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Effects of cocaine combined with a social cue on conditioned place preference and nucleus accumbens monoamines after isolation rearing in rats

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Abstract

Rationale—Social interaction during drug exposure can potentiate cocaine reward. Isolation rearing (ISO) during adolescence increases social interaction and may amplify this potentiation.

Objectives—The objectives of this study are to determine whether ISO alters conditioned place preference (CPP) for cocaine when combined with a social cue and to determine whether ISO alters the effects of cocaine when combined with social cue on nucleus accumbens shell (NAcS) dopamine (DA) and serotonin (5-HT).

Methods—Male and female rats were either ISO or group (GRP) reared for 4 weeks during adolescence. CPP was performed using a low dose of cocaine (2 mg/kg or saline) with or without exposure to a novel same-sex conspecific during conditioning. In vivo microdialysis was performed using the same parameters.

Results—ISO rats engaged in more social and aggressive behaviors during conditioning relative to GRP. Cocaine reduced social and aggressive behaviors in all rats. CPP was not influenced by rearing condition. Cocaine produced significant CPP, and a social cue produced CPP only in

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males. In contrast, the interaction of cocaine and a social cue on NAcS DA and 5-HT differed depending upon rearing condition. In isolates, cocaine-induced DA was attenuated, while cocaine plus a social cue produced potentiated DA and 5-HT.

Conclusions—Exposure to a low dose of cocaine in the presence of a social cue produced additive effects on CPP while producing synergistic effects on DA and 5-HT in the NAcS of ISO rats. The aversive effects of this compound stimulus may negate the rewarding effects in isolates.

Keywords

Accumbens; Cocaine; Reward; Dopamine; Serotonin

Introduction

Early-life adversity, including during adolescence, produces a variety of developmental changes that can result in vulnerability to (Hicks et al. 2009; Logan and Rose 2004) or, conversely, resistance to (Skinner et al. 2009) psychopathologies including substance abuse disorders. Adolescence represents the transition from childhood to adulthood and, in the rat, has been defined broadly as consisting of early [postnatal day (P)21–P30], middle (P30–P45), and late (P45–P60) periods (Tirelli et al. 2003) and more conservatively as P28–P42 (Spear 2000). These periods are thought to correspond to similar developmental stages in humans (McCormick and Mathews 2009). During adolescence, social interactions with peers become increasingly important, and social deprivation during this period (also known as isolation rearing) has been used as a model of early adversity in rats and other social species (Fone and Porkess 2008; Hall 1998; Lapid et al. 2003). Isolation rearing consists of housing rats in isolation from weaning throughout the adolescent period. This results in a constellation of outcomes, coined “isolation syndrome” (Hatch et al. 1965), which includes changes in patterns of social interaction (Ferdman et al. 2007; Wall et al. 2012; Wongwitdecha and Marsden 1996a; Zhao et al. 2009), hypofrontality (Baarendse et al. 2013; Wall et al. 2012; Zhao et al. 2009), and dysfunction of monoamine systems (Hall 1998; Hall et al. 1998; Jones et al. 1992; Lukkes et al. 2009).

Isolation rearing can also produce altered responses to drugs of abuse, including psychostimulants such as cocaine (Morutto and Phillips 1997; Phillips et al. 1994b; Schenk et al. 1986; Schenk et al. 1987; Smith et al. 2005; Wongwitdecha and Marsden 1995). Importantly, unlike in most laboratory conditions, in real-life situations, drugs are frequently not consumed in isolation, especially during the initiation phase. Rather, drug taking is often inextricably linked to the experience of social interaction with peers (Epstein et al. 2007), which in itself can be highly reinforcing, particularly during adolescence (Douglas et al. 2004; Varlinskaya and Spear 2008). Recent research by Thiel et al. (2008) has demonstrated that the rewarding effects of cocaine are potentiated in adolescent male rats by exposure to a conspecific during conditioned place preference (CPP). We (Wall et al. 2012) and others (Vale and Montgomery 1997; Wongwitdecha and Marsden 1996b) have observed increases in social interaction after isolation rearing, suggesting that social interaction may be more rewarding to isolation-reared rats. Isolation rearing may increase the salience of social interactions, producing vulnerability to the social context dimension of addiction. Thus, isolation rearing may produce either resistance or vulnerability to drug addiction via effects

on two different dimensions of the drug experience: the intrinsic effects of the drug and the social context in which the drug is administered.

