Published in final edited form as:

Drug Alcohol Depend. 2008 August 1; 96(3): 202–212.

Social Reward-Conditioned Place Preference: A Model Revealing an Interaction between Cocaine and Social Context Rewards in Rats

Kenneth J. Thiel^a, Alec C. Okun^a, and Janet L. Neisewander^{a,*}

^aDepartment of Psychology, Arizona State University, P.O. Box 871104, Tempe, AZ 85287

Abstract

A recent thrust in drug abuse research is the influence of social interactions on drug effects. Therefore, the present study examined conditioned place preference (CPP) as a model for assessing interactions between drug and social rewards in adolescent rats. Parameters for establishing social reward-CPP were examined, including the number of conditioning sessions/day (1 or 2), the total number of sessions (2, 4, or 16), and the duration of sessions (10 or 30 min). Subsequently, the effects of cocaine or dextromethorphan on social reward-CPP and play behavior were examined. The results demonstrate that social reward-CPP (i.e., preference shift for an environment paired previously with a rat) was similar using either 1 or 2 conditioning sessions/day and either 10 or 30 min sessions; however, social reward-CPP increased as the number of social pairings increased. Additionally, a low dose of cocaine (2 mg/kg, IP) and a low number of social pairings (2 pairings) failed to produce CPP when examined alone, but together produced a robust CPP, demonstrating an interaction between these rewards. The non-rewarding drug, dextromethorphan (30 mg/kg, IP), failed to enhance social reward-CPP, suggesting that drug-enhanced social reward-CPP is specific to rewarding drugs. Surprisingly, there was no relationship between play behaviors and preference shift in drug-naïve animals. Furthermore, cocaine inhibited play behavior despite enhancing social reward-CPP, suggesting that aspects of social interaction other than play behavior likely contribute to social reward. The findings have important implications for understanding the influence of social context on cocaine reward during adolescence.

Keywords

Social Interaction; Conditioned Place Preference; Adolescence; Cocaine; Drug reward

1. Introduction

Adolescence is a developmental time period of enhanced social cue salience (Spear, 2000; Vanderschuren et al., 1997). Social play in adolescent rats is thought to serve an essential role in fostering healthy development (Einon et al., 1978; Meaney & Stewart, 1979; Van den Berg, Hol, et al., 1999; Vanderschuren et al., 1997). Numerous studies suggest that nature has imbued the opportunity to engage in social play with intrinsic natural reward. For example, rats will traverse a T-maze for access to another rat (Humphreys & Einon, 1981; Normansell &

*Corresponding author. Tel: +1 480 965 0209; fax: +1 480 965 8544., E-mail address: Janet.Neisewander@asu.edu (J.L. Neisewander).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Panksepp, 1990; Werner, 1976) and demonstrate robust approach towards another rat if given free access (Panksepp et al., 1984). Additionally, rats will spend more time in an environment previously paired with a playmate (Calcagnetti & Schecter, 1992; Crowder & Hutto, 1992; Douglas et al., 2004; Van den Berg, Pijlman, et al., 1999). This latter measure is referred to as social reward-conditioned place preference (CPP).

Adolescence is also a time of enhanced sensitivity to drugs of abuse (Spear, 2000). Enhanced sensitivity of adolescent rats to the rewarding effects of drugs of abuse has been demonstrated with the CPP model for alcohol (Philpot et al., 2003), nicotine (Belluzzi et al., 2004; Shram et al., 2006; Torrella et al., 2004; Vastola et al., 2002), and cocaine (Badanich et al., 2006; but see Campbell et al., 2000).

Several studies suggest that social context influences the affective valence of drugs of abuse and vice versa. For example, an ethanol place aversion is attenuated when rats are given the opportunity to engage in social interaction (Gauvin et al., 1994). Among adolescent rats, low doses of alcohol facilitate social interaction and social preference (Varlinskaya & Spear, 2002; Varlinskaya & Spear, 2006). In addition, Normansell & Panksepp (1990) demonstrate that morphine paired with a playmate at the end of a T-maze will result in greater resistance to extinction of the T-maze task when a play partner is no longer available. These findings suggest that drugs and social context interact and mutually contribute to each other's rewarding effects.

The primary goal of this study was to further develop social reward-CPP as a model for examining interactions between social and drug rewards. CPP is thought to be established via classical conditioning (for reviews see Bardo & Bevins, 2000; Bardo et al., 1995; Carr et al., 1989; Hoffman, 1989). In this model, environmental stimuli (i.e., conditioned stimuli, CS) paired with a drug or the opportunity for social interaction (i.e., unconditioned stimuli, US) become associated with the rewarding effects of the US. This is reflected as incentive motivation to approach, and maintain contact with, the environmental stimuli (Schneirla, 1959). The present study first examined whether sensitivity of social reward-CPP varies with parameters that influence classical conditioning, including the number and duration of conditioning sessions with and without pre-exposure to a playmate, as well as the effects of conditioning once versus twice/day. Subsequently, we verified that CS-US pairings are necessary for preference shifts as an explicitly unpaired control group receiving equal exposure to a playmate and the conditioning environments, but not paired together, failed to exhibit preference shift. These experiments were conducted using the biased CPP procedure, in which the US is paired with the initially non-preferred side of a two-compartment apparatus. This procedure provides greater sensitivity to detect changes in preference shifts relative to an unbiased procedure. To rule out the potential problem with the biased procedure that preference shifts may be due to reduction of initial aversion to the non-preferred compartment rather than social reward-CPP, we next verified that social reward-CPP is evident using an unbiased conditioning procedure, in which the compartment paired with social interaction was counterbalanced. Next, we selected sub-threshold parameters for establishing social reward- and cocaine-CPPs in order to assess whether these rewards interact to influence overall affective valence of the US-paired environment. Finally, we examined whether drug enhancement of social reward-CPP would occur with a non-rewarding psychoactive drug, such as dextromethorphan. Dextromethorphan is an over-the-counter antitussive medication that binds to sigma receptors in the brain (Craviso & Musacchio, 1980; Jaffe & Martin, 1990) and supports drug discrimination learning, but not CPP (Holtzman, 1994; Gavend et al., 1995; Huang et al., 2003).

A secondary aim of this study was to examine the relationship between specific play behaviors and the degree of preference shift amongst both rats in a play-pair. Previous research has shown

that the rat in a play-pair that scores the most pins, considered to be the quintessential measure of play and one of the easiest behaviors to identify (e.g., Panksepp, 1981), finds social play to be rewarding (Calcagnetti & Schecter, 1992). The present study examined the degree to which the rat that scores the least pins in a play-pair (i.e., is pinned the most) finds social play to be rewarding. We predicted that the rats that scored the least pins would exhibit a lower magnitude CPP relative to rats that scored the most pins based on previous findings of Douglas et al. (2004) demonstrating a positive correlation between play fighting and social reward-CPP.

2. Method

2.1. Animals

Male Sprague-Dawley rats (Charles River, San Diego, CA) arrived at Arizona State University on postnatal day (PND) 22 (i.e., 22 days old). All rats were individually housed in a climate-controlled facility with a 12-h light/dark cycle (lights on at 6 PM) with *ad libitum* access to food and water. Housing and care were conducted in accordance with the Guide for the Care and Use of Laboratory Rats (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996). The experiments were designed such that conditioning occurred during the rats' adolescent age period (i.e., PND 28–42; Spear, 2000), since social reward is most robust during this developmental stage (Douglas et al., 2004). Prior to conditioning, animals were acclimated to handling for 5 days (i.e., PND 23–27). On each of these days, each rat was removed from his cage and handled for at least 2 minutes. Rats remained isolated except when paired together during conditioning. Such conditions likely optimize CS salience and US reward (Panksepp & Beatty, 1980; Panksepp et al., 1984; Varlinskaya, Spear, & Spear, 1999).

