 347.52±26.81; saline day 1 157.82±6.90, day 7 169.66± 7.78; main effect of day $F(1, 42)=17.278$, $p<.0001$). A significant *day* × *pre-exposure* interaction was also revealed ($F(1, 42)=7.546$, $p=.009$). A follow-up analysis of simple effects showed an effect of *pre-exposure* on both day 1 ($F(1, 42)=45.097$, $p<.0001$) and day 7 ($F(1, 42)= 50.753$, $p<.0001$). Cocaine rats exhibited higher locomotor

activity on both days 1 and 7. Furthermore, the analysis revealed a simple effect of *day* for cocaine ($F(42)=23.831$, $p<.0001$) but for not saline ($F(42)=.994$, $p=.325$) rats. Bonferoni post hoc analyses revealed that cocaine rats exhibited higher locomotor activity on day 7 than on day1 ($p<.0001$), while saline rats did not show a change in activity ($p=.325$).

Experiment 1: effect of cocaine pre-exposure on mixed-valence conditioning

Tests 1–3: conditioned cue preference/avoidance

Test 1 The first test was given after the completion of three mixed-valence conditioning sessions. Figure 2a shows time spent exploring the appetitive, aversive, and neutral cued arms. ANOVA revealed a significant main effect of *valence* on exploration time ($F(2, 52)=24.586$, $p<.0001$), indicating that the amount of time spent exploring each arm differed and was dependent upon the arm's motivational valence. An analysis of contrasts was conducted to compare the amount of time spent exploring the appetitive and aversive arms relative to the time spent exploring the neutral arm. This analysis revealed that, overall, rats spent significantly more time exploring the appetitive than neutral arm ($F(1, 26)=31.560$, $p<.0001$) indicating effective acquisition of cue-reward association. However, the amount of time spent in the aversive arm did not significantly differ from time spent in the neutral arm ($F(1, 26)=1.862$, $p=.184$). A significant *valence* × *pre-exposure* interaction was also observed ($F(2, 52)=3.688$, $p=.032$). A follow-up analysis of simple effects revealed that this effect was due to a significant *pre-exposure* effect in the aversive arm ($F(1, 26)=5.960$, $p=.022$) but not the appetitive ($F(1, 26)=.309$, $p=.583$) or neutral arms ($F(1, 26)=1.168$, $p=.290$). The analysis further revealed a simple effect of *valence* for both saline-pre-exposed ($F(25)=12.879$, $p<.0001$) and cocaine-pre-exposed ($F(25)=8.455$, $p=.002$) groups. Bonferoni post hoc comparisons revealed that saline-pre-exposed rats spent significantly more time in the appetitive compared to neutral ($p=.002$) and appetitive compared to aversive arms ($p<.0001$) and significantly less time in the aversive compared to neutral arm ($p=.023$), indicating effective acquisition of all valence cue associations. On the other hand, although cocaine-pre-exposed rats spent significantly more time in the appetitive compared to neutral arm ($p=.001$) and appetitive compared to aversive arm ($p=.040$), there was no difference in the time spent in the aversive compared to neutral arm ($p=.845$). Thus, cocaine, unlike saline, pre-exposed rats did not show evidence of aversive cue learning in test 1.

Test 2 The second test was conducted after the completion of the sixth conditioning session. As in test 1, ANOVA revealed a significant main effect of *valence* on exploration time ($F(2, 52)=42.471$, $p<.0001$). Rats spent significantly more time exploring the appetitive arm than the neutral arm ($F(1, 26)=33.041$, $p<.0001$) and significantly less time exploring the aversive arm than the neutral arm ($F(1, 26)=15.809$, $p<.0001$). In contrast to test 1, no significant *valence* × *pre-exposure* interaction was observed ($F(2, 52)=.190$, $p=.828$). Thus, in test 2, both cocaine- and saline-pre-exposed rats showed both appetitive and aversive cue learning (*data not shown*).