The purpose of the present study was to explore the effects of isolation rearing on the rewarding effects of cocaine in combination with a social cue, in order to mimic drug taking in a social context. Male and female rats were exposed to 4 weeks of either isolation or group rearing beginning at weaning (P21) throughout adolescence, then tested with a low dose of cocaine either in the presence or absence of a novel same-sex conspecific (social cue). A low-dose regimen was selected in order to remain near the threshold for CPP, to avoid ceiling effects with cocaine alone, and to maintain adequate levels of social interaction, as cocaine has been demonstrated to dose-dependently reduce social behaviors including affiliation, aggression, and play behavior (Achterberg et al. 2013; Miczek and O'Donnell 1978; Rademacher et al. 2002). In separate experiments, we assessed reward-seeking behavior using CPP and nucleus accumbens shell (NAcS) monoamines [dopamine (DA) and serotonin (5-HT)] using in vivo microdialysis coupled with high-performance liquid chromatography (HPLC). We predicted that isolation-reared rats would develop greater CPP for the combination of cocaine and a social cue than group-reared rats and that this would be mirrored by greater NAcS dopamine DA and 5-HT release produced by this compound stimulus.

Materials and methods

Animals

Male and female Sprague–Dawley rats were purchased (Harlan Laboratories, Indianapolis, IN, USA) immediately after weaning at P21 (a period corresponding to the early adolescent period) and housed for 4 weeks either singly [isolation-reared (ISO)] or in same-sex groups [group-reared (GRP)] of three (microdialysis) or four (CPP) in standard Plexiglas cages before experimentation began. Experimentation took place during P51–P55, a period corresponding to late adolescence. Rats were on a 12-h light/dark cycle and maintained at 23 °C with food and water freely available in an AAALAC-accredited facility. Rats were weighed weekly but otherwise unhandled during the 4 weeks of group or isolation rearing. No effects of rearing condition were observed on animals' weights. Vaginal smears were not performed on females because regular handling during the isolation period is known to reduce the effects of isolation rearing (Krebs-Thomson et al. 2001; Rosa et al. 2005), and the stress of monitoring cycle status has been shown to alter 5-HT and adrenal responses to subsequent stressor exposure (Sfikakis et al. 1996). All procedures were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

Drugs

Cocaine HCl (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in sterile 0.9 % saline at a concentration of 2, 5, or 10 mg/ml. Injection volumes (IP) of cocaine and saline were 1 ml/kg.

Dose dependency of cocaine's effects on social exploration

Because social interaction is a crucial variable in the present studies, it is important that adequate levels of social interaction are maintained throughout the conditioning period. Thus, we assessed whether commonly used doses of cocaine (Tzschentke 2007) would affect the amount of time actively engaged in social exploration with a novel same-sex conspecific using GRP males. Each rat was placed in a clear Plexiglas cage with fresh bedding, identical to the cages used for housing. After a 10-min habituation period rats ($n=4$ per group) were injected with either 0.9 % saline or with 2, 5, or 10 mg/kg cocaine IP, doses that are commonly used in CPP studies (Tzschentke 2007). A novel, naive male rat, slightly younger and smaller than the experimental rat to increase social exploration and decrease fighting (Christianson et al. 2008), was then placed in the cage for 10 min. Sessions were digitally recorded using a webcam and later scored for the amount of time spent in active social exploration by the experimental rat by an experimenter blinded to the treatment conditions. Social exploration was defined as the overall time the experimental rat spent actively interacting (e.g., sniffing, following, grooming, pinning, pouncing; initiated by or engaged in by the experimental rat) with the stimulus rat (File and Seth 2003).

Conditioned place preference

A timeline of the CPP experiment is shown in Fig. 1a. CPP was performed as previously described (Der-Avakian et al. 2006) with the addition of a social cue during conditioning in some groups. The Plexiglas place preference apparatus measures 72 cm×30 cm×30 cm (length, width, and height) and is large enough to easily accommodate two rats. The apparatus consists of three distinct environments: two conditioning environments and a neutral area. Each conditioning environment measures 30 cm×30 cm×30 cm. The environments differ from each other in olfactory, visual, and tactile cues. One conditioning environment is striped horizontally with alternating 2-cm black and white electrical tape on the walls, while the other conditioning environment is striped vertically in the same manner. The floor of one environment consists of metal bars 5 mm in diameter spaced 1.5 cm apart, and the floor of the second environment is a wire mesh grate. A tray underneath the floors contains either pine or cedar bedding. The neutral area measures 12 cm×30 cm×30 cm and is painted gray with a sanded black Plexiglas floor. During the conditioning phase, vertically and horizontally striped Plexiglas partitions were inserted on the respective sides of the neutral area to restrict the rats to their conditioning environment. The activity of each rat was monitored via Logitech Quickcam Pro 5000 webcams (Fremont, CA, USA) mounted 1.0 m above the center of the conditioned place preference apparatus and sent to a computer running AnyMaze software (Stoelting Co., Wood Dale, IL, USA) to record the time a subject spends within each of the three compartments as well as locomotor activity. Each test session was video-recorded with AnyMaze for later analysis of social behavior.