2.2. Apparatus

All conditioning was conducted in rectangular Plexiglas chambers containing two equal-sized compartments divided by a removable partition. Each compartment measured $36 \times 24 \times 30$ cm high. Initial studies in our laboratory established that the compartments were equally preferred by adult rats (i.e., unbiased apparatus; O'Dell et al., 1996). One compartment had pine-scented bedding beneath a wire mesh floor and all but the front wall were white. The other compartment had cedar-scented bedding beneath a bar grid floor and all but the front wall were black. The front wall of both compartments was transparent to allow direct observation of the rats' behavior. The conditioning room had an overhead fluorescent light. In addition, there was a small fluorescent light suspended 32 cm above each of the black compartments, such that light intensity measured from the floor of the black and white sides were equal. On the CPP test days the solid partition was replaced by a partition that contained an opening in the center (8×8 cm high), allowing the rats free-access to both compartments. A third plastic chamber placed in a room separate from the CPP room was used as an alternate environment. It measured $34 \times 22 \times 26$ cm, and contained recycled paper bedding placed on top of a clear plastic bottom.

2.3. Drugs

Dextromethorphan hydrobromide (Sigma, St. Louis, MO) and cocaine hydrochloride (RTI International, Research Triangle Park, NC) were dissolved in saline and injected IP.

2.4. Specific Experiments

2.4.1. Experiment 1: Number of sessions/day—On PND 28, rats were transported to the CPP room, placed into the CPP apparatus, and allowed to explore for 10 min in order to acclimate them to these procedures; preference was not measured. On PNDs 29 and 30, baseline preference was assessed by allowing the rats free-access to the entire CPP apparatus for 10 min. Starting compartment was counterbalanced and entry into a compartment was

operationally defined as the rats' two forepaws in contact with the floor of that compartment and continued to be recorded as such until the rats' two forepaws contacted the floor of the other compartment. Total time that rats spent in each compartment was averaged across the two baseline days and the side in which the rat spent the least amount of time was designated as the initially non-preferred side. The rats were then divided into pairs matched for initial compartment preference and body weight (within 10 g). Rats that failed to demonstrate at least five compartment crossovers during either baseline test day were excluded due to inadequate expression of choice behavior, but were still used as playmates for rats that remained in the study.

In order to determine if magnitude of social reward-CPP would be affected by the number of conditioning sessions given per day, rat pairs were divided into 2 groups that were equated for overall total number of conditioning sessions (i.e., 8 sessions), but differed in terms of number of conditioning sessions/day. For both groups, half the conditioning sessions consisted of the rats being placed into their initially non-preferred side with their assigned playmate, and the other half of the sessions consisted of the rats being placed alone into their initially preferred side. The Once/day group (n=8) underwent 8 consecutive days of conditioning (i.e., PNDs 31–38) in which the rats were placed in one side of the CPP apparatus on one day, and were placed in the opposite side of the apparatus on the next day. The Twice/day group (n=8) underwent 4 consecutive days of conditioning (i.e., PNDs 35–38) in which each day the rats were placed in one side of the CPP apparatus during the morning session, and were placed in the opposite side of the apparatus during the afternoon session. These sessions were separated by 6 h. For both groups, starting side on the first conditioning session (i.e., initially preferred or non-preferred side) was counterbalanced. In summary, there were a total of 8 conditioning sessions for both groups consisting of 4 social pairing sessions alternating with 4 sessions spent alone. A particular rat always began a conditioning session at the same time each day. CPP was assessed on PND 39 during a 10-min test conducted as described for baseline tests. The general procedure is summarized in Table 1 and was subsequently used for Experiments 2–4, and 6.

2.4.2. Experiment 2: Number and duration of sessions—Results from Experiment 1 indicated that there was no significant difference in social reward-CPP using 1 session/day versus 2 sessions/day, so the 2 session/day procedure was utilized for efficiency, with the exceptions of Experiments 5 and 7. Rats were tested for baseline preferences in the CPP apparatus and assigned to pairs as described in Experiment 1. The pairs were then assigned to 1 of 6 groups (n=10 or 11), counterbalanced for magnitude of preference for their initially non-preferred side. The groups either received 1, 4, or 8 social pairings in the initially non-preferred side of the apparatus for either 10 or 30 min, alternating with placement alone in the initially preferred side as described previously (i.e., total of 2, 8, or 16 total conditioning sessions, respectively). To control for overall social exposure, all groups received a total of 8 opportunities to interact with their partner for 10 or 30 min, depending on group assignment. For the 1 and 4 Pairings groups, the first 7 and 4 social pairings, respectively, took place in the alternate environment once the 8 social pairings group began conditioning (see Table 1). Panksepp (1981) suggests that stable play-pinning relationships emerge after as little as 4 play sessions. In addition, Taylor (1977) suggests that these relationships can endure across a variety of play-contexts. Therefore, holding total number of social interactions constant allowed for social play relationships to develop equally across groups and also controlled for isolation stress across groups. The alternate environment sessions took place at a time of day between the morning and afternoon conditioning sessions (typically starting at 11:00 a.m.). At the end of these sessions, the rats were returned to their individual home cages. Rats were tested for CPP on PND 39.

Play behavior in the CPP apparatus was videotaped for 10 min on the last day of conditioning (i.e., PND 38). Nape attacks and pins were later quantified from the tapes by an observer blind

to group assignment. A nape attack was operationally defined as a rat lunging forward and directing the tip of its snout toward the nape of his playmate, a behavior associated with play initiation (Pellis & Pellis, 1987). The number of pins each rat scored was used to assess play fighting. A pin was operationally defined as standing above the pinned rat with the latter lying on his dorsal surface with his ventral surface exposed.

2.4.3. Experiment 3: Conditioning without social access pre-exposure—A single playmate pairing failed to produce social reward-CPP in the previous experiment. This experiment examined whether US pre-exposure may have retarded acquisition of single pairing CPP. All rats were tested for baseline preferences in the CPP apparatus and assigned to pairs as described in Experiment 1. Rat pairs were divided into 2 groups (n=11), counterbalanced for magnitude of preference for their initially non-preferred side, that underwent either 1 or 4 social pairings in the CPP apparatus using an identical procedure as in Experiment 2 except that exposure to the alternate environment was omitted to eliminate playmate (i.e., US) pre-exposure. The rats were handled on days they were not conditioned, but remained isolated. Rats were tested for CPP on PND 39.

2.4.4. Experiment 4: Influence of non-associative factors on preference shift—Rat pairs were assigned to either a social reward-conditioned group (i.e., Conditioned, n=9) or an explicitly unpaired control group (i.e., Unpaired Control, n=10) counterbalanced for magnitude of preference for their initially non-preferred side. The Conditioned group underwent the identical procedure as the 8 pairings for 10 min group described in Experiment 2. The Unpaired Control group differed from rats in the Conditioned group in that they were placed alone in both their initially non-preferred and preferred sides during conditioning. All rats were given a third 10-min session in the alternate environment 1 h after the last session in the CPP apparatus. The Conditioned group was placed alone in the alternate environment, whereas the Unpaired Control group was placed with their playmate. Thus, exposure to the CPP apparatus, the alternate environment, and social interaction was equal in both groups, with the only difference being where the social interaction took place. Rats were tested for CPP on PND 39.

2.4.5. Experiment 5: Social reward-CPP using a counterbalanced procedure—The present experiment was conducted due to discovering a slight bias of the rats to spend more time in the white side during baseline tests (i.e., across the previous experiments, 65% demonstrated an initial preference for the white side). To ensure that the preference shifts observed using the biased design were not the result of aversion reduction to the initially non-preferred side, this experiment utilized a counterbalanced design. There was no baseline testing for initial preferences. Instead, rats were randomly assigned to receive a playmate paired with either the white side of the apparatus (n=8) or the black side of the apparatus (n=8). Rats were handled from PND 23–30, then received 2 conditioning sessions/day for a total of 8 days (PND 31–38) as described previously, and subsequently were tested for CPP on PND 39.

