Prosocial Influences on Nicotine Reinforcement, Reward, and Neural Signaling in Rodent Models

by

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ABSTRACT

Social influences are important determinants of drug initiation in humans, particularly during adolescence and early adulthood. My dissertation tested three hypotheses: 1) conditioned and unconditioned nicotine and social rewards elicit unique patterns of neural signaling in the corticolimbic neurocircuitry when presented in combination versus individually; 2) play behavior is not necessary for expression of social reward; and 3) social context enhances nicotine self-administration. To test the first hypothesis, Fos protein was measured in response to social and nicotine reward stimuli given alone or in combination and in response to environmental cues associated with the rewards in a conditioned place preference (CPP) test. Social-conditioned environmental stimuli attenuated Fos expression in the nucleus accumbens core. A social partner elevated Fos expression in the caudate-putamen, medial and central amygdala, and both nucleus accumbens subregions. Nicotine decreased Fos expression in the cingulate cortex, caudate-putamen, and the nucleus accumbens core. Both stimuli combined elevated Fos expression in the basolateral amygdala and ventral tegmental area, suggesting possible overlap in processing both rewards in these regions. I tested the second hypothesis with an apparatus containing compartments separated by a wire mesh barrier that allowed limited physical contact with a rat or object. While 2 pairings with a partner rat (full physical contact) produced robust CPP, additional pairings were needed for CPP with a partner behind a barrier or physical contact with an object (i.e., tennis ball). The results demonstrate that physical contact with a partner rat is not necessary to establish social-reward CPP. I tested the third hypothesis with duplex operant conditioning chambers separated either by a solid or a wire mesh barrier to allow for

social interaction during self-administration sessions. Nicotine (0.015 and 0.03 mg/kg, IV) and saline self-administration were assessed in male and female young-adult rats either in the social context or isolation. Initially, a social context facilitated nicotine intake at the low dose in male rats, but suppressed intake in later sessions more strongly in female rats, suggesting that social factors exert strong sex-dependent influences on self-administration. These novel findings highlight the importance of social influences on several nicotine-related behavioral paradigms and associated neurocircuitry.

DEDICATION

To all of my teachers, mentors, and colleagues over the years,

for inspiring me, challenging me,

and giving me the confidence to achieve my goals.

To all the female scientists before me,

who broke down barriers and paved the way, making the path

much smoother for women like me to pursue an education.

To my entire family,

for providing unrelenting and vital support along the way.

To the love of my life, Patrick,

and our beautiful son, Liam,

for being the reasons I feel so fortunate every single day.

Finally, I would like to dedicate this dissertation

in memory of the late Suzanne Weber.

You helped me so much along the way and

I am forever grateful for your friendship.

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CHAPTER 1

GENERAL OVERVIEW

Social factors are important determinants of drug initiation in humans, yet little is known about social influences on drug-related behavior in pre-clinical animal models of drug abuse. Alas, there is a growing interest in the role of social influences on the development of drug abuse and dependence in the field of neuroscience. An important feature of tobacco use in humans that has been largely overlooked in animal models is that initiation of use typically occurs in a social setting in which peer interaction serves to reinforce the behavior (Baker, Brandon, & Chassin, 2004; Geckova et al., 2005; Sussman, 2005; West, Sweeting, & Ecob, 1999).

Experimentation with drug use most frequently occurs during adolescence to early-adulthood (Breslau & Peterson, 1996; Kandel & Logan, 1984). This is particularly troublesome since adolescents are known to engage in more risk-taking behaviors despite negative consequences (see Willoughby, Good, Adachi, Hamza, & Tavernier, 2013 for review), perhaps due to under-developed cortical areas important for judgment, planning and decision-making in the adolescent brain (see Spear, 2000 for review). In animal models, adolescents typically display enhanced sensitivity to the rewarding effects and reduced sensitivity to the aversive effects of nicotine relative to adults (Belluzzi, Lee, Oliff, & Leslie, 2004; Kota, Martin, Robinson, & Damaj, 2007; O'Dell, Bruijnzeel, Ghozland, Markou, & Koob, 2004; Shram, Funk, Li, & Le, 2006). In fact, earlier onset of nicotine self-administration in rats leads to escalated intake, which persist into adulthood (Levin et al., 2011).