Conditioning was performed using a biased design. Rats were handled for 2 min/day for 2 days prior to conditioning. Then, rats were habituated to the CPP apparatus by allowing free access to the entire apparatus for 10 min/day across 3 days. On day 3, baseline preference was recorded. Next, four conditioning sessions were conducted with two 10-min sessions/day for 2 days in a counterbalanced fashion. Thiel et al. (2008) have reported that

these parameters, which were subthreshold for either cocaine or social cue conditioning, are optimal for cocaine plus social cue conditioning. The starting side for conditioning on the first day was the preferred side for half of the rats and the nonpreferred side for the other half. This order was reversed on the second day. In addition, half of the rats received drug in the morning session and half in the afternoon session, and this order was reversed on the second day. Rats received saline injections on alternate trials. Rats were injected IP with cocaine or saline and placed within the appropriate environment within 3 min. In trials that included a social cue, a same-sex group-housed naive stimulus rat was placed in the environment immediately after the experimental rat. Slightly younger (P24–P34), naive, group housed rats were used as social stimuli in order to be less threatening to the experimental rats which reduces aggressive behavior (Christianson et al. 2008). Rats were exposed to the same stimulus rat for both social-cue paired trials. Conditioning sessions were performed at the same time each day, with 4 h between sessions to allow drug clearance. Preference was tested the day after the last session. Rats were exposed to the entire apparatus in a drug-free state with no stimulus rat for 10 min and time spent in each chamber was recorded. Data for the CPP experiments are expressed as a difference score consisting of time (seconds) spent in the paired chamber posttest minus time spent in the paired chamber pretest. One hundred forty-seven rats completed the CPP experiment, with final group sizes of 9–12.

Social behavior was assessed by analyzing the digital video files recorded by the AnyMaze software by experimenters blind to the treatment conditions. The following behaviors were assessed: *social exploration*—overall time the experimental rat spends actively interacting (e.g., sniffing, following, grooming) with the stimulus rat (File and Seth 2003); *pinning*—standing over/holding down the stimulus rat while it is in a supine posture (Hurst et al. 1999); *aggressive grooming*—vigorous grooming by the experimental rat of the stimulus rat when it is standing, crouching, supine, or trying to escape (Hurst et al. 1999).

In vivo microdialysis

Surgery

All surgeries were performed using aseptic procedures under isoflurane anesthesia and using the coordinates (from bregma) of Paxinos and Watson (2007). Guide cannulae (MAB 9.14.2, SciPro/Microbiotech, Stockholm) were aimed at the NAcS shell: for males, AP+1.8, LM±0.7, DV–5.8; for females, AP+1.7, LM±0.6, DV–5.5, adapted from Philpot et al. (2001). The cannulae and tether screws were anchored to the skull using skull screws and dental acrylic. After surgery, rats were returned to their presurgery housing conditions. A protective aluminum cap (SciPro/Microbiotech, Stockholm, Sweden) allowed postsurgery group housing of group-reared rats. Rats were handled as for the CPP experiments, once before surgery and once 3 days after surgery.

Microdialysis

A timeline of the microdialysis experiment is shown in Fig. 1b. Microdialysis was performed using similar procedures to those previously reported (Bland et al. 2003). On the afternoon before microdialysis, rats were transferred to a separate dialysis room on the same

light–dark cycle. Micro-dialysis probes (MAB 9.14.2, SciPro; M_r cut-off, 6 kDa, 2 mm active membrane) were inserted into the guide cannulae, and rats were placed in separate clear Plexiglas boxes (30 cm× 30 cm×30 cm) with food and water freely available. Artificial cerebrospinal fluid (145 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl, 1.0 mM KCl) was perfused through the probes using a Stoelting infusion pump at a flow rate of 0.2 μ l/min overnight. The flow rate was increased to 2.0 μ l/min the next morning, and, after a 2 h equilibration period, sample collection began. Dialysates were collected manually every 20 min and placed on dry ice until after the experiment, then transferred to a –80 °C freezer until HPLC was performed.

Three baseline samples were collected. Rats were then injected IP with either cocaine (2 mg/kg) or equivolume saline. In trials that included a social cue, a same-sex, slightly smaller group-housed naive rat was then placed in the infusion bowls for 10 min. Sampling continued for 120 min. Following the experiment, brains were removed for histological verification of probe placement using cresyl violet and light microscopy. Only rats with at least 75 % of the probe in the NAcS were included in the analysis. Figure 2 shows representative probe placements. HPLC procedures were similar to those previously described (Bland et al. 2009). Briefly, DA in the dialysates was analyzed using an ESA 5600A Coularray detector with an ESA 5014B analytical cell and an ESA 5020 guard cell connected to an ESA HD-80 column (C18, 3 μ m, 80×3 mm), which is maintained at 37 °C. The mobile phase was 150 mM sodium dihydrogen phosphate monohydrate, 4.76 mM citric acid monohydrate, 3 mM sodium dodecyl sulfate, 50 μ M EDTA, 10 % methanol, and 15 % acetonitrile, pH=5.60. The potentials were set at –75 (reduction) and +220 mV (oxidation). Data were expressed as percent of baseline (the mean of the first three samples) and analyzed using mixed ANOVAs with time as a within-subjects factor and sex, housing, social cue, and drug as between-groups factors.