Test 3 The third test was conducted after the completion of the eighth conditioning session. As in tests 1 and 2, ANOVA of exploration time in test 3 revealed a significant main effect of *valence* ($F(2, 52)=31.369$, $p<.0001$), with time spent exploring the appetitive arm being significantly greater than the time spent exploring the neutral arm ($F(1, 26)=26.960$, $p<.0001$) and time spent exploring the aversive arm being significantly lower than the neutral arm ($F(1, 26)=14.012$, $p=.001$). Again, a significant *valence* × *pre-exposure* interaction was not observed ($F(2, 52)=.033$, $p=.967$). Thus, both cocaine and saline-pre-exposed rats continued to exhibit both appetitive and aversive cue learning after eight conditioning sessions (Fig. 2b).

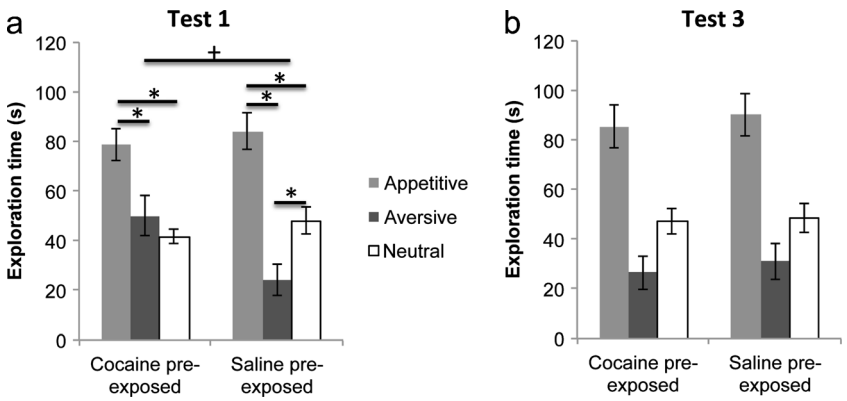

Fig. 2 Conditioned cue preference/avoidance measured as mean exploration time (±SEM) for **a** test 1 and **b** test 3. Cocaine-pre-exposed rats ($n=15$) showed attenuated aversive cue learning compared to saline-pre-exposed rats ($n=13$). *$p<0.05$ within group, +$p<0.05$ between groups

Conflict test In this test, time spent exploring an arm in which appetitive and aversive cues were superimposed was compared to time spent exploring the neutral arm. Given that the purpose of the test was to assess preference for the arm imbued with both appetitive and aversive valences, all rats that failed to exhibit appetitive and/or aversive conditioning at test 3 were removed from the analysis. On the basis of this criterion, eight subjects were removed from the analysis (four cocaine and four saline).

Data for the remaining subjects are presented in Fig. 3. ANOVA revealed a significant main effect of *valence* ($F(1, 18)=10.115$, $p=.005$); rats spent more time exploring the conflict arm than the neutral arm. Furthermore, there was a significant *valence* × *pre-exposure* interaction effect ($F(1, 18)=5.783$, $p=.027$). A follow-up analysis of simple effects revealed an effect of *pre-exposure* on time spent in the conflict arm ($F(1, 18)=8.724$, $p=.009$) and no effect of *pre-exposure* for time spent in the neutral arm ($F(1, 18)=.035$, $p=.854$). Moreover, there was a significant simple effect of *valence* for cocaine-pre-exposed ($F(18)=14.953$, $p=.001$) but not saline-pre-exposed ($F(18)=.944$, $p=.344$) rats. Bonferroni post hoc comparisons revealed that saline-pre-exposed rats did not differ in time spent exploring the conflict and neutral arms ($p=.607$); on the other hand, cocaine-pre-exposed rats spent significantly more time exploring the conflict compared to neutral arm ($p=.001$). The data suggest that cocaine pre-exposure altered conflict resolution in favor of an appetitive incentive drive.