2.4.6. Experiment 6: Interaction between cocaine and social rewards—Rats were handled from PND 23–28, habituated to transportation to the CPP apparatus on PND 29, and then tested for initial preferences on PNDs 30–31. Conditioning took place over 2 days (i.e., PND 33–34). The rats were divided into 4 groups that received the following upon placement into their initially non-preferred compartment of the CPP apparatus: Control received saline and were placed alone (n=9); Social Only received saline paired with a playmate (n=9); Cocaine Only received cocaine and were placed alone (n=9); Cocaine/Social received cocaine paired with a playmate (n=9). All groups were given saline and placed alone in their initially preferred compartment. Across each conditioning day, groups were given 2 conditioning sessions in the CPP apparatus as well as 1 control session in the alternate environment. Order of starting side

in the CPP apparatus was counterbalanced such that half the rats began their first session in their initially non-preferred side, and half began in their initially preferred side. These 2 conditioning sessions in the CPP apparatus were at least 6 h apart to allow time for cocaine clearance, and the alternative environment session took place at least 1 h after the last conditioning session of the day. For each 10 min session, rats received an injection of either saline or cocaine (2 mg/kg, IP) and were then immediately placed into their assigned compartment either alone or with a playmate that received an identical injection. As illustrated in Table 1, all groups were equated for total drug and social play exposure across each day, but the location of these exposures varied. The parameters (i.e., two social pairings across two days and a low 2 mg/kg, IP dose of cocaine) were selected such that the Cocaine Only and Social Only groups would demonstrate sub-threshold CPP in order to allow sensitivity for detecting a synergistic interaction between cocaine and social interaction in the Cocaine/Social group while avoiding potential ceiling effects. Play behavior was videotaped during the last conditioning session for the Social Only and the Cocaine/Social groups and was later scored for nape attacks and pins.

2.4.7. Experiment 7: Interaction between dextromethorphan and social rewards

—Rats underwent the same procedures described in Experiment 6 with the exception that conditioning sessions were conducted over 6 consecutive days with only 1 session/day (see Table 1). The latter modification was necessary to achieve drug clearance between sessions given the relatively long half-life of dextromethorphan. As in Experiment 6, the rats received a total of 2 exposures to each of the 3 compartments (i.e., initially preferred/non-preferred sides of the CPP apparatus and alternate environment), but rats were placed in only 1 of the compartments each day (rather than being exposed to all 3 compartments in one day as in Experiment 6) for 10 min across PNDs 32–37, with initial starting compartment counterbalanced. The same design as that of Experiment 6 was used resulting in 4 groups that received the following paired with their initially non-preferred compartment: Control received saline and were placed alone (n=8); Social Only received saline paired with a playmate (n=10); DXM Only received dextromethorphan (30 mg/kg, IP) and were placed alone (n=9); DXM/Social received dextromethorphan paired with a playmate (n=10). All groups were given saline and placed alone in their initially preferred compartment. The dose of dextromethorphan used was selected based on previous studies which demonstrate that rats can discriminate 30 mg/kg of dextromethorphan from vehicle, suggesting that it produces distinct interoceptive stimulus effects (Holtzman, 1994; Gavend et al., 1995). Play behavior in the DXM/Social and Social Only groups was measured as described above. Additionally, the effect of dextromethorphan on locomotor activity was assessed for the DXM Only group.

2.5. Data analysis

For experiments using the biased design (i.e., Experiments 1–4 & 6–7), social reward-CPP was operationally defined as a significant increase in time spent in the initially non-preferred side (i.e., playmate-paired side) post-conditioning relative to pre-conditioning baseline and was analyzed using a mixed factor ANOVA with Day (baseline vs. test day) as the repeated measures factor and group as the between subjects factor. Although this operational definition of CPP is the most powerful measure since it involves a within subjects analysis, in some cases such as correlations requiring a single unit of measure, a difference score of time spent in the US-paired side post-conditioning minus pre-conditioning was used. Also, for Experiment 5 (i.e., counterbalanced design) in which there was no pre-conditioning baseline, the amount of time spent in the playmate-paired side was compared to the alternate side using a nonparametric Wilcoxin signed-ranks test. A nonparametric statistic was necessary for this analysis because time spent on one side of the apparatus on test day is not entirely independent of time spent in the other side. In addition, crossovers between compartments in the CPP apparatus on the test

day were also analyzed with ANOVAs or t-test depending on the design. *Post-hoc* Tukey's HSD tests were conducted to further analyze significant effects.

For the play behaviors, pins and nape attacks, inter-rater agreement was 94% on average. For Experiment 2, the rats were categorized depending on whether they scored the most or least number of pins during the observation period. For example, if Rat 1 and Rat 2 were a play-pair, and Rat 1 scored more pins than Rat 2, then Rat 1 was included in the Most Pins condition, and Rat 2 was included in the Least Pins condition. Separate analyses were performed for each condition because measures of play behavior in rat pairs are not completely independent (i.e., behavior of one rat influences behavior of the other rat). Correlation analyses were performed using Pearson's correlation to determine whether pinning was related to preference shift in either of these conditions. Some of the rat pairs failed to exhibit any pinning and were designated as the No Pins condition. Videos were scored a second time for nape attacks. Rats were separated into Most Attacks and Least Attacks groups as described above for pinning. Importantly, rats designated to the Most Pins group from above were not necessarily designated to the Most Attacks group. In Experiments 6 and 7, pins and nape attacks were also scored, and comparisons between total pins and nape attacks/rat in groups receiving drug versus saline were conducted using t-tests.

Locomotor activity in Experiment 7 was measured by photo beam interruptions in the CPP apparatus during the last 10-min conditioning sessions with dextromethorphan and saline in the DXM Only group and was analyzed using a paired samples t-test.

3. Results

3.1. Conditioned place preference

In Experiment 1, the ANOVA of time in playmate-paired side revealed an effect of Day ($F(1, 14)=30.09, p < .001$), but no Day $\times$ Sessions/day interaction. The mean time spent in the playmate-paired side ($s \pm SEM$) increased following conditioning, regardless of whether the rats received 1 or 2 conditioning sessions per day (Figure 1). Furthermore, there was no group difference among rats conditioned once versus twice per day on either day.

In Experiment 2, the ANOVA of time spent in the playmate-paired side failed to reveal a significant Day $\times$ Pairings $\times$ Duration interaction. Surprisingly, there was no main effect of duration of conditioning sessions for either time spent in the playmate-paired side or difference scores of time spent on the playmate-paired side minus time spent on the preferred side (Figure 2A). However, there was a Day $\times$ Pairings interaction ($F(2, 57)=6.63, p<.001$). Analysis of the simple main effects collapsed across duration of conditioning sessions revealed that time spent in the playmate-paired side of the chamber increased following conditioning for the rats given 4 and 8 social pairings, but not for rats given only 1 social pairing ($p<.05$, Tukey's HSD, Figure 2B). Additionally, rats given 8 social pairings spent more time in their playmate-paired side on test day than rats given only 1 social pairing ($p<.05$, Tukey's HSD). Finally, a trend analysis of preference shift (time spent in the playmate-paired side post-conditioning minus preconditioning) as a function of the number of social pairings revealed a significant linear trend ($p<.001$), indicating greater preference shift with greater numbers of social pairings.

In Experiment 3, the ANOVA of time spent in the playmate-paired side revealed a Day $\times$ Group interaction ($F(1,20)=14.12, p<.001$). Rats in the 4 Pairings group, but not the 1 Pairing group, demonstrated CPP. In addition, the 4 Pairings group spent a greater amount of time in the playmate-paired side on test day relative to the 1 Pairing group (Tukey's HSD, $p<.05$; Figure 3).

In Experiment 4, the ANOVA of time spent in the playmate-paired side revealed a Day $\times$ Group interaction ($F(1,17)=9.77, p<.01$). Rats in the Conditioned group, but not in the Unpaired Control group, demonstrated CPP. Furthermore, the Conditioned group spent a significantly greater amount of time in the playmate-paired side on test day relative to the Unpaired Control group (Tukey's HSD, $p<.05$; Figure 4).

As mentioned earlier, we found that overall there was a slight bias for rats to initially prefer the white side of the apparatus that was likely due to the adolescent rats slipping on the bar floor of the black side more often than observed previously in adult rats who show no side bias. To determine whether the white bias affected degree of preference shift, we first conducted an analysis across Experiments 1–4 to determine if there was a difference in CPP depending on whether rats initially preferred the white side or the black side. Only groups that demonstrated CPP were included in this analysis ($n=26$ for rats with an initial black preference, $n=54$ for rats with an initial white preference, 32.5 versus 67.5%, respectively). This analysis revealed only a main effect of Day ($F(1,78)=123.8, p<.001$) with no effects of Experiment nor Day $\times$ Black versus White US-paired Side interaction. The mean time spent ($s \pm SEM$) in the initially non-preferred (US-paired) side for animals with an initial white versus black preference, respectively, was 227.4 ± 5.7 and 259.7 ± 7.2 during baseline and 330.0 ± 6.7 and 340.0 ± 11.4 on the test day.