Statistical analysis

Statistics were performed using PASW Statistics 18.0. Social exploration in the dose–response study was analyzed with one-way ANOVA. CPP data [difference scores: time spent in the paired chamber posttest minus pretest (s)] were analyzed using 2 (rearing)×2 (drug)×2 (sex)×2 (social cue) factorial ANOVA. In addition, to assess whether place preference had occurred, single-sample *t* tests were performed on each group to determine whether the difference score was significantly different than 0. Behavioral data were analyzed using 2 (rearing)×2 (drug)×2 (sex) factorial ANOVA. Fisher's least significant difference post hoc tests were performed when significant interactions were observed. DA and 5-HT basal data (pg/40 μ l sample) were analyzed using 2 (rearing)× 2 (Drug)×2 (sex)×2 (social cue) factorial ANOVA. DA and 5-HT (percent baseline) data were analyzed using mixed ANOVA with rearing, drug, sex, and social cue as between-groups variables and time as a within subjects variable. Simple effects tests were performed post hoc at each time point when significant interactions with time were observed. Alpha was set at .05 for all experiments.

Results

Dose dependency of cocaine's effects on social exploration

In order to determine the effects of three different doses of cocaine on social exploration, we assessed the amount of time that group-reared, late adolescent male rats spent actively engaged with a novel conspecific during 10-min trials. Cocaine dose dependently decreased the amount of time spent actively engaged in social exploration, with higher doses having profoundly suppressive effects on social exploration (Fig. 3). One-way ANOVA revealed a highly significant effect of cocaine dose on social exploration [$F(3,12)=27.87, p<.0001$]. Compared to saline controls, 2 mg/kg cocaine did not significantly decrease the amount of time engaged in social exploration. However, cocaine at both 5 and 10 mg/kg produced a marked reduction in social exploration compared to both saline and 2 mg/kg cocaine (both $p<.01$).

Isolation rearing did not affect CPP for cocaine, a social cue, or the combination of cocaine and a social cue

We assessed the effects of isolation rearing on CPP to 2 mg/kg cocaine, either with or without a social cue during the conditioning trials, in males and females. Figure 4 shows difference scores (posttest minus pretest) during a 10-min test period. Both cocaine and a social cue produced reliable CPP. ANOVA revealed highly significant main effects of drug [$F(1, 131)=15.43, p<.001$] and social cue [$F(1, 131)=23.68, p<.001$]. In addition, there was a modest but significant sex difference in CPP [$F(1, 131)=3.94, p<.05$], reflecting greater CPP in males than females. There were no significant interactions of rearing, drug, sex, or social cue. Because there was a main effect of sex, we analyzed the CPP data separately for males and females. Significant main effects of both drug ($p<.01$) and social cue ($p<.0001$) were observed for males; however, a main effect for drug ($p<.05$) but not for social cue ($p=.16$) was observed for females. Thus, it is likely that the main effect of sex was driven by the robust CPP in males to a social cue, whether in the presence or absence of cocaine. Contrary to our predictions, rearing condition did not significantly influence CPP to cocaine [rearing $\times$ drug, $F(1, 31)=.07, p=.79$], social cue [rearing $\times$ social cue, $F(1, 31)=1.02, p=.31$], or the combination of cocaine plus a social cue [rearing $\times$ drug $\times$ social cue, $F(1, 31)=.17, p=.68$]. Furthermore, there was no drug $\times$ social cue interaction, $F(1, 31)=.001, p=.98$. The effects of drug and social cue were additive. Thus, overall, in the absence of a social cue, the increase in preference for the cocaine-paired side was 37 s greater than saline, while in the presence of a social cue, the increase in preference above that produced by the social cue alone was 40 s. Single-sample t tests indicated that significant CPP (significant difference from 0) was observed for cocaine alone in GRP males and females, for social cue alone in GRP and ISO males, and for cocaine plus social cue in GRP and ISO males and females.

Isolation rearing increased, while cocaine decreased, social behavior

We assessed social behavior during the conditioning trials in rats that had a social cue present.

Social exploration—There were significant main effects of sex, rearing, and drug on overall time spent in social exploration, as shown in Fig. 5a. Males spent more time engaging in social exploration than females [$F(1, 65)=9.02, p<.01$]. ISO spent more time engaging in social exploration than GRP [$F(1, 65)=70.89, p<.0001$], and cocaine reduced the amount of social exploration [$F(1, 65)=14.78, p<.001$]. This is in contrast to our dose-response experiment, in which the decrease in social exploration was not significant. This is likely due to the large number of rats and the increased power in the present experiment, as the magnitude of the decrease in social exploration is similar in both studies.