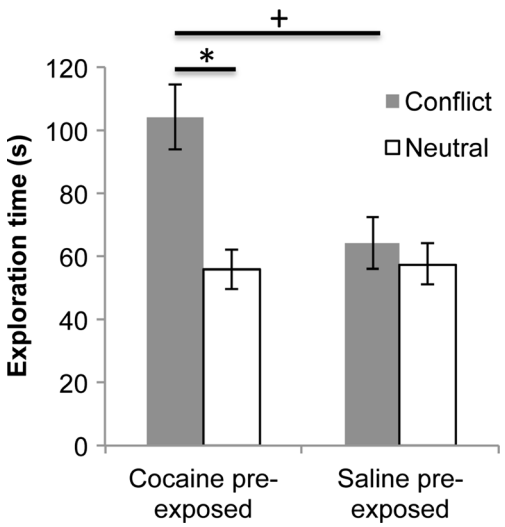

Fig. 3 Conditioned cue preference/avoidance measured as mean exploration time (±SEM) in the conflict test. After removal of data from all rats that did not demonstrate cue learning, cocaine-pre-exposed rats ($n=11$) showed enhanced preference for the mixed-valence arm compared to saline-pre-exposed rats ($n=9$). *$p<0.05$ within group, + $p<0.05$ between groups

Experiment 2: effect of cocaine pre-exposure on mixed-valence conditioning using a modified runway procedure

Acquisition phase Latency values for trials 1 and 2 were averaged separately for each of the six sessions. These average latency values were then combined into three separate bins, such that bin 1 was an average of latency values from sessions 1 to 2, bin 2 from sessions 3 to 4, and bin 3 from sessions 5 to 6.

ANOVA of latency to reach the goal compartment revealed a significant main effect for *bin* ($F(2, 34)=57.846$, $p<.0001$) but not a significant *bin* × *pre-exposure* interaction ($F(2, 34)=.100$, $p=.905$). An analysis of contrasts revealed that goal latency for bin 2 was significantly lower than for bin 1 ($F(1, 17)=36.801$, $p<.000$), and goal latency for bin 3 was significantly lower than that for bin 2 ($F(1,17)=33.525$, $p<.000$) (Table 1). These data indicate that the rate and degree of acquisition of sucrose reinforcement learning were comparable in saline and cocaine-pre-exposed rats. The amount of sucrose consumed during the 1-min period of confinement in the goal compartment was then assessed, with data arranged in the same manner as for the assessment of goal latency values. Similar to the outcome of the analysis of goal latency, ANOVA revealed a significant main effect for *bin* ($F(2, 34)=91.577$, $p<.0001$) but no *bin* × *pre-exposure* interaction ($F(2, 34)=.176$, $p=.839$). Sucrose consumption in bin 2 was significantly higher than that in bin 1 ($F(1, 17)=87.359$, $p<.0001$), and consumption in bin 3 was greater than that in bin 2 ($F(1, 17)=18.335$, $p<.001$) (Table 1). These data indicate that the rate and degree of sucrose consumption across sessions were comparable in saline and cocaine-pre-exposed rats.

Conflict phase Goal latency values for every trial over the subsequent 16 days of foot shock delivery were averaged to determine a single conflict-phase goal latency value for each rat. These averaged latency values were then compared with overall averaged latency values in the acquisition phase by *pre-exposure* condition (Fig. 4a). ANOVA revealed a significant main effect for *phase* ($F(1, 17)=9.136$, $p=.008$); overall, latency values to reach the goal compartment were higher in the conflict phase than in the acquisition phase. Moreover, the ANOVA revealed a significant interaction of *phase* by *pre-exposure* ($F(1, 17)=4.939$, $p=.040$). An analysis of simple effects revealed no *pre-exposure* effect for the acquisition phase ($F(1, 17)=2.801$, $p=.113$) but a significant effect for the conflict phase ($F(1, 17)=5.430$, $p=.032$). The analysis further revealed a simple effect of *phase* within the saline-pre-exposed ($F(17)=14.519$, $p=.001$) but not the cocaine-pre-exposed ($F(17)=.304$, $p=.588$) group. Bonferroni post hoc comparisons revealed that saline-pre-exposed rats exhibited significantly longer latency values to reach the goal