In Experiment 5 using the counterbalanced design, rats paired with their playmate on either side of the apparatus (i.e., black or white side) spent more time in that side relative to the alternate side during the CPP test (Wilcoxin signed-ranks test, $p<.05$; Figure 5).

In Experiment 6, the ANOVA of the time spent in the playmate- and/or cocaine-paired side revealed a Day $\times$ Group interaction ($F(3,32)=4.18, p<.05$). Analysis of simple main effects revealed that only the Cocaine/Social group demonstrated CPP on test day ($p<.05$, Tukey's HSD, Figure 6). Additionally, rats in the Cocaine/Social group spent more time in their playmate-paired side on test day relative to all other groups ($p<.05$, Tukey's HSD) and there were no differences among the other three groups.

In Experiment 7, the ANOVA of the time spent in the playmate- and/or dextromethorphan-paired side revealed a main effect of day ($F(1,33)=7.87, p<.01$) due to small increases in time spent on the US-paired side on the test day regardless of group. There were no main effects or interactions involving social condition or drug, suggesting that in contrast to cocaine, dextromethorphan does not interact with social reward (Figure 7).

In each of the above experiments, one-way ANOVAs of crossovers on test day revealed no differences between groups as summarized in Table 2.

3.2. Play behavior

In Experiment 2, there was no correlation between pinning and preference shift in the Most Pins group ($r=0.13, p=.54$), although there was a strong trend towards a negative correlation between pinning and preference shift in the Least Pins group ($r=-0.38, p=.06$). Additional one-factor repeated measures ANOVAs revealed increases in time spent in the playmate-paired side on test day relative to baseline for both the Most Pins ($F(1,25)=29.02, p<.001$) and the Least Pins ($F(1,25)=24.51, p<.001$) conditions. Even when a correlation between number of pins and preference shift was conducted for all rats regardless of Most or Least Pins group, no significant relationship emerged ($r=-0.11, p=.37$). Additional analyses found no correlations between number of pins and preference shift for rats within a given group, suggesting the lack of correlation found with all animals is evident regardless of number of social interactions experienced in the CPP apparatus. Mean difference scores and pins are presented in Table 3. Interestingly, the No Pins condition demonstrated the greatest difference score.

Nape attacks also failed to correlate with preference shift in either the Most Attacks ($r = -0.09$, $p = .63$) or the Least Attacks ($r = 0.09$, $p = .65$) conditions with both spending more time in the playmate-paired side on test day relative to baseline ($F(1,33) = 38.47$ and $F(1,28) = 29.64$, respectively, $p < .001$). Again, no significant relationship emerged when all rats were included in the analysis ($r = 0.006$, $p = .96$) nor when individual groups were analyzed. The mean difference scores and nape attacks are presented in Table 4.

In Experiment 6, rats given saline (i.e., Social Only) exhibited more nape attacks ($t = 2.11$, $p < .05$) and a trend toward more pins ($t = 1.96$, $p = .08$) than rats given cocaine (i.e., Cocaine/Social, Figure 8).

In Experiment 7, rats given dextromethorphan (i.e., DXM/Social) exhibited less nape attacks ($t = 4.90$, $p < .001$) and pins ($t = 4.20$, $p < .001$) than rats given saline (i.e., Social Only; Figure 9). Dextromethorphan also reduced locomotor activity (as measured by total photo beam breaks in the CPP apparatus) in the DXM Only group when they were given dextromethorphan compared to when they were given saline (means $\pm$ SEM of 6.7 ± 1.6 and 17.9 ± 1.3 , respectively, $t = 7.53$, $p < .001$).

4. Discussion

The most important and novel finding in the present study is evidence that cocaine and social rewards interact to produce CPP. Specifically, CPP was established by pairing both a playmate and cocaine with an environment, but not by pairing these same stimuli individually with the environment. We chose to use sub-threshold parameters for establishing CPP with the individual stimuli in order to detect an interaction (i.e., 2 mg/kg cocaine and 2 playmate exposures). We reasoned that CPP established with a combination of stimuli that are not rewarding enough alone to produce CPP would suggest these stimuli interact synergistically in producing reward rather than via a simple additive effect. Additionally, the results of Experiments 6 and 7 suggest that it is the rewarding effects of cocaine, rather than nonspecific psychoactive stimulus effects, that mediate the enhancement of social reward-CPP. Specifically, we demonstrated that dextromethorphan failed to interact with social reward to produce a CPP despite its ability to produce discriminative stimulus effects (Holtzman, 1994; Gavend et al., 1995) and the present findings that it attenuated play behaviors and locomotor activity.

Further research is needed to draw firm conclusions regarding the precise nature of the interaction between drug and social rewards; however in any case, these findings have important implications for understanding drug abuse given that many adolescents first begin to experiment with drugs while in a social setting. The findings contribute to an existing literature that suggests social context plays an important role in the subjective effects of drugs. For instance, in humans alcohol consumption in the presence of peers is more pleasurable than drinking alone (Adesso, 1985; Pliner & Cappell, 1974; Smith et al., 1975). In animals, social interaction can attenuate an alcohol aversion (Gauvin et al., 1994), and can influence the general responsiveness and sensitivity to alcohol (Varlinskaya et al., 2001). Furthermore, pairing an ethanol sipper with social interaction enhances ethanol intake in rats (Tomie et al., 2004). Collectively, these findings highlight a growing interest in the influence of social context on drug addiction and support the use of CPP for this line of investigation.

In this regard, the present study extends previous findings (Calcagnetti & Schechter, 1992; Douglas et al., 2004; Van den Berg, Pijlman, et al., 1999) by providing additional information regarding choice of conditioning parameters, as well as support for the interpretation that social reward-CPP is established via Pavlovian conditioning. Preference shift increased as the number of social pairings in the CPP apparatus increased, as expected from increasing the number of

CS-US pairings in Pavlovian conditioning. Furthermore, the results rule out the possibility of non-associative factors accounting for the preference shifts since the Unpaired Control rats failed to exhibit CPP despite the same repeated exposure to the CS and US as the Conditioned rats, but in an explicitly unpaired manner. The results also suggest that there is no advantage in using 1 conditioning session/day since this produced social reward-CPP that was similar to that obtained using 2 sessions/day. This outcome is important because more conditioning sessions can be conducted during the relatively short time period of adolescence in rats. Interestingly, the duration of conditioning sessions also had no effect on preference shift, suggesting that longer sessions also present no advantage for producing social reward-CPP.

Previous findings with drug-CPP have found that the optimal length of conditioning sessions varies depending on the duration of US effects of the drug (e.g., Bardo et al., 1995; O'Dell et al., 1996). One explanation for the lack of benefit from using longer 30-min sessions is that rats may habituate to the rewarding effects of social interaction within 10 min. For instance, Douglas et al. (2004) report that playful behavior declines after the first 10 min of interaction. Although we did not quantify play behavior beyond 10 min in the present study, we did observe that play behavior almost completely ceased following the first 15 min in the conditioning chamber.

Unlike some drug-CPPs that can be demonstrated using a single drug-environment pairing (Bardo & Neisewander, 1986; Bardo et al., 1986; Neisewander et al., 1990; Zavala et al., 2003), the present findings suggest that establishment of social reward-CPP in adolescent rats likely requires additional pairings since a single pairing failed to produce social reward-CPP regardless of whether rats were pre-exposed to their playmate. We had predicted that the 7 playmate pairings in the alternate environment prior to the single pairing in the CPP apparatus in Experiment 2 may have inhibited acquisition of social reward-CPP through a US pre-exposure effect. US pre-exposure is thought to reduce the salience of the US in subsequent CS-US pairing(s), and the inhibitory effects of US pre-exposure on Pavlovian conditioning have been well documented (for review see Mis & Moore, 1973; Randich & LoLordo, 1979; Taylor 1956). Although the results from Experiment 3 failed to support a US pre-exposure effect, it is interesting to note that the animals receiving 4 pairings during conditioning without prior playmate exposure (Experiment 3) expressed a relatively more robust CPP than those conditioned previously after 4 playmate pre-exposures (Experiment 2). Thus, it is possible that US pre-exposure does produce an inhibitory effect on social reward-CPP.