Peer interaction during drug administration generally enhances drug reward and reinforcement (see Bardo, Neisewander, & Kelly, 2013; and Neisewander, Peartree, & Pentkowski, 2012 for review). Therefore, gaining a better understanding of how social factors

promote drug-related behaviors during a vulnerable developmental period (i.e., adolescence to early adulthood) will likely contribute to the development of prevention and intervention strategies for tobacco dependence.

Importance of Social Interactions and Social Rewards

During adolescence in humans and rodents alike, adequate social interaction is crucial for normal development and formation of appropriate social behaviors in adulthood (Einon, Morgan, & Kibbler, 1978; Panksepp, 1981; Spear, 2000). Since rats are a highly-social species, social interaction serves as a robust natural reward for adolescents and adults, measured by both operant and classical conditioning paradigms. For instance, adolescent rats will readily traverse a T-maze to gain access to another rat (Humphreys & Einon, 1981; Normansell & Panksepp, 1990; Werner & Anderson, 1976). In addition, conditioned place preference (CPP) studies demonstrate that both adolescent and adult rats will spend more time in an environment paired with a conspecific, however social motivation and reward are more robust during adolescence relative to adulthood (Calcagnetti & Schechter, 1992; Douglas, Varlinskaya, & Spear, 2004; Thiel, Okun, & Neisewander, 2008; Van den Berg et al., 1999). Further, a single re-exposure to a social partner reinstates an extinguished preference for that environment in adolescent rats (Trezza, Damsteegt, & Vanderschuren, 2009). Recently, Fritz and colleagues found that opportunity for social reward in adults rats reverses a previously-established cocaine CPP when the 2 rewards compete for preference on opposite sides of the apparatus, thus demonstrating that reward-strength associated with social interaction supersedes cocaine reward (Fritz et al., 2011).

Several researchers suggest that the primary rewarding feature of a social context is the ability to engage in rough-and-tumble play behavior (i.e. play fighting) (Douglas, et al., 2004; Panksepp & Beatty, 1980; Panksepp, Siviy, & Normansell, 1984). However, our lab and others

have shown that play behavior may not be solely responsible for the rewarding aspects of social interaction for male adolescent rats. In some cases, play behavior is insufficient for establishing CPP. For instance, we have shown that there is no relationship between the social reward-CPP and the amount of play behavior that occurs during exposure to the social partner (Thiel, et al., 2008). Furthermore, social reward is observed in adolescent rats that have the same social experience histories of group housing, however social avoidance occurs in socially-housed rats paired with a previously socially-isolated partner (Douglas, et al., 2004; Varlinskaya, Spear, & Spear, 1999), suggesting that previous social experience affects the hedonic value of subsequent social interactions. Social behaviors apart from play (i.e., crawling over, grooming and sniffing another conspecific) increase when motivation to play is decreased by chronic social exposure, such as group housing (Varlinskaya, et al., 1999) or through pharmacological inactivation of the drive for play behavior (Deak & Panksepp, 2006; Pellis & McKenna, 1995), suggesting that other components of social interactions contribute to the rewarding aspects of social interaction in rodents. The intense drive to engage in social interaction appears to be highly imprinted upon humans and rodents alike. Therefore, it is crucial that we begin to incorporate social factors into our pre-clinical research models of human drug abuse.

Nicotine in Animal models

Smoking is a major health hazard, with 1 out of every 5 deaths in the United States resulting from deleterious health issues resulting from tobacco use (U.S. Dept. of Health & Human Services, 2014). Nicotine is the primary active pharmacological ingredient in tobacco products that is thought to be responsible for its dependence liability. Paradoxically, nicotine possesses relatively weak intrinsic reinforcing effects compared to other drugs of abuse despite having a high addiction liability evidenced by reported difficulty with cessation.