Table 1 This table shows goal latency (±standard error of the mean (SEM)) and sucrose consumption (±SEM) across bins in the acquisition phase of experiment 2

	Latency to enter goal box (s)			Sucrose consumption (g)		
	Bin 1	Bin 2	Bin3	Bin 1	Bin 2	Bin 3
Saline pre-exposure (n=11)	10.57±1.11	6.81±0.63	4.37±0.60	1.57±0.20	2.62±0.15	3.05±0.17
Cocaine pre-exposure (n=9)	8.80±1.03	5.20±0.78	3.10±0.35	1.86±0.24	3.05±0.15	3.39±0.20

Cocaine-pre-exposed (n=9) and saline-pre-exposed rats (n=11) showed an equivalent rate of acquisition of runway behavior and sucrose consumption

compartment during the conflict phase compared to the acquisition phase (p=.001), whereas cocaine-pre-exposed rats exhibited comparable latency values in the two phases (p=.588). Consistent with the findings from experiment 1, these results suggest that cocaine pre-exposure had an effect on conflict resolution, favoring appetitive incentive drive.

A similar analysis comparing sucrose consumption in the acquisition and conflict phases did not reveal significant main effects or interactions (Fig. 4b).

Locomotor sensitization

Cocaine-induced locomotor sensitization was determined by comparing the total distance traveled in the 2 h following administration of the challenge dose (10 mg/kg cocaine, i.p.) with the baseline measurement of total distanced traveled during the 2 h prior to the challenge. Repeated-measures ANOVA revealed a main effect of the *challenge* (F(1, 44)=83.527, p<.0001), indicating a cocaine-induced enhancement in locomotor activity. The analysis further revealed a significant *challenge* by *pre-exposure* interaction (F(1, 44)=14.123, p=.001). Subsequent analysis of simple effects revealed no effect of *pre-exposure* on baseline locomotion (F(1, 44)=.248, p=.621) but a significant effect on cocaine-induced locomotion (F(1, 44)=9.2, p=.004). The analysis further revealed a simple effect of the cocaine challenge in both cocaine-pre-exposed (F(1, 44)=83.172, p<.0001) and saline-pre-exposed rats (F(1, 44)=14.479, p<.0001). Bonferoni post hoc comparisons show that cocaine-pre-exposed (p<.0001) and saline-pre-exposed (p<.0001) rats both exhibited greater locomotion following the cocaine challenge relative to baseline locomotion (Fig. 5).

Elevated plus maze

ANOVA was carried out to compare the time spent exploring the closed and open arms (Fig. 6). Overall, rats spent more time exploring the open than closed arm (main effect of arm, F(1, 45)=35.638, p<.0001), but there was no effect of *pre-exposure* or interaction between factors (F(1, 45)=.052, p=.820).

Discussion

The purpose of the present study was to examine the effects of cocaine pre-exposure upon behavioral responding in approach-