There are a few methodological issues regarding the CPP procedure used in the present study that need to be addressed. First, relative novelty affects preference behavior in favor of spending time in the more novel side (Bardo & Bevins, 2000; Bardo et al., 1993; Bardo et al., 1996; Bevins et al., 2002; Carr et al., 1989). Thus, it is possible that social interaction in the playmate-paired compartment may have interfered with the processes that render that context familiar. In addition, exposure to this compartment on test day without the presence of a playmate changes the overall stimulus conditions and may also render the previously playmate-paired side novel relative to the side experienced in isolation. Several arguments mitigate the possibility that relative novelty influenced CPP. First, one would expect more exploratory behavior with increasing relative novelty (Bardo et al., 1993), yet there were no group differences in crossovers on the test day. Second, previous research demonstrates that rats still process environmental features while interacting with novel objects that are present during conditioning (Bevins et al., 2002). Third, rats in the present study were given three days of access to the entire apparatus in the absence of a playmate prior to conditioning, and therefore, exposure to the initially non-preferred compartment on test day without a playmate was not an entirely novel experience.

The second methodological issue is the use of a biased procedure in which playmates were only paired with the initially non-preferred side of the apparatus; therefore, preference shifts may reflect reduction of an aversion to the initially non-preferred side of the apparatus rather than social reward-CPP. We did not expect this to be an issue since we have reliably found no initial preference bias in adult rats in our apparatus. We were surprised to find that with adolescent rats, more initially preferred the white side (65%) relative to the black side. Nevertheless, there are two compelling arguments that favor the interpretation that preference shifts in this study were due to the rewarding effects of the US(s) rather than reduction of initial aversion. First, the vast majority of conditioned animals spent greater than 50 percent of the test period in their initially non-preferred side, suggesting a true preference shift rather than a reduction of an aversion. Second, CPP was evident in Experiment 5 using a counterbalanced conditioning procedure which controls for the influence of potential bias.

The important secondary aim of this study was to determine whether the rough-and-tumble play behaviors, pinning and nape attacks, correlate with the degree of reward derived from the social experience. We chose to focus on pins and nape attacks because of the relative ease in operationally defining and detecting these behaviors during a play bout. In addition, nape attacks are thought to reflect initiation of play and pinning correlates highly with a number of other more ambiguous play behaviors (Panksepp & Beatty, 1980), suggesting that this measure may offer an efficient indicator of play overall. In Experiment 2, neither pinning nor nape attacks correlated with preference shift, defined as time in the playmate-paired side postconditioning minus pre-conditioning, for either those scoring the most or least of either behavior in each play pair. Interestingly, rats exhibiting no pins on the last day of conditioning demonstrated the greatest preference shifts. The play-reducing effects of cocaine in Experiment 6 are in line with previous reports that psychomotor stimulants cause reductions in play behaviors (Beatty et al., 1982; Beatty et al., 1984; Ferguson et al., 2000; Humphreys & Einon, 1981; Sutton & Raskin, 1986). Although cocaine inhibited play relative to saline, it interacted with the social context to produce greater CPP than either cocaine or social pairings given alone. Inhibition of play behavior *per se* is not the cause of cocaine-enhanced social reward-CPP since dextromethorphan also inhibited play but failed to alter place preference. Taken together, these findings suggest that social play is rewarding for both rats in a pair. Furthermore, the degree to which a social encounter is rewarding is not solely dependent upon the amount of rough-and-tumble play behavior that takes place during the encounter; other aspects of the social interaction likely contribute to its rewarding effects.

The present findings are in agreement with Panksepp et al. (1984) suggesting that both rats in a play-pair should find the interaction rewarding, but are discrepant with other research suggesting that play is actually necessary for social interaction to be rewarding. For instance, previous research suggests that rats only find exposure to another rat rewarding if they are able to play, as determined by preventing play pharmacologically (e.g., using scopolamine or haloperidol) in one of the rats of a pair (Calcagnetti & Schechter, 1992; Humphreys & Einon, 1981; Pellis & McKenna, 1995). In contrast, the results from the present study demonstrated that rats that did not engage in pinning behavior under more naturalistic conditions (i.e., No Pins condition in Experiment 2) still found the opportunity for social interaction to be rewarding. Thus, the absence of play fighting in the non-drugged state does not preclude social reward. Also, inhibiting play behavior pharmacologically with cocaine not only failed to prevent social reward, but actually facilitated social reward. It is unlikely that the rewarding effects of cocaine simply overtook social interaction given that the dose of cocaine used was not rewarding by itself. Instead, cocaine interacted with social context to produce a rewarding state that was greater than either the cocaine or social interaction alone.

The fact that cocaine interacted with the social context to produce CPP despite decreasing play, as well as the lack of a relationship between CPP and play behaviors in drug naive rats,

challenges the notion that the degree of social reward is related to the amount of rough-and-tumble play behavior that takes place during conditioning as suggested previously (Douglas et al., 2004). The reason for this discrepancy is unclear; however, there are a number of methodological differences to consider. One difference is that the present study used only male rats. Douglas et al. (2004) reported that although both male and female adolescents demonstrate social reward-CPP, the effect is most pronounced among the isolation-housed males. In addition, a number of studies have shown that males generally engage in more play and other dominance-related behaviors than females (Douglas et al., 2004; Meaney & Stewart, 1981; Pellis et al., 1997; Pellis & Pellis, 1990; Thor & Holloway, 1983, 1986). Given that Douglas et al. included both sexes in their correlation analyses, whereas the present study did not include females, it is possible that females may have contributed to the positive correlations observed previously. Another difference between studies is that Douglas et al. utilized a different measure of CPP for which preference coefficients were calculated that excluded the time that rats spent in between the compartments. Although it not clear how these approaches compare, it is clear that our procedure provides an adequate measure of social reward-CPP that is sensitive enough to detect differences in preference shift depending on number of CS-US pairings. Perhaps the most important difference between studies is that Douglas et al. (2004) summed the number of pins, nape attacks, and chases together in order to form a single measure called “play fighting.” It is possible that individual components of play are not strong enough to detect a relationship between play and preference shift. However, even when we analyzed the sum of pins and nape attacks together, play failed to correlate with preference shift. It is surprising that no relationship between play behaviors and preference shift was found in the present study even when considering the experimental differences discussed above. If any of these experimental differences are the reason for the discrepant findings, then perhaps a relationship between amount of play and overall social reward can only be observed under specific conditions. Indeed, the present results clearly demonstrate that this relationship does not hold while animals are under the influence of cocaine.

In conclusion, the present findings are consistent with the idea that social reward-CPP is established via Pavlovian association between the environment and rewarding effects of social interaction, similar to that of drug-CPPs. Furthermore, the additional parametric data obtained in this study will aid in designing future social reward-CPP experiments. The present findings also challenge the traditional view that the degree of reward derived from a social interaction is related to the amount of rough-and-tumble play behavior that takes place during the interaction, and further suggest that other aspects of the social encounter likely contribute to social reward. The present study also demonstrates the importance of examining drug reward in a social context. The fact that a sub-threshold dose of cocaine administered to an adolescent in a social situation produces a robust CPP has important implications for understanding drug initiation. Further elucidating the nature of this interaction may aid in developing more relevant models of adolescent drug abuse that can be used to examine neural changes that underlie social influences on drug reward.