Aggressive grooming—There were significant main effects of sex, rearing, and drug on aggressive grooming, as shown in Fig. 5b. Males spent more time engaging in aggressive grooming than females [$F(1, 65)=7.46, p<.01$]. ISO spent more time engaging in aggressive grooming than GRP [$F(1, 65)=39.99, p<.0001$], and cocaine reduced the amount of time spent in aggressive grooming [$F(1, 65)=21.23, p<.001$]. For pinning, ISO rats exhibited significantly more pins than GRP rats in the saline condition, but not after cocaine, as shown in Fig. 5c. This was supported by a significant rearing by drug interaction [$F(1, 65)=4.46, p<.05$], indicating that cocaine reduced pinning more in ISO rats than in GRP rats.

Isolation rearing attenuated cocaine-induced NAcS DA but potentiated NAcS DA when cocaine was combined with a social cue

We assessed the effects of a combination of cocaine and a social cue on NAcS DA in male and female rats after isolation or group rearing. There were no significant group differences in basal levels of DA. Table 1 shows basal levels of DA and 5-HT collapsed across drug and social cue.

Dopamine—One hundred thirteen rats completed the microdialysis experiment, with final group sizes of 6–8. Figure 6 shows NAcS DA levels (percent baseline) in male and female GRP and ISO rats exposed to saline or cocaine in the presence or absence of a social cue. There was a significant rearing $\times$ drug $\times$ social cue $\times$ time interaction [$F(8, 752)=2.98, p<.01$]. Post hoc simple effects tests performed at each time point indicated that cocaine increased DA levels at time 20 (the first sample after injections of cocaine or saline) in ISO rats regardless of sex, but only in the presence of a social cue ($p<.05$). This increase was greater than that of GRP rats receiving cocaine ($p<.05$). Cocaine increased dopamine in GRP rats at time 20, regardless of the presence or absence of a social cue ($p<.05$). Cocaine did not produce a significant increase in dopamine in ISO rats in the absence of a social cue. A social cue alone did not influence DA in the NAcS in any group, and there were no main effects or interactions of Sex on NAcS DA.

Analysis of lower-order effects indicated that cocaine increased NAcS DA [main effect of drug, $F(1, 89)=22.46, p<.0001$; drug $\times$ time interaction, $F(8, 712)=12.42, p<.0001$]. Significant interactions were also obtained for social cue $\times$ time [$F(8, 712)=3.39, p<.01$] and rearing $\times$ social cue $\times$ time [$F(8, 712)=2.5, p<.01$], likely reflecting the large increase in DA in ISO rats in the presence of cocaine and a social cue.

Isolation rearing potentiated cocaine-induced 5-HT when cocaine was combined with a social cue

We also assessed the effects of a combination of cocaine and a social cue on NAcS 5-HT in male and female rats after isolation or group rearing. There were no significant group differences in basal levels of 5-HT, although there was a trend for decreased 5-HT in ISO rats [$F(1, 81)=3.61, p=.06$]. Table 1 shows basal levels of DA and 5-HT collapsed across drug and social cue.

Figure 7 shows NAcS 5-HT levels (percent baseline) in male and female GRP and ISO rats exposed to saline or cocaine in the presence or absence of a social cue. Several rats had undetectable basal levels of NAcS 5-HT; thus, data are from 98 rats (5–7 per group). There was a significant rearing $\times$ drug $\times$ sex $\times$ social cue $\times$ time interaction [$F(8, 656)=2.27, p<.05$]. There was a marked synergistic effect of cocaine and a social cue on NAcS 5-HT in male ISO rats. This was supported by post hoc simple effects tests indicating that the combination of cocaine and a social cue produced increases in NAcS 5-HT in male ISO rats more than all other groups at times 20 and 40 ($p<.01$). The combination of cocaine and a social cue also produced an increase in NAcS 5-HT at time 20 in ISO females relative to ISO females with either cocaine or a social cue or saline alone ($p<.05$). Thus, cocaine plus a social cue produced an increase in NAcS 5-HT in both male and female ISO rats, but this increase was greater in males. Analysis of lower-order effects indicated that cocaine increased NAcS 5-HT [main effect of drug, $F(1, 78)=8.73, p<.01$; drug $\times$ time interaction, $F(8, 624)=8.156, p<.0001$] and that a social cue increased NAcS 5-HT [main effect of social cue, $F(1, 78)=4.76, p<.05$]. A significant interaction was also obtained for rearing $\times$ social cue $\times$ sex $\times$ time [$F(8, 624)=3.09, p<.01$], likely reflecting the large increase in 5-HT in ISO male rats in the presence of cocaine and a social cue.

Discussion

We hypothesized that the increase in social exploration produced by 4 weeks of isolation rearing would potentiate both CPP for a social cue and CPP for a combined social cue and cocaine challenge, and that this would be associated with potentiation of NAcS monoamines. To explore this idea, we used low doses of both social exposure (Calcagnetti and Schechter 1992; Douglas et al. 2004; Kummer et al. 2011) and cocaine. Here, we report a dissociation between behavioral and neurochemical responses to a low dose of cocaine combined with a social cue in isolation-reared rats. We observed additive effects of the combination of social cue and cocaine reward on CPP that was not affected by rearing condition or by sex. However, we observed synergistic effects of the combination of social cue and cocaine reward on both DA and 5-HT in the NAcS of isolation-reared rats.