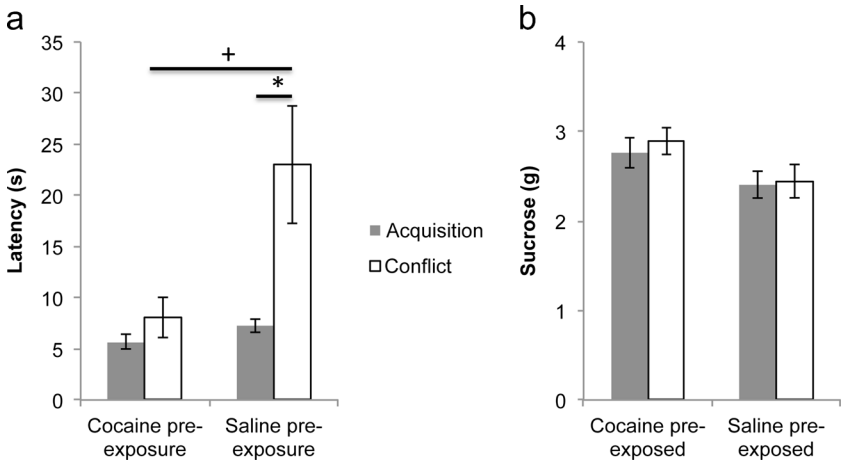

Fig. 4 Mean goal latency (±standard error of the mean (SEM)) and sucrose consumption (±SEM) compared between acquisition and conflict phases. Cocaine-pre-exposed rats (n=9) showed attenuated avoidance of the mixed-valence goal compartment in the conflict phase compared to saline-pre-exposed rats (n=10). Sucrose consumption did not differ between the phases for either group. **a** Acquisition phase compared to conflict phase goal latency. **b** Acquisition phase compared to conflict phase sucrose consumption. *p<0.05 within group, +p<0.05 between groups

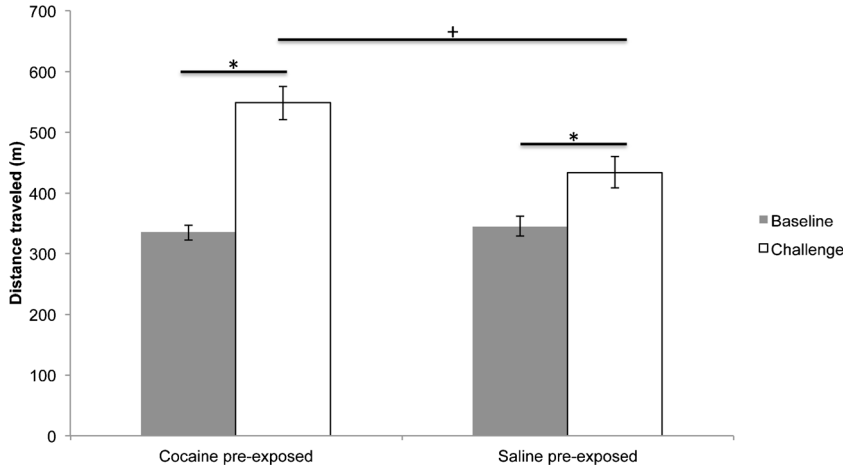

Fig. 5 Cocaine pre-exposure (n=23), compared to saline pre-exposure (n=23), enhanced locomotor activity as measured by mean total distance traveled (±SEM) following a low-dose cocaine challenge (10 mg/kg). *p<0.05 within group, +p<0.05 between groups

avoidance conflict tasks. Findings from experiment 1, utilizing our novel mixed-valence conditioning paradigm, suggest that rats with a history of cocaine exposure were slower to learn the association for the cue predictive of foot shock punishment, while also displaying greater preference for an arm cue imbued with both positive and negative valences. Consistent with these findings, experiment 2 utilizing our mixed-valence runway paradigm revealed that cocaine-pre-exposed rats displayed no significant changes in latency to enter the goal compartment for sucrose reinforcement, despite the consequences of impending foot shock. Taken together, the findings of the present study provide evidence for cocaine-induced alterations that attenuate learning of aversive associations, as well as enhance positive incentive drive in tasks requiring the resolution of conflicting motivational processes.