References

- Adesso, VJ. Cognitive factors in alcohol and drug use. In: Galizio, M.; Mattio, SA., editors. *Determinants of Substance Abuse: Biological, Psychological, and Environmental Factors*. Plenum Press; New York: 1985. p. 179-208.
- Badanich KA, Adler KJ, Kirstein CL. Adolescents differ from adults in cocaine conditioned place preference and cocaine-induced dopamine in the nucleus accumbens septi. *Eur J Pharmacol* 2006;550 (1-3):95-106. [PubMed: 17011546]
- Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl)* 2000;153(1):31-43. [PubMed: 11255927]

- Bardo MT, Bowling SL, Robinet PM, Rowlett JK, Lacy M, Mattingly BA. Role of D₁ and D₂ receptors in novelty-maintained place preference. *Exper Clin Psychopharm* 1993;1:101–109.
- Bardo MT, Donohew RL, Harrington NG. Psychobiology of novelty seeking and drug seeking behavior. *Behav Brain Res* 1996;77(1–2):23–43. [PubMed: 8762157]
- Bardo MT, Neisewander JL. Single-trial conditioned place preference using intravenous morphine. *Pharmacol Biochem Behav* 1986;25(5):1101–1105. [PubMed: 3786364]
- Bardo MT, Neisewander JL, Miller JS. Repeated testing attenuates conditioned place preference with cocaine. *Psychopharmacology (Berl)* 1986;89(2):239–243. [PubMed: 3088643]
- Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. *Neurosci Biobehav Rev* 1995;19(1):39–51. [PubMed: 7770196]
- Beatty WW, Costello KB, Berry SL. Suppression of play fighting by amphetamine: effects of catecholamine antagonists, agonists and synthesis inhibitors. *Pharmacol Biochem Behav* 1984;20(5):747–755. [PubMed: 6539920]
- Beatty WW, Dodge AM, Dodge LJ, White K, Panksepp J. Psychomotor stimulants, social deprivation and play in juvenile rats. *Pharmacol Biochem Behav* 1982;16(3):417–422. [PubMed: 6123118]
- Belluzzi JD, Lee AG, Oliff HS, Leslie FM. Age-dependent effects of nicotine on locomotor activity and conditioned place preference in rats. *Psychopharmacology (Berl)* 2004;174(3):389–395. [PubMed: 14740150]
- Bevins RA, Besheer J, Palmatier MI, Jensen HC, Pickett KS, Eures S. Novel-object place conditioning: behavioral and dopaminergic processes in expression of novelty reward. *Behav Brain Res* 2002;129(1–2):41–50. [PubMed: 11809493]
- Campbell JO, Wood RD, Spear LP. Cocaine and morphine-induced place conditioning in adolescent and adult rats. *Physiol Behav* 2000;68(4):487–493. [PubMed: 10713288]
- Calcagnetti DJ, Schechter MD. Place conditioning reveals the rewarding aspect of social interaction in juvenile rats. *Physiol Behav* 1992;51(4):667–672. [PubMed: 1594664]
- Carr, GD.; Fibiger, HC.; Phillips, AG. Conditioned place preference as a measure of drug reward. In: Lieberman, JM.; Cooper, SJ., editors. *The Neuropharmacological Basis of Reward*. Oxford University Press; New York: 1989. p. 264–319.
- Craviso GL, Musacchio JM. High affinity binding of the antitussive dextromethorphan to guinea-pig brain. *Eur J Pharmacol* 1980;65:451–453. [PubMed: 7408950]
- Crowder WF, Hutto CW Jr. Operant place conditioning measures examined using two nondrug reinforcers. *Pharmacol Biochem Behav* 1992;41(4):817–824. [PubMed: 1594650]
- Douglas LA, Varlinskaya EI, Spear LP. Rewarding properties of social interactions in adolescent and adult male and female rats: impact of social versus isolate housing of subjects and partners. *Dev Psychobiol* 2004;45(3):153–162. [PubMed: 15505797]
- Einon DF, Morgan MJ, Kibbler CC. Brief periods of socialization and later behavior in the rat. *Dev Psychobiol* 1978;11(3):213–225. [PubMed: 658602]
- Ferguson SA, Frisby NB, Ali SF. Acute effects of cocaine on play behaviour of rats. *Behav Pharmacol* 2000;11:175–179. [PubMed: 10877123]
- Gauvin DV, Briscoe RJ, Goulden KL, Holloway FA. Aversive attributes of ethanol can be attenuated by dyadic social interaction in the rat. *Alcohol* 1994;11(3):247–251. [PubMed: 8060526]
- Gavend M, Mallaret M, Dematteis M, Baragatti G. Discriminative stimulus properties of dextromethorphan in rats. *Biomed Pharmacother* 1995;49(10):456–464. [PubMed: 8746072]
- Hoffman DC. The use of place conditioning in studying the neuropharmacology of drug reinforcement. *Brain Res Bull* 1989;23(4–5):373–387. [PubMed: 2556210]
- Holtzman SG. Discriminative stimulus effects of dextromethorphan in the rat. *Psychopharmacology (Berl)* 1994;116:249–254. [PubMed: 7892413]
- Huang EY, Liu TC, Tao PL. Co-administration of dextromethorphan with morphine attenuates morphine rewarding effect and related dopamine releases at the nucleus accumbens. *Naunyn Schmiedeberg Arch Pharmacol* 2003;368(5):386–392. [PubMed: 14564449]
- Humphreys AP, Einon DF. Play as a reinforcer for maze learning in juvenile rats. *Anim Behav* 1981;29:259–270.

- Jaffe, GH.; Martin, WR. Opioid analgesics and antagonists. In: Gilman, AG.; Rall, TW.; Nies, AS.; Taylor, P., editors. *The Pharmacological Basis of Therapeutics*. Pergamon Press; New York: 1990. p. 485-521.
- Meaney MJ, Stewart J. Environmental factors influencing the affiliative behavior of male and female rats (*Rattus norvegicus*). *Anim Learn Behav* 1979;7:397-405.
- Meaney MJ, Stewart J. A descriptive study of social development in the rat (*Rattus norvegicus*). *Anim Behav* 1981;29:34-45.
- Mis RW, Moore JW. Effects of preacquisition UCS exposure on classical conditioning of the rabbit nictitating membrane response. *Learn Motiv* 1973;4:108-114.
- Neisewander JL, Pierce RC, Bardo MT. Naloxone enhances the expression of morphine-induced conditioned place preference. *Psychopharmacology (Berl)* 1990;100(2):201-205. [PubMed: 2305008]
- Normansell L, Panksepp J. Effects of morphine and naloxone on play-rewarded spatial discrimination in juvenile rats. *Dev Psychobiol* 1990;23(1):75-83. [PubMed: 2160387]
- O'Dell LE, Khroyan TV, Neisewander JL. Dose-dependent characterization of the rewarding and stimulant properties of cocaine following intraperitoneal and intravenous administration in rats. *Psychopharmacology (Berl)* 1996;123(2):144-153. [PubMed: 8741937]
- Panksepp J. The ontogeny of play in rats. *Dev Psychobiol* 1981;14(4):327-332. [PubMed: 7250521]
- Panksepp J, Beatty WW. Social deprivation and play in rats. *Behav Neural Biol* 1980;30(2):197-206. [PubMed: 7447871]
- Panksepp J, Siviy S, Normansell L. The psychobiology of play: theoretical and methodological perspectives. *Neurosci Biobehav Rev* 1984;8(4):465-492. [PubMed: 6392950]
- Pellis SM, Field EF, Smith LK, Pellis VC. Multiple differences in the play fighting of male and female rats. Implications for the causes and functions of play. *Neurosci Biobehav Rev* 1997;21(1):105-120. [PubMed: 8994213]
- Pellis SM, McKenna M. What do rats find rewarding in play fighting?--an analysis using drug-induced non-playful partners. *Behav Brain Res* 1995;68(1):65-73. [PubMed: 7619307]
- Pellis SM, Pellis VC. Play-fighting differs from serious fighting in both target of attack and tactics of fighting in the laboratory rat *Rattus norvegicus*. *Aggress Behav* 1987;13:227-242.
- Pellis SM, Pellis VC. Differential rates of attack, defense, and counterattack during the developmental decrease in play fighting by male and female rats. *Dev Psychobiol* 1990;23(3):215-231. [PubMed: 2379760]
- Philpot RM, Badanich KA, Kirstein CL. Place conditioning: age-related changes in the rewarding and aversive effects of alcohol. *Alcohol Clin Exp Res* 2003;27(4):593-599. [PubMed: 12711921]
- Pliner P, Cappell H. Modification of affective consequences of alcohol: a comparison of social and solitary drinking. *J Abnorm Psychol* 1974;83(4):418-425. [PubMed: 4414699]
- Randich A, LoLordo VM. Associative and nonassociative theories of the UCS preexposure phenomenon: implications for Pavlovian conditioning. *Psychol Bull* 1979;86(3):523-548. [PubMed: 377357]
- Schneirla, T. An evolutionary and developmental theory of biphasic processes underlying approach and withdrawal. In: Jones, M., editor. *Nebraska Symposium on Motivation*. University of Nebraska Press; Nebraska: 1959. p. 27-58.
- Shram MJ, Funk D, Li Z, Le AD. Periadolescent and adult rats respond differently in tests measuring the rewarding and aversive effects of nicotine. *Psychopharmacology (Berl)* 2006;186(2):201-208. [PubMed: 16586088]
- Smith RC, Parker ES, Noble EP. Alcohol and affect in dyadic social interaction. *Psychosom Med* 1975;37(1):25-40. [PubMed: 1091942]
- Spear LP. The adolescent brain and age-related behavioral. *Neurosci Biobehav Rev* 2000;24:417-563. [PubMed: 10817843]
- Sutton ME, Raskin LA. A behavioral analysis of the effects of amphetamine on play and locomotor activity in the post-weaning rat. *Pharmacol Biochem Behav* 1986;24(3):455-461. [PubMed: 3703882]
- Taylor GT. Affiliation in highly aggressive domesticated rats. *J Comp Physiol Psychol* 1977;94:953-961.