We were surprised that two conditioning trials with a social cue, even in the absence of cocaine, produced significant CPP, and although this was most pronounced in males, there was no impact of rearing condition. Previous reports of social CPP have used four or eight trials (Calcagnetti and Schechter 1992; Douglas et al. 2004; Kummer et al. 2011) of social cue exposure. We assessed social exploration during the conditioning trials in which a social cue was present and found that social exploration was greatest in ISO males. In agreement with our previous findings (Wall et al. 2012) as well as those of others (Vale and

Montgomery 1997; Wongwitdecha and Marsden 1995), 4 weeks of isolation rearing increased the amount of time male and female rats spent interacting with a novel same-sex conspecific relative to group-reared rats. The increase in social interaction typically observed after 4–6 weeks of isolation rearing contrasts with decreased social interaction produced by longer periods of isolation rearing (Hermes et al. 2010; Moller et al. 2010). In addition, and also consistent with previous work (Johnston and File 1991; Stack et al. 2010; Wall et al. 2012), males spent more time interacting with a novel conspecific than females. Cocaine reduced social exploration as well as aggressive grooming, supporting previous reports of decreased aggressive behavior produced by cocaine (Miczek and O'Donnell 1978; Rademacher et al. 2002). Cocaine also reduced pinning, which is typically considered a play behavior. Pinning behavior was greater in ISO males and females, and cocaine reduced these levels more in ISO than GRP rats. Thus, cocaine also reduced play behavior, but it should be noted that the higher number of pins elicited by ISO rats may indicate age-inappropriate behavior. Pinning normally decreases dramatically across adolescence (Panksepp 1981), and this decrease appears to be hindered in ISO rats.

It has previously been reported that in male rats, the combination of 2 mg/kg cocaine and a social cue produced CPP with two 10-min pairings, while neither produced CPP alone (Thiel et al. 2008). However, this finding was not observed in the present study using the same cocaine dose, number of exposures to a social cue, and time in the conditioning chamber. Rather, we observed an additive effect of cocaine and social cue. This was especially pronounced in male rats, which developed robust CPP to a social cue alone. This discrepancy may be due to the differences in the ages of the rats in the two studies. In the study of Thiel et al. (2008), CPP commenced at P28 (early adolescence), rather than at P50 (late adolescence) in the present study necessitated by the 4 weeks of differential housing during adolescence. One possibility is that early, rather than late, adolescence is a critical period for drug/social cue synergy. Both social interaction (Douglas et al. 2004) and cocaine (Zakharova et al. 2009b) are more rewarding during adolescence than adulthood, but to the best of our knowledge, differences across different periods of adolescence have not been explored. Using concurrent CPP for social interaction vs cocaine (15 mg/kg) in young adult male rats, Fritz et al. (2011) demonstrated that social interaction and cocaine were equally rewarding. In accordance with this, we observed significant CPP to both cocaine and a social cue when each was administered separately. There is evidence that females are more sensitive to the rewarding effects of cocaine, including during adolescence (Zakharova et al. 2009a). However, under the conditions in the present study, females did not exhibit greater CPP to cocaine than males.

Although 4 weeks of isolation rearing produced an increase in time spent engaged in social exploration during the conditioning trials, CPP for a social cue was not increased in ISO rats, contrary to our prediction. Thus, isolation rearing may increase the motivational effects, but not the hedonic effects of social interaction. However, Douglas et al. (2004) have reported that 10 days of social isolation increased social CPP. It is interesting to note that in the present study aggressive behavior was increased in rats after 4 weeks of isolation rearing; thus, it may be that the novel social interaction contained an aversive component that was greater for rats that had experienced extended social isolation during adolescence. Thus, social interactions may be more stressful or anxiogenic to isolation-reared rats. Hall et

al. (1997) have shown that the greater preference for a novel environment produced by isolation rearing was no longer observed when the environment was highly arousing. The greater hyper-reactivity known to be a component of the isolation syndrome may produce animals that are hyper-aroused by a novel conspecific.

Published reports on the effects of isolation rearing on cocaine CPP reveal divergent results. Schenk et al. (1986) observed that isolation rearing decreased cocaine (but not amphetamine) CPP. Green et al. (2010) have reported that relative to socially-reared rats, isolation-reared rats exhibited no difference in cocaine CPP, though when compared to enriched rats cocaine CPP was reduced. In females, isolation rearing reduced cocaine CPP (Smith et al. 2009). Decreased CPP to other classes of drugs, including opioids (Schenk et al. 1983; Smith et al. 2005; Wongwitdecha and Marsden 1996a), amphetamine (Wongwitdecha and Marsden 1995) and sulpiride (Morutto and Phillips 1997) have also been observed in isolation-reared rats.