In both adolescent and adult rodents, nicotine produces reward across a range of doses in CPP models (Belluzzi, et al., 2004; Shram, et al., 2006; Thiel, Sanabria, & Neisewander, 2009; Vastola, Douglas, Varlinskaya, & Spear, 2002), as well as self-administration across multiple doses and schedules of reinforcement (H. Chen, Matta, & Sharp, 2007; Corrigall & Coen, 1989; Cox, Goldstein, & Nelson, 1984; Donny et al., 1998; Donny et al., 2000; Latiff, Smith, & Lang, 1980; Watkins, Epping-Jordan, Koob, & Markou, 1999). However, nicotine is less readily self-administered in rodent models compared to other drugs of abuse (Manzardo, Stein, & Belluzzi, 2002; Palmatier et al., 2006). This is likely due to nicotine's dual role as a weak primary reinforcer, but a robust enhancer of other reinforcers, such as non-pharmacological stimuli (Caggiula et al., 2001; Palmatier, et al., 2006). In fact mildly reinforcing response-contingent cues are typically paired with nicotine infusions to establish and maintain nicotine self-administration because the behavior is much less reliable without this procedure (Caggiula et al., 2002; Palmatier, et al., 2006). Non-contingent nicotine enhances responding for such cues (Palmatier, et al., 2006), demonstrating that nicotine can enhance the reinforcing effects of other non-pharmacological stimuli. The reinforcement-enhancing effects of nicotine are more robust with stimuli possessing greater natural incentive value (Chaudhri, Caggiula, Donny, Booth, et al., 2006; Palmatier et al., 2007).

Drugs of Abuse and Social Interactions

In humans, social affiliation is a major factor influencing initiation of drug and alcohol use. Social pressure and desire for group membership are cited as the most prevalent factors contributing to the initiation of tobacco use among adolescents and young adults (Geckova, et al., 2005; Jackson, 1997; Pierce, Choi, Gilpin, Farkas, & Merritt, 1996; Sussman, 2005; West, et al., 1999). In humans, alcohol consumption is consistently rated as more pleasurable when it

takes place in a social context than when alone (Pliner & Cappell, 1974; R. C. Smith, Parker, & Noble, 1975). Similarly in rats, oral ethanol intake is facilitated by a conspecific (Tomie, Burger, Di Poce, & Pohorecky, 2004) and social context attenuate aversive effects of alcohol (Gauvin, Briscoe, Goulden, & Holloway, 1994; Varlinskaya, Spear, & Spear, 2001). Prosocial interactions in rodents impact drug-related behavioral outcomes largely by increasing the rewarding and reinforcing effects of the drugs themselves (Bardo, et al., 2013; Neisewander, et al., 2012). For instance, our lab has found that a social partner enhances both nicotine and cocaine CPP (Thiel, et al., 2008; Thiel, et al., 2009). Recently, several laboratories have begun to investigate the influence of the presence of a social conspecific on stimulant self-administration. Specifically, Chen and colleagues (2011) demonstrated that social transmittance of food preference via access to a social partner attenuates nicotine-induced taste aversion to a palatable olfactory gustatory cue paired with response-contingent intravenous (IV) nicotine infusions, suggesting social context attenuates aversive nicotine effects. Similarly, both a conspecific behind a Plexiglas barrier and social housing within the operant self-administration chamber increase d-amphetamine and cocaine self-administration, respectively (Gipson et al., 2011; M. A. Smith, 2012).

Given that social stimuli enhance drug reward and reinforcement, it is important to examine the neural circuitry involved in these effects. It is known that both nicotine and social rewards each independently activate mesocorticolimbic pathways (Insel & Fernald, 2004; Ochoa, 1994; Panksepp, et al., 1984; Siviy & Panksepp, 2011; Young, Gobrogge, & Wang, 2011); however, little is known about the neural mechanisms involved in the *interaction* of these two stimuli when they are presented together. A useful approach for addressing this gap in knowledge is to examine Fos protein expression, which has been widely used as a functional

marker of neuronal signaling in response to drug- and drug-associated stimuli (see Chao & Nestler, 2004; and Nestler, 2001 for review).

Sex differences and Nicotine

Sex differences in drug abuse are well established (see Carroll, Lynch, Roth, Morgan, & Cosgrove, 2004; and Roth, Cosgrove, & Carroll, 2004 for review), but the involvement of sex and gonadal hormones on nicotine-related behaviors appears to be confounded by other factors (i.e., age and drug-abuse model). For instance, sex differences have been documented using nicotine CPP in rodents (Torres, Natividad, Tejeda, Van Weelden, & O'Dell, 2009) (Isiegas, Mague, & Blendy, 2009; Pogun & Yararbas, 2009; Yararbas, Keser, Kanit, & Pogun, 2010). However, neither sex nor estrous cycle phase appear to have a consistent effect on nicotine self-administration (Chaudhri et al., 2005; H. Chen, Sharp, Matta, & Wu, 2011; Donny, et al., 2000; Feltenstein, Ghee, & See, 2012; Levin, et al., 2011; Lynch, 2009). Additionally, other studies have failed to detect sex differences or estrous cycle effects on cue or stress-primed reinstatement of nicotine-seeking behavior (Feltenstein, et al., 2012) or nicotine-induced hyperlocomotion (Kuo et al., 1999) in young adult rats.