- Taylor JA. Level of conditioning and intensity of the adaptation stimulus. *J Exp Psychol* 1956;51:127–130. [PubMed: 13295499]
- Thor DH, Holloway WR Jr. Play soliciting in juvenile male rats: effects of caffeine, amphetamine and methylphenidate. *Pharmacol Biochem Behav* 1983;19(4):725–727. [PubMed: 6139827]
- Thor DH, Holloway WR Jr. Social play soliciting by male and female juvenile rats: effects of neonatal androgenization and sex of cagemates. *Behav Neurosci* 1986;100(2):275–279. [PubMed: 3964428]
- Tomie A, Uveges JM, Burger KM, Patterson-Buckendahl P, Pohorecky LA. Effects of ethanol sipper and social opportunity on ethanol drinking in rats. *Alcohol* 2004;39(3):197–202.
- Torrella TA, Badanich KA, Philpot RM, Kirstein CL, Wecker L. Developmental differences in nicotine place conditioning. *Ann N Y Acad Sci* 2004;1021:399–403. [PubMed: 15251917]
- Van den Berg CL, Hol T, Van Ree JM, Spruijt BM, Everts H, Koolhaas JM. Play is indispensable for an adequate development of coping with social challenges in the rat. *Dev Psychobiol* 1999;34(2):129–138. [PubMed: 10086231]
- Van den Berg CL, Pijlman FT, Koning HA, Diergaarde L, Van Ree JM, Spruijt BM. Isolation changes the incentive value of sucrose and social behaviour in juvenile and adult rats. *Behav Brain Res* 1999;106(1–2):133–142. [PubMed: 10595429]
- Vanderschuren LJ, Niesink RJ, Van Ree JM. The neurobiology of social play behavior in rats. *Neurosci Biobehav Rev* 1997;21(3):309–326. [PubMed: 9168267]
- Varlinskaya EI, Spear LP. Acute effects of ethanol on social behavior of adolescent and adult rats: role of familiarity of the test situation. *Alcohol Clin Exp Res* 2002;26(10):1502–1511. [PubMed: 12394283]
- Varlinskaya EI, Spear LP. Differences in the social consequences of ethanol emerge during the course of adolescence in rats: social facilitation, social inhibition, and anxiolysis. *Dev Psychobiol* 2006;48(2):146–161. [PubMed: 16489593]
- Varlinskaya EI, Spear LP, Spear NE. Social behavior and social motivation in adolescent rats: role of housing conditions and partner's activity. *Physiol Behav* 1999;67(4):475–482. [PubMed: 10549884]
- Varlinskaya EI, Spear LP, Spear NE. Acute effects of ethanol on behavior of adolescent rats: role of social context. *Alcohol Clin Exp Res* 2001;25(3):377–385. [PubMed: 11290848]
- Vastola BJ, Douglas LA, Varlinskaya EI, Spear LP. Nicotine-induced conditioned place preference in adolescent and adult rats. *Physiol Behav* 2002;77(1):107–114. [PubMed: 12213508]
- Werner CM, Anderson DF. Opportunity for interaction as reinforcement in a T-maze. *Pers Soc Psychol Bull* 1976;2:166–169.
- Zavala AR, Weber SM, Rice HJ, Alleweireldt AT, Neisewander JL. Role of the prelimbic subregion of the medial prefrontal cortex in acquisition, extinction, and reinstatement of cocaine-conditioned place preference. *Brain Res* 2003;990(1–2):157–164. [PubMed: 14568340]

Figure 1.

Influence of number of conditioning sessions/day on social reward-CPP illustrated as time (mean $s \pm SEM$) spent in the playmate-paired (i.e., initially non-preferred) side preconditioning (Baseline) vs. post-conditioning (Test) in groups receiving either 1 (closed triangle, $n=8$) or 2 (closed circle, $n=8$) conditioning sessions per day. Asterisk (*) indicates significant main effect of Day ($p < .001$). The dotted line represents 50% of the total test period (i.e., 300 s).

Figure 2.

Influence of duration and number of conditioning sessions on social reward-CPP shown as difference score (i.e., amount of time spent in the playmate-paired side postconditioning minus pre-conditioning) for groups receiving either 10-min (open bars) or 30-min (closed bars) conditioning sessions (panel A) and as time (mean $\pm$ SEM) spent in the playmate-paired side pre-conditioning (Baseline) vs. post-conditioning (Test) following either 1 (open square, $n=20$), 4 (closed circle, $n=21$), or 8 (closed square, $n=22$) social pairings collapsed across the duration (i.e., 10- and 30-min) of the pairings (panel B). Baselines ($s \pm$ SEM) did not differ across groups and ranged from 233.0 ± 12.7 to 250.3 ± 12.7 . There was no main effect of duration of sessions or interaction with number of sessions (panel A). There was an interaction

between number of social pairings and Day (panel B). Note that all rats received 8 opportunities for social interaction, but the groups are labeled to reflect only the number of social pairings in the CPP apparatus. Asterisk (*) indicates significant increase in amount of time spent in the playmate-paired side on Test day relative to Baseline ($p < .05$, Tukey's HSD). Cross (+) indicates a significantly greater amount of time spent in playmate-paired side on Test day for the 8 social pairings group relative to the 1 pairing group ($p < .05$, Tukey's HSD). The dotted line represents 50% of the total test period (i.e., 300 s).

Figure 3.

Social reward-CPP without pre-exposure to the playmate prior to conditioning shown as time (mean s ± SEM) spent in the playmate-paired side pre-conditioning (Baseline) vs. postconditioning (Test) for the 1 Pairing group (open square, n=11) and the 4 Pairings group (closed circle, n=11). Asterisk (*) indicates a significant increase in time spent in the playmate-paired side on Test day relative to Baseline ($p < .05$, Tukey's HSD). Cross (+) indicates a significantly greater amount of time spent in the playmate-paired side on Test day for the 4 Pairings group relative to the 1 Pairing group ($p < .05$, Tukey's HSD). The dotted line represents 50% of the total test period (i.e., 300 s).

Figure 4.

Non-associative factors fail to influence place preference as shown by time (mean $s \pm \text{SEM}$) spent in the initially non-preferred side pre-conditioning (Baseline) vs. post-conditioning (Test) for the Unpaired Control (open circle, $n=10$) and the Conditioned group (closed square, $n=9$) that experienced equal exposure to the apparatus and social interaction but not paired together in the apparatus. The initially non-preferred side was the playmate-paired side for the Conditioned group. Asterisk (*) indicates significant increase in time spent in the initially non-preferred side on Test day relative to Baseline ($p < .05$, Tukey's HSD). Cross (+) indicates a significantly greater amount of time spent in the initially non-preferred side on Test day for the Conditioned group relative to the Unpaired Control group ($p < .05$, Tukey's HSD). The dotted line represents 50% of the total test period (i.e., 300 s).