Several studies have investigated the effects of isolation rearing on cocaine self-administration, but these results are also equivocal. Some groups have reported that isolation-reared rats self-administered more cocaine than group housed controls (Schenk et al. 1987; Yajie et al. 2005), yet it has also been reported that isolation rearing reduced cocaine self-administration (Phillips et al. 1994a). Escalation of cocaine self-administration was enhanced in isolation-reared rats, but only at a low dose (Gipson et al. 2010). Baarendse et al. (2013) have recently demonstrated that rats that were isolated during the juvenile and early adolescent period, then socially housed until adulthood, self-administered more cocaine and had higher breakpoints, though extinction and reinstatement were not impacted. In self-administration studies without breakpoint data the interpretation of increased drug-taking can be ambiguous, as rats may self-administer less to obtain the same hedonic effects if they are more sensitive to the drug (enhanced hedonic efficacy); thus, an increase in self-administration may suggest a decreased sensitivity to the drug. In support of this, because the inter-reinforcement intervals for cocaine lever presses were shorter for isolated adolescents in the study by Yajie et al. (2005), the authors speculated that adolescent isolation may decrease the reinforcing effects of cocaine. Moreover, Smith et al. (2009) have noted that while isolation-reared rats had a higher breakpoint for cocaine than enriched rats, responding for the inactive lever was also greatly increased, consistent with the hyper-reactivity frequently observed in isolates (Fone and Porkess 2008; Hall 1998). When expressed as a percent of total responding, breakpoints were markedly lower in isolates (Smith et al. 2009). Taken together, it remains unclear whether isolation rearing increases, decreases, or has no effect on the reinforcing effects of cocaine, and many variables including strain, time spent in isolation, and procedural details may make important contributions to these effects.

In addition to assessing the behavioral effects of social cue and cocaine in isolation and group reared rats, we assessed NAcS monoamines using *in vivo* microdialysis. Using a low dose of cocaine with or without a social cue, isolates required the combination of cocaine and a social cue to exhibit a significant DA increase in the NAcS. This effect was synergistic in that neither cocaine nor a social cue alone produced an increase in NAcS DA, while the combination produced a robust increase in both males and females. In contrast, cocaine

increased NAcS DA in GRP rats regardless of the presence of a social cue. Several groups have observed increases in DA release in the NAcS of isolates in response to various challenges. For instance, acute footshock stress as well as reexposure to the stress environment (conditioned emotional response) produced robust increases in NAcS DA release in isolates that were greater than those of group-reared rats (Fulford and Marsden 2007; Lapiz et al. 2003). In a slice voltammetry study, both electrically evoked DA release and DA reuptake were increased in isolation-reared rats (Yorgason et al. 2013). Furthermore, and in contrast with the present findings, cocaine produced a greater increase in NAc DA (core and ventrolateral shell) in Lister Hooded rats after 8 weeks of isolation rearing (Howes et al. 2000). This is consistent with an earlier study that revealed enhanced amphetamine-induced NAc DA in isolates (Jones et al. 1992). The use of different strains and lengths of isolation, methodological differences include probe placements, doses, and the route of drug administration may account for the discrepancy. Moreover, an important procedural difference in our study is the return of group-reared rats postsurgery to social housing conditions, which is not standard in microdialysis studies. Future studies will be required to determine the impact of post-surgical housing conditions on mesolimbic DA function.

In the present study, exposure to a social cue alone did not produce significantly elevated NAcS DA in any group; although there was a significant social cue $\times$ time interaction, this may have been driven by the interaction with cocaine. This was surprising, as a social cue alone produced CPP in males, and suggests that social reward is independent of NAcS DA and may rely more on mediation by endogenous peptides such as opioids (Panksepp et al. 1980) or endocannabinoids (Trezza et al. 2012). Alternatively, social-cue-induced CPP may involve phasic activation of DA neurons in the ventral tegmental area, and it is generally accepted (e.g., Floresco et al. 2003) that microdialysis detects tonic, but not phasic, levels of neurotransmitters, including DA.

We assessed NAcS 5-HT because it has been shown that isolation rearing can influence 5-HT neurotransmission (Fone and Porkess 2008; Hall 1998; Lukkes et al. 2009) and because of the known role of 5-HT as a modulator of DA in the NAcS. 5-HT modulation of NAcS DA is complex and involves multiple receptor subtypes as well as numerous brain regions including the prefrontal cortex (Bland et al. 2004; Liegeois et al. 2002). 5-HT has been shown to exert inhibitory control on DA release in the NAcS via both 5-HT_{2B} (Auclair et al. 2010) and 5-HT_{2C} (Navailles et al. 2006) receptors. Behavioral evidence for an inhibitory role for 5-HT in reward includes the finding that a 5-HT_{2C} receptor agonist attenuated cocaine CPP (Craig and Unterwald 2013). We observed a synergistic effect of cocaine and a social cue on levels of extracellular 5-HT in the NAcS in isolates; neither cocaine nor a social cue alone produced significant increases in NAcS 5-HT, but the combination produced a robust increase, especially in male isolates. High levels of NAcS 5-HT are associated with stress (Bland et al. 2003; Fulford and Marsden 2007), and the present results suggest that the experience of cocaine in the presence of a social cue is more stressful to ISO rats, particularly males. This may in part explain the absence of a synergistic effect of cocaine and social cue on CPP in isolates as predicted: The aversive effects of this combination may override the reinforcing effects. Consistent with this idea, the known anxiogenic effects of cocaine (Ettenberg and Geist 1991; Ettenberg et al. 1999) have been