Aims of Research

The goal for the first part of my dissertation was to explore neural signaling correlates that parallel the synergistic social and nicotine interaction found previously by our laboratory (Thiel, et al., 2009). In chapter two, we examined immediate early gene expression (i.e., Fos protein expression) in response to environmental cues associated with nicotine and social rewards, as well as in response to sub-threshold social and nicotine stimuli given alone or in combination in male adolescent rats. A secondary aim of this chapter was to measure social and nicotine-reward thresholds in our new CPP apparatus that was designed specifically for use of

smaller rodents, including adolescent rats. The goal for the second part of my dissertation was to validate novel social paradigms used in both CPP and self-administration models. In chapter three, I sought to determine whether social reward CPP could be established by a partner rat behind a mesh screen, which restricted physical contact and eliminated rough-and-tumble play behavior in male adolescent rats. The results from chapter three validated limited physical contact as a rewarding social stimulus, which compelled me to integrate limited physical contact into the self-administration paradigm. Limited physical contact through a mesh screen during self-administration sessions is rewarding and therefore should serve as a method to examine social influence on acquisition of nicotine self-administration in rats transitioning into young adulthood (i.e., beginning post-natal day 60). Furthermore, the barrier protects and preserves each rats surgically-implanted cannula ports used for intravenous infusions and prevents non-contingent lever pressing by the partner rat that would likely occur if two rats were placed in the same chamber with full physical access to one another. Thus, in chapter four, I used custom-built operant self-administration chambers with a removable partition that contained either a mesh screen or a solid partition, allowing social interaction or isolation between the adjoining chambers, respectively. The aim of chapter four was to examine social influences on nicotine self-administration in male and female young-adult rats.

CHAPTER 2

FOS PROTEIN EXPRESSION AFTER EXPOSURE TO SOCIAL AND NICOTINE REWARDS OR REWARD-CONDITIONED ENVIRONMENTS IN ADOLESCENT MALE RATS

Smoking is a major societal concern, with one out of every 5 deaths in the United States resulting from detrimental health effects of smoking (U.S. Dept. Health & Human Services, 2014). Initiation of smoking most commonly occurs during adolescence (Breslau & Peterson, 1996; Kandel & Logan, 1984; Olds & Thombs, 2001; Taioli & Wynder, 1991), and adolescent initiation of smoking is associated with a faster rate of dependence development and increased difficulty with cessation compared to adult initiation (Breslau & Peterson, 1996; J. Chen & Millar, 1998; Colby, Tiffany, Shiffman, & Niaura, 2000; Kandel & Chen, 2000). The effects of nicotine, the component of tobacco that is thought to underlie its abuse potential, vary age-dependently. Nicotine has rewarding effects across a range of doses in conditioned place preference (CPP) models (Belluzzi, et al., 2004; Thiel, et al., 2009; Vastola, et al., 2002) and adolescent rodents demonstrate greater sensitivity to the rewarding and reinforcing effects of nicotine compared to adults (Adriani, Macri, Pacifici, & Laviola, 2002; Belluzzi, et al., 2004; Levin et al., 2007; Torres, Tejeda, Natividad, & O'Dell, 2008). In contrast, adolescents are less sensitive to the aversive properties of nicotine and nicotine withdrawal (Kota, et al., 2007; O'Dell, et al., 2004; Shram, et al., 2006). Thus, adolescence is a developmental period of increased vulnerability to nicotine abuse potential.

Nicotine is less readily self-administered in rodent models compared to other drugs of abuse (Manzardo, et al., 2002; Palmatier, et al., 2006; Palmatier, et al., 2007) and in humans social reinforcement is a major factor in initiating tobacco use in adolescents. Indeed ‘group