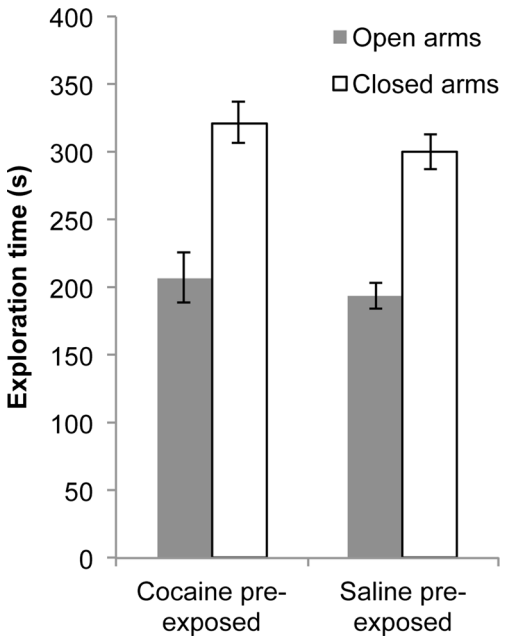

Fig. 6 Anxiety measured as exploration time (±SEM) in open and closed arms in an elevated plus maze. Cocaine-pre-exposed (n=24) and saline-pre-exposed (n=23) rats displayed no differences in anxiety

Novel mixed-valence conditioning paradigm

Our novel mixed-valence conditioning paradigm is unique in that it allows for the behavioral analysis of competing motivational processes. In traditional paradigms for conditioned place preference (CPP) and conditioned place avoidance (CPA), analyses are limited to specifically observing either positive or negative incentive motivation and may be prone to ceiling or floor effects, respectively. In a solely CPA task, for example, animals that have reached asymptote for the aversive association will know very well to avoid negative valence locations. Thus, the task may not be particularly sensitive to manipulations intended to further alter negative incentive motivation.

Our mixed-valence conditioning paradigm, on the other hand, presents an opportunity to assess conflicting motivational processes, such as those characteristic of addiction pathology. Our data from the saline-pre-exposed control group showed that under normal circumstances, exploration of the mixed-valence cue is equivalent to that of the neutral cue, which suggests an equivalent balance of control over behavioral output by both positive and negative incentive motivational processes. In contrast, repeated cocaine pre-exposure resulted in a relative increase in time spent exploring the mixed-valence cue, indicating a shift in favor of positive incentive control over behavior. Of note, these results are consistent with the results that we obtained using the mixed-valence runway task, wherein latency to enter the mixed-valence compartment was significantly shorter following repeated cocaine pre-exposure.

The mixed-valence conditioning paradigm further allows for comparative analyses of the rate and degree to which positive and negative associations are acquired. Since conditioning of both positive and negative cues occurs concurrently in the training sessions, we can observe the rate of gradual acquisition across the three preference tests that precede the conflict test. Here, we found that control animals effectively acquired all cue associations by the first preference test and retained the associations at both subsequent tests. Thus, our training parameters are effective in conditioning positive and negative cue

associations at an equivalent rate and strength and are not prone to extinction after multiple preference tests. This validation further increases our confidence that the attenuated acquisition of the aversive association that we observed in cocaine-pre-exposed rats was indeed an effect of the pre-exposure regimen.

Cocaine pre-exposure enhances positive incentive motivation in tests of motivational conflict

Our finding of cocaine-pre-exposed rats displaying increased exploration of a mixed-valence cue and lower latency values to enter a mixed-valence compartment is consistent with the view that cocaine pre-exposure augments the incentive value of positive rewards and reward-associated stimuli. According to the incentive sensitization hypothesis of drug addiction, the prospect of reward not only becomes more salient but also becomes more desired or "wanted" (Berridge 2007; Robinson and Berridge 1993). As such, cues predictive of reward availability evoke more salient experiences of craving and enhanced motivation to obtain the reward, and therefore, the cocaine-pre-exposed animal exhibits enhanced reward-seeking behaviors. Such an effect has been postulated to be attributable to increased sensitivity of neuronal activity in the brain's mesolimbic reward system, such as enhanced dopamine signaling in response to psychostimulant drugs or drug-associated stimuli (Harmer et al. 1997; Harmer and Phillips 1998, 1999). Thus, in the case of addiction, persistent approach and administration of drugs, despite negative consequences, may be at least partially driven by enhanced processing of reward-related information.