Figure 5.

Social reward-CPP obtained using a counterbalanced design shown as time (mean $s \pm SEM$) spent in the playmate-paired side versus alternate side of the apparatus in rats receiving social pairings in either the black (i.e., Paired On Black, $n=8$) or the white side (i.e., Paired On White, $n=8$). White and black bars represent the amount of time rats spent in the white and black sides of the apparatus on test day, respectively. Asterisk (*) indicates significantly more time spent in the playmate paired-side relative to the alternate side ($p<.05$, Wilcoxin signed-ranks test). The dotted line represents 50% of the total test period (i.e., 300 s).

Figure 6. Interaction between cocaine (2 mg/kg, IP) and social rewards illustrated as time (mean $\pm$ SEM) spent in the playmate-paired (i.e., initially non-preferred) side pre-conditioning (Baseline) vs. post-conditioning (Test) for the Control group (open circle, n=9), Social Only group (downward closed triangle, n=9), Cocaine Only group (closed square, n=9), and the Cocaine/Social group (open square, n=9). Asterisk (*) indicates significant increase in time spent in the initially non-preferred side on Test day relative to Baseline ($p < .05$, Tukey's HSD). Cross (+) indicates a significantly greater amount of time spent in the initially non-preferred side on Test day for the Cocaine/Social group relative to the all other groups ($p < .05$, Tukey's HSD). The dotted line represents 50% of the total test period (i.e., 300 s).

Figure 7.

Lack of interaction between dextromethorphan (DXM; 30 mg/kg, IP) and social reward illustrated as no group differences in time (mean $\pm$ SEM) spent in the playmate-paired (i.e., initially non-preferred) side pre-conditioning (Baseline) vs. post-conditioning (Test) among the Control group (open circle, n=8), Social Only group (downward closed triangle, n=10), Dextromethorphan (DXM) Only group (closed diamond, n=9), and the DXM/Social group (open diamond, n=10). The dotted line represents 50% of the total test period (i.e., 300 s).

Figure 8.

Cocaine produced a strong trend toward a decrease in Pinning behavior (i.e., Pins) and a decrease in Nape Attacks shown as the mean number of each behavior scored on the last day of conditioning in the Social Only group (white bar, $n=9$) versus the Cocaine/Social group (black bar, $n=9$). Asterisk (*) represents significant decrease relative to Social Only group ($p < .05$).

Figure 9.

Dextromethorphan (DXM) produced a decrease in Pinning behavior (i.e., Pins) and Nape Attacks shown as the mean number of each behavior scored in the Social Only group (white bar, n=10) versus the DXM/Social group (black bar, n=10). Asterisk (*) represents significant decrease relative to Social Only group ($p < .001$).

Table 1

Timeline of procedures across post-natal days (PND) and the stimuli paired with each context during the conditioning phase; stimuli included the playmate and injections of saline (Sal), cocaine (Coc), or dextromethorphan (DXM) given just prior to context pairing.

Exp.	Group	n	Baseline (PND)	Conditioning Sessions (PND)	Stimulus Conditions Paired With Each Context During Conditioning Sessions ¹			Test (PND)
					Non-preferred	Preferred	ALT environment	
1	Once/day	8	29-30	31-38	Playmate	Alone	N.A.	39
	Twice/day	8	29-30	35-38	Playmate	Alone	N.A.	39
	1 Pairing	20	29-30	38	Playmate	Alone	Playmate (PNDs 31-37)	39
2	4 Pairings	21	29-30	35-38	Playmate	Alone	Playmate (PNDs 31-34)	39
	8 Pairings	22	29-30	31-38	Playmate	Alone	N.A.	39
	1 Pairing	11	29-30	38	Playmate	Alone	N.A.	39
3	4 Pairings	11	29-30	35-38	Playmate	Alone	N.A.	39
	Unpaired Control	10	29-30	31-38	Alone	Alone	Playmate	39
	Conditioned Control	9	29-30	31-38	Playmate	Alone	Alone	39
4	Control	9	30-31	33-34	Alone + Sal	Alone + Sal	Playmate + Coc	35
	Cocaine Only	9	30-31	33-34	Alone + Coc	Alone + Sal	Playmate + Sal	35
	Social Only	9	30-31	33-34	Playmate + Sal	Alone + Sal	Alone + Coc	35
5	Cocaine/Social	9	30-31	33-34	Playmate + Coc	Alone + Sal	Alone + Sal	35
	Control	8	30-31	32-37	Alone + Sal	Alone + Sal	Playmate + DXM	38
	DXM Only	9	30-31	32-37	Alone + DXM	Alone + Sal	Playmate + Sal	38
6	Social Only	10	30-31	32-37	Playmate + Sal	Alone + Sal	Alone + DXM	38
	DXM/Social	10	30-31	32-37	Playmate + DXM	Alone + Sal	Alone + Sal	38

¹ On a given day, rats received pairings in each side of the CPP apparatus with 6 h intervening, except for the Once/day group in Exp. 1 and all groups in Exp. 7 who received a pairing in only one side of the CPP apparatus on a given day. Where applicable, rats also received pairings in the alternate (ALT) environment either during conditioning or on PNDs specified above.

Table 2Crossovers (mean $\pm$ SEM) on the test day

Experiment	Group (n)	Crossovers
1	Once/day (8)	21.1 $\pm$ 1.7
	Twice/day (8)	22.1 $\pm$ 2.8
2	1 Social Pairing (20)	23.4 $\pm$ 1.4
	4 Social Pairings (21)	25.5 $\pm$ 1.6
	8 Social Pairings (22)	26.6 $\pm$ 1.3
3	1 Pairing (11)	14.2 $\pm$ 1.1
	4 Pairings (11)	17.6 $\pm$ 1.7
4	Conditioned (9)	23.0 $\pm$ 2.2
	Unpaired Control (10)	23.0 $\pm$ 2.3
5	Paired On White (8)	26.7 $\pm$ 2.8
	Paired On Black (8)	29.3 $\pm$ 3.3
6	Control (9)	18.8 $\pm$ 3.2
	Social Only (9)	19.0 $\pm$ 2.7
	Cocaine Only (9)	20.6 $\pm$ 3.6
	Cocaine/Social (9)	17.4 $\pm$ 2.3
7	Control (8)	20.1 $\pm$ 1.7
	Social Only (10)	19.9 $\pm$ 2.2
	DXM Only (9)	17.7 $\pm$ 1.9
	DXM/Social (10)	17.3 $\pm$ 2.4

Table 3

Lack of correlation between mean number of pins ($\pm$ SEM) and mean preference shift, expressed as a difference score (seconds in playmate-paired side post-conditioning minus pre-conditioning $\pm$ SEM), in animals exhibiting either the most or least pins of a rat pair or exhibiting no pins.

Group (n)	Pins	CPP Difference Score	r	p
Most Pins (26)	16.8 $\pm$ 2.5	68.6 $\pm$ 12.7	0.13	0.54
Least Pins (26)	9.2 $\pm$ 1.5	55.5 $\pm$ 11.1	-0.38	0.06
No Pins (11)	0.0 $\pm$ 0.0	104.7 $\pm$ 25.7	N.A.	N.A.

Table 4

Lack of correlation between mean number of nape attacks ($\pm$ SEM) and mean preference shift, expressed as a difference score (seconds in playmate-paired side post-conditioning minus preconditioning $\pm$ SEM), in animals exhibiting either the most or least nape attacks of a rat pair.

Group (n)	Nape Attacks	Difference Score	r	p
Most Nape Attacks (34)	17.5 $\pm$ 1.5	73.3 $\pm$ 12.1	-0.09	0.63
Least Nape Attacks (29)	11.1 $\pm$ 1.4	64.7 $\pm$ 11.7	0.09	0.65