membership' and 'peer encouragement' are cited as the most prevalent reasons for initiation of tobacco use among adolescents and young adults (Geckova, et al., 2005; Jackson, 1997; Pierce, et al., 1996; Sussman, 2005; West, et al., 1999). During adolescence in humans and rodents alike, social interaction fosters healthy development and appropriate social behavior in adulthood (M.J. Meaney & Stewart, 1979; Panksepp, 1981; Spear, 2000). In rodents, the robust rewarding effects of social interaction is measured by both operant (Angermeier, Schaul, & James, 1959; Humphreys & Einon, 1981; Werner & Anderson, 1976) and classical conditioning paradigms (Calcagnetti & Schechter, 1992; Thiel, et al., 2009; Trezza, Damsteegt, et al., 2009). Prosocial interactions exert a substantial influence on drug-related behaviors largely by increasing the rewarding and reinforcing effects of the drugs themselves (Bardo, et al., 2013; Neisewander, et al., 2012). For instance, social interaction enhances both nicotine and cocaine CPP (Thiel, et al., 2008; Thiel, et al., 2009). Additionally, the presence of a conspecific also enhances stimulant self-administration (H. Chen, et al., 2011; Gipson, et al., 2011; M. A. Smith, 2012).

Since adolescence is a period of enhanced vulnerability for initiating smoking and this behavior is largely influenced by social factors, the modulatory role that social interaction plays in the initial drug experience is crucial for understanding neural processes involved in the development of nicotine addiction. Both nicotine and social reward each independently activate mesocorticolimbic pathways (Insel & Fernald, 2004; Ochoa, 1994; Panksepp, et al., 1984; Siviy & Panksepp, 2011; Young, et al., 2011); however, little is known about the neural mechanisms involved in the interaction of these stimuli when they are presented together. A useful approach for addressing this gap in knowledge is to examine Fos protein expression, which has been widely used as a functional marker of neuronal signaling in response to drug- and drug-associated stimuli (Chao & Nestler, 2004; Nestler, 2001). Specifically, Fos is the protein product

of the immediate early gene *c-fos*, which is a transcription factor transiently induced in response to physiological or psychological stimuli, and initiates many signal transduction pathways (Chaudhuri, 1997; Curran & Morgan, 1995; Harlan & Garcia, 1998). Fos protein is rapidly expressed (i.e., peaks around 90-120 min following stimulus exposure), (Chaudhuri, 1997; Nestler, 2001), which makes it a viable candidate for measuring immediate early gene activity in response to an acute stimulus.

In the present study, we used Fos protein expression to examine the neural circuitry involved in the conditioned and unconditioned rewarding effects of nicotine and social rewards using the CPP model. In experiment 1, we measured Fos protein expression in response to environmental cues associated with social and nicotine rewards conditioned separately or in combination in adolescent male rats. In experiment 2, we measured Fos protein expression in response to sub-threshold social and nicotine reward stimuli given alone or in combination. Rats were conditioned using previously established experimental parameters from our laboratory (Thiel et al., 2009), however we utilized in a new apparatus that was designed for the small size of the adolescent rats and the procedure was modified in experiment 2 to utilize longer time intervals between US presentations. We hypothesized that nicotine- and social-reward *unconditioned* and *conditioned environmental stimuli* would elicit a more robust increase in functional activation within the cortical, striatal and limbic circuitry when presented in combination than when presented individually.

Methods

Animals

Male Sprague–Dawley rats (Charles River, San Diego, CA) (N=130) arrived at Arizona State University on postnatal day (PND) 22 (i.e., 22 days old) for both experiments. They were

individually housed in a climate-controlled facility with a 12-h light/dark cycle (lights on at 7 PM) with ad libitum access to food and water. Housing and care were conducted in accordance with the 8th ed. Guide for the Care and Use of Laboratory Animals (National Research Council (US) Committee for the update of the Guide for the Care and Use of Laboratory Animals 2011). All experiments were conducted within the conservative estimated timeframe of rodent adolescence of PNDs 28–42 (Spear, 2000). Prior to baseline testing, animals were acclimated to handling for 9–11 days. On each of these days, rats were handled for at least 2 min/day.

Drug Preparation

(−)Nicotine hydrogen tartrate (Sigma, St. Louis, MO) was dissolved in 0.9% sterile saline and pH was adjusted to 7.2. All injections were given subcutaneously (s.c.) at a volume of 1 mL/kg. Dose is reported as nicotine base.