The cocaine-pre-exposure-induced bias for positive incentive control that we observed using the mixed-valence conditioning paradigm may also involve drug-induced adaptations in cortico-limbic-striatal circuits involved in inhibition control and decision making (Everitt and Robbins 2013; Volkow and Baler 2014). Although our task does not vary the probability of reward or punishment, which is a component that is traditionally emphasized in decision-making paradigms, exposure to the superimposition of the positive and negative cues may impose a situation in which the outcome for entering the arm becomes subjected to uncertainty. Here, the appetitive cue signals the possibility of obtaining reward, while the aversive cue, at the same time, signals the risk of receiving foot shock. The process of valuation for costs and benefits and the inhibition of risky goal-directed behavior are importantly influenced by activity in the prefrontal cortex (PFC), an area that has been shown to undergo structural, physiological, and functional abnormalities following chronic cocaine exposure (Hearing et al. 2013; Peters et al. 2008; Rushworth et al. 2011; Volkow and Baler 2014). Research on human cocaine addicts have shown decreased metabolic activity in the PFC, which is thought to represent a down-regulation of the PFC's inhibitory influence on subcortical structures that are crucial for reward processing and the elicitation of appetitive urges, such as the VTA and NAc (Volkow and Baler 2014). These cocaine-induced adaptations in cortico-striatal circuits can have implications for increased impulsivity, enhanced sensitivity to the prospect of reward, and decreased sensitivity to the prospect of punishment, which together may serve to attenuate the suppression of approach behavior in contexts wherein the appraisal of valence is ambivalent, such as the one encountered in our mixed-valence conditioned conflict test.

Cocaine pre-exposure attenuates learning of aversive associations

Our data also corroborate the notion that cocaine pre-exposure can lead to decreased sensitivity to punishment. In our mixed-valence conditioning task, cocaine-pre-exposed rats demonstrated attenuation in the acquisition of aversive conditioning to the foot shock cue, as indicated by a slower acquisition of the aversive association by cocaine, relative to saline-pre-exposed rats. Indeed, whereas saline-pre-exposed rats had acquired the association by test 1, cocaine-pre-exposed rats took until test 2. Importantly, the comparable strengths of cue conditioning observed between saline- and cocaine-pre-exposed rats in tests 2 and 3 allowed for a comparative analysis of behavior in the conflict test.

The attenuated acquisition of aversive associations is consistent with the notion that addicts persist in using drugs despite negative consequences. One interpretation is that the negative outcomes do not readily become associated with the drug experience. This interpretation is consistent with studies that have demonstrated cocaine-induced aberrations in dopaminergic signaling within the NAc, specifically in relation to the relative excitability of medium spiny neurons (MSNs) that express D1 or D2 receptors (Bordreau and Wolf 2005; Conrad et al. 2008; Kourrich et al. 2007; Luscher and Malenka 2011). Together with evidence that D1 receptor activation in the NAc augments reward-seeking behaviors, whereas activation of NAc D2 receptors attenuate reward-seeking behaviors (Hikida et al. 2010; Lobo et al. 2010; Lobo and Nestler 2011), it is conceivable that in the present study, the shift in the balance of behavioral control that we observed in cocaine-pre-exposed rats could potentially be attributed to a shift in the excitability of NAc D1 and D2 receptor MSNs. This is an important question for future research.

Conclusion

The present study utilized novel approach-avoidance paradigms to demonstrate that cocaine pre-exposure alters incentive motivational processing involved in motivational conflict resolution. Not only have we shown that repeated cocaine pre-exposure induces a shift in balance of motivational control that favors appetitive behavioral output but also we have

demonstrated, for the first time, that cocaine-pre-exposed animals exhibit attenuated acquisition of an aversive association. These findings help disentangle the individual contributions of positive and negative incentive processes in cocaine addiction, as well as highlight the importance of dysregulation in negative incentive motivational processes as a key contributor to cocaine addiction pathology.

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