attributed to serotonin (Ettenberg et al. 2011). Interestingly, results from a recent investigation of the play-suppressive effects of psychostimulants in adolescent rats suggest that heightened activity of both 5-HT and DA are responsible for the reduction in social play produced by cocaine (Achterberg et al. 2013).

In summary, the combination of cocaine and a novel social cue produced an additive effect on CPP, regardless of rearing condition or sex. Cocaine also reduced the amount of time spent engaged in social interaction. However, exposure to cocaine in the presence of a social cue exerted synergistic effects on both DA and 5-HT in the NAcS of isolation-reared rats, suggesting that the rewarding and aversive effects of this compound stimulus may oppose each other.

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a Conditioned place preference **b** *In vivo* microdialysis

Fig. 1. Timelines of the CPP (a, top) and microdialysis (b, bottom) experiments

Fig. 2.

Representative microdialysis probe placements in the NAcS. Left: photomicrographs of cresyl violet stained probe tracks in the NAcS with midline (*ML*) and anterior commissure (*AC*) as landmarks. *Right* Image adapted from Paxinos and Watson (2007) showing representative placements. Values on the *right* indicated distance from bregma. Not all 113 placements are shown due to overlapping placements

Fig. 3.

Dose dependent effects of cocaine on social interaction with a novel conspecific in male rats. Values represent the total amount of time (s, means $\pm$ SEMs) that experimental rats spent actively engaged in social interaction with a novel conspecific during a 10 min trial. ** $p < .01$, less than saline; ## $p < .01$, < 2 mg/kg cocaine

Fig. 4.

Conditioned place preference (CPP) to cocaine (2 mg/kg) with or without a social cue in isolation reared (ISO) and group-reared (GRP) female (*top*) and male (*bottom*) rats. Values represent difference scores: time (s, means $\pm$ SEMs) spent in the drug/social cue-paired chamber posttest minus time spent in that chamber pretest. [#] $p < .05$, ^{*} $p < .01$, difference scores significantly different from the hypothesized mean of 0

Fig. 5.

Social behavior with a novel same-sex conspecific during the conditioning trials in rats exposed to a social cue during place preference, averaged across both paired trials. **a** Time (s) engaged in social interaction. **b** Time (s) spent in aggressive grooming by the experimental rat. Cocaine reduced aggressive grooming overall (main effect of drug, $p < .001$). **c** Pinning (number of pins) by the experimental rat. * $p < .05$, less than ISO saline

Fig. 6.

DA in the NAcS of GRP (**a, c**) and ISO (**b, d**) male and female rats exposed to cocaine either in the presence or absence of a social cue. Cocaine produced increases in NAcS DA regardless of the presence or absence of a social cue in GRP rats (**a, c**). Cocaine produced increases in NAcS DA only in the presence of a social cue in ISO rats (**b, d**). The *arrows* indicate injection of either saline or 2 mg/kg cocaine, and the *horizontal bars* indicate the presence of a novel same-sex conspecific in the dialysis chamber. * $p < .05$, GRP rats with cocaine with or without a social cue different from GRP rats with saline, with or without social experience, at this time point. # $p < .05$, ISO rats with cocaine and social cue different from all other ISO groups at this time point

Fig. 7.

5-HT in the NAcS of GRP (**a, c**) and ISO (**b, d**) male and female rats exposed to cocaine either in the presence or absence of a social cue. Cocaine and a social cue interacted to increase NAcS 5-HT preferentially in male ISO rats. The *arrows* indicate injection of either saline or 2 mg/kg cocaine, and the *horizontal bars* indicate the presence of a novel same-sex conspecific in the dialysis chamber. \$ $p < .01$, ISO males with cocaine and social cue different from all other groups at this time point

Table 1
Basal levels of monoamines (pg/sample; the average of the first 3 samples) in the NAc shell of GRP and ISO reared males and females

	GRP	ISO
Dopamine		
Female	4.25±0.99	4.91±1.07
Male	4.11 ± 1.06	5.91 ±1.58
Serotonin		
Female	1.14±0.37	0.70±0.09
Male	0.89±0.27	0.47±0.08

There were no significant differences in basal levels of extracellular monoamines between any groups; thus, values are shown collapsed across social cue and drug conditions. Values are means±SEMs of 26–30 rats/group