Apparatus

Conditioning took place in rectangular Plexiglas chambers as previously described (Peartree, Hood, et al., 2012). Each chamber contained a removable solid partition that separated the chamber into two equal-sized compartments, each measuring 35 × 24 × 31 cm high. One compartment had corn cob bedding beneath a wire 1 × 1 cm grid floor and alternating black and white vertical stripes on the walls. The other compartment had pine-scented bedding beneath a parallel bar floor (5 mm diameter) and alternating black and white horizontal stripes on the walls. On the pre- and post-conditioning test days, the removable center partition of the apparatus was replaced by a similar partition that contained an opening in the center (28 × 6 cm), allowing the rats free-access to the adjacent compartments simultaneously. A rectangular tower measuring 70 × 24 × 74 cm high of clear Plexiglas was used as an extension of the apparatus to prevent the rats from escaping from the chamber while maintaining the ability to record their behavior via an

overhanging video camera. The conditioning room was dimly lit with two overhead lamps, each containing a 25 Watt light bulb providing equal light distribution for each conditioning chamber. Unpublished data from our laboratory established that adolescent and adult experimentally naïve rats showed no preference for a particular compartment (i.e., unbiased apparatus). A camera (Panasonic WV-CP284, color CCTV, Suzhou, China) used to record testing sessions was mounted 101 cm above the center of the apparatus. A WinTV 350 personal video recorder (Hauppage, NJ, USA) captured live video and encoded it to MPEG streams. A modified version of TopScan Software (Clever Sys., Inc. Reston, VA, USA) used the orientation of an animal's body parts to track its location, which yielded measures of time spent in each compartment.

The alternate environment was an opaque plastic container measuring 34 × 22 × 26 cm high with sani-chip bedding covering the plastic bottom and a perforated blue plastic top to prevent escape while allowing for ventilation. It was located in a separate room away from the dedicated CPP conditioning room.

Baseline Preference

On the first day of the procedure (see Figure 1A for timeline), rats were placed individually into their assigned CPP apparatus where they had free access to both compartments for a 10-min habituation period. This procedure was repeated across the next 2 consecutive days with the starting compartment counterbalanced across days and the time spent in each compartment recorded to assess initial baseline preference. Time in a particular compartment was determined by the software based on the location of the rat's forepaws. Time spent in each compartment was averaged across the two baseline tests to determine each rat's initial side preference. Rats that failed to demonstrate at least five compartment crossovers during either baseline day were excluded from the experiments due to inadequate environmental exploration;

however, they were assigned as a physical play partner for experimental rats when initial preferences and body weights did not allow for pairing experimental rats together.

Conditioning and Testing

For Experiment 1, conditioning took place over 2 consecutive days on PNDs 38-39 and for Experiment 2, conditioning took place over 4 consecutive days on PNDs 36-39. During conditioning sessions rats were confined either to the initially non-preferred side of the apparatus for 10 min with their assigned unconditioned stimulus (US; i.e., partner rat and/or nicotine) or were confined in the initially preferred side of the apparatus with no US. The initially non-preferred side of the apparatus served as a conditioned stimulus (CS+) that was exclusively paired with the US and the initially preferred side of the apparatus served as a CS- that was never paired with the US; these session types alternated. For CS^{+}-US pairings, rats received either saline (Sal) or nicotine (Nic; 0.1 mg/kg/mL, s.c.) and were immediately confined to their initially non-preferred compartment of the chamber either while socially isolated (Iso) or with a social partner (Soc) resulting in 4 groups: 1) Nic+Soc; 2) Nic+Iso; 3) Sal+Soc and 4) Sal+Iso. All groups received saline and were immediately confined to their initially preferred side alone during their CS- session. Rats also received 10-min sessions during which they were placed into an alternate environment. Rats received exposure to reward(s) that they had not received during conditioning, so that all groups received identical amounts of nicotine and social reward exposure and only the timing and location varied (see Fig 1B).

For Experiment 1 (n=40), each conditioning session type (i.e., CS+, CS- and ALT sessions) occurred on the same day, repeated across 2 consecutive days. The order of the session type was counterbalanced across animals and 6 h intervened between the CS+ and CS- sessions. The ALT sessions occurred at least 2h after the last CS conditioning session. For Experiment 2

(n=80), the CS+ and CS- sessions occurred on separate days in an 'ABBA' fashion (see timeline in Figure 1A). The CS+ sessions occurred on the first and fourth day of conditioning to reduce the nicotine (Nic+Iso) and social (Sal+Soc) parameters to sub-threshold for producing reward relative to nicotine plus a social partner (Nic+Soc) in our new apparatus. The CS- and ALT sessions occurred on the second and third day of conditioning. Thus, each rat received only 2 exposures to each of the environments. This timing also allowed the brains to be harvested after the last CS+ session on the last day. Socially-conditioned rats were assigned to pairs that were matched for initial compartment preference and body weight within 10 g. All rat partners were unfamiliar with each other prior to conditioning, but remained constant throughout conditioning.

After the last conditioning session, all rats were returned to their home cages in the colony. Experiment 1 was designed to examine Fos protein changes resulting from *expression of CPP*. Rats in this experiment were given a 10-min place preference test the following day and then were once again returned to their home cages. They were sacrificed 90-min after their CPP expression *test* as described below. The 90-min time-point was chosen because it is when Fos protein expression peaks following c-Fos induction (Nestler, 2001). Experiment 2 was designed to examine Fos protein changes resulting from *US exposure.* We used sub-threshold nicotine (Nic+Iso) and social (Sal+Soc) reward parameters in order to observe the unique pattern of Fos expression resulting from their synergistic combination (i.e., Nic+Soc; Thiel et al 2009). Rats in this experiment were placed back into their home cages and were either sacrificed 90-min after the end of the last CS+ session (n=40) or remained in their home cages until the following day when they were given a 10-min place preference test (n=40) to verify establishment of CPP.

Tissue Preparation

Rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.). Approximately 200 mL of ice-cold 0.1 M phosphate-buffered saline (PBS, pH 7.4) was perfused through the circulatory system transcardially followed by 250 mL of ice-cold 4% paraformaldehyde in PBS. Brains were then removed and post-fixed in 4% paraformaldehyde for ~24 h and then transferred to 15% and 30% sucrose for ~24 h each. The brains were then sectioned using a microtome (Microm International, Walldorf, Germany) connected to a filtered water freezing stage (Physitemp, Clifton, NJ). Serial coronal 40 µm sections were collected, separated by 160 µm, centered at anatomical locations +1.6, -2.56, and -5.6 mm relative to bregma (Paxinos and Watson, 1998). The tissue sections were then placed in 0.02 M PBS cryoprotectant solution comprised of 30% sucrose, 10% polyvinyl pyrrolidone and 30% ethylene glycol and stored at 4° C.

Fos Protein Immunohistochemistry

Fos immunohistochemistry was carried out as previously described (Bastle et al. 2012). Briefly, free floating tissue sections were first washed in 0.1 M PB (7×10 min). The tissue was next incubated for 30 min in 1% H_2O_2 and then rinsed in 0.1 M PB (3×10 min) followed by incubation for 30 min in 0.1 M PB containing 3% normal goat serum (NGS) (Vector Laboratories, Burlingame, CA, USA). The tissue was then incubated for 48 h at 4°C with anti-Fos rabbit polyclonal antibody (sc-52, Santa Cruz Biotechnology, Santa Cruz, CA), diluted 1:2,000 in PBH solution containing 2% NGS, 0.1% bovine serum albumin (Sigma, St. Louis, MO, USA, #A9647) and 0.2% Triton X-100 (Sigma, St. Louis, MO, USA). Following incubation, tissue sections were washed in 0.01 M PB (3×10 min) and then incubated for 1 h in biotinylated goat anti-rabbit IgG antibody (Vector Laboratories), diluted 1:500 in PBH solution.

The tissue was then washed in 0.01 M PB (3×10 min) and then incubated for 1 h in avidin-biotinylated horseradish peroxidase complex (ABC Elite Kit; Vector Laboratories) diluted 1:1000 in PBH. The sections were again washed in 0.1 M PBS (9×10 min) and incubated for 20 min in 0.1 M PB containing 0.02% 3,3'-diaminobenzidine (DAB; Sigma) containing 2% nickel ammonium sulfate, 20% D-glucose, and 0.4% ammonium chloride. Fos immunolabeling was visualized with glucose oxidase (1 □L/mL) for 10 min and then the DAB reaction was terminated by rinsing the tissue in 0.01 M PB (6×10 min). An orbital shaker set at 90 rpm was used for all of the washes and incubations described above. Stained tissue sections were immediately mounted onto gelatin-coated slides, air-dried, and dehydrated before cover slipping.

Fos Immunolabeling Analysis

Figure 2A illustrates the brain regions analyzed. Sections taken at +1.6 mm contained the Cg1 and Cg2 regions of the anterior cingulated cortex, the dorsal lateral (dlCPu) and dorsal medial caudate putamen (dmCPu), nucleus accumbens core (NAcC) and shell (NAcSh); sections taken at −2.56 mm contained the medial amygdala (MeA), central amygdala (CeA), and basolateral amygdala (BlA); and sections taken at −5.6 mm contained the ventral tegmental area (VTA). Fos immunolabeling was examined using a Nikon Eclipse E600 (Nikon Instruments, Melville, NY) microscope set at 20× magnification and counted by an observer blind to treatment conditions using the ImageTool software package (Version 3.0, University of Texas Health Science Center, San Antonio, TX). A range of 4-10 bilateral sample areas were counted per region of interest for each subject (i.e., 1 sample area/2 hemispheres/5 sections maximum), depending on tissue quality and preservation. Fos immunolabeling was identified by a brown-black oval-shaped nucleus distinguishable from background (see Fig. 2B) and quantified using Image J software (U.S. National Institutes of Health, Bethesda, MA). Counts were averaged per

subject for each region to provide a mean number of immunolabeled nuclei per sample area (0.26 mm^2).

Data Analysis

CPP was operationally defined as a significant increase in time spent in the initially non-preferred side (i.e., US-paired side) on the post-conditioning test relative to the average of the pre-conditioning tests (i.e., baseline), with more than half of the total test time (i.e., >300 seconds) spent in the US-paired side. For each experiment, time spent in the initially non-preferred side of the CPP apparatus was analyzed using a mixed-factor ANOVA with Day (baseline vs. test day) as a within-subjects factor and Drug (saline vs. nicotine), and Social Condition (isolation vs. social partner) as between-subjects factors. Significant interactions were further analyzed using smaller ANOVAs and tests of simple effects. Additionally, *a priori* Bonferroni planned comparison analyses using independent samples t-tests were used to examine hypotheses regarding preference for nicotine and/or a social partner (i.e., Nic+Soc, Nic+Iso, and Sal+Soc) compared to negative controls (Sal+Iso). Fos-positive nuclei were analyzed using two-way ANOVAs with Drug (saline vs. nicotine) and Social Condition (isolation vs. social partner) as between-subjects factors. Significant interactions were further analyzed using post-hoc Newman-Keuls tests. Some tissue samples were not able to be analyzed in the amygdala and VTA in both experiments due to tissue quality or staining artifacts, resulting in variation in n/group across regions. For Experiment 1, the Nic+Soc group had 1 subject excluded for the MeA, CeA, and BlA, and 2 subjects excluded for the VTA. The Nic+Iso group had 3 subjects excluded from the VTA and the Sal+Iso group had 1 subject excluded from the VTA. For Experiment 2, the Sal+Soc group had 1 subject excluded from the BlA and the Nic+Iso group had 2 subjects excluded from the VTA.

Results

Conditioned Place Preference

The CPP results for both experiments are shown in Fig. 3. For Experiment 1, the ANOVA of time spent in the initially non-preferred side revealed a main effect of Day ($F(1,36) = 90.78$, $p < 0.001$), a main effect of Drug ($F(1,36) = 9.10$, $p < 0.01$) and a Day × Drug interaction ($F(1,36) = 8.81$, $p < 0.01$). Subsequent simple effects tests revealed that the Day× Drug interaction was due to an increased amount of time spent in CS+ side on test day in nicotine-conditioned groups relative to their saline-conditioned counterparts, $t(38) = 3.17$, $p < 0.01$. *A priori* planned comparisons revealed that the Sal+Soc, Nic+Iso and Nic+Soc groups all spent significantly more time in the CS+ side on test day relative to the Sal+Iso negative control group [Sal+Iso vs. Sal+Soc, $t(18) = 3.79$, $p < 0.01$; Sal+Iso vs. Nic+Iso $t(17) = 5.34$, $p < 0.001$; Sal+Iso vs. Nic+Soc, $t(18) = 3.79$, $p < 0.01$]. For Experiment 2, the ANOVA of time spent in the CS+ side revealed a main effect of Day ($F(1,36) = 35.32$, $p < 0.001$), a main effect of Social Condition ($F(1,36) = 6.38$, $p < 0.05$) and a marginally significant Day × Social Condition interaction ($F(1,36) = 90.78$, $p = 0.052$). The *a priori* planned comparisons revealed that only the Nic+Soc group spent significantly more time in the CS+ side on test day relative to the Sal+Iso group [Sal+Iso vs. Nic+Soc, $t(18) = 3.64$, $p < 0.01$]. The lack of a difference between the Sal+Iso group and Sal+Soc and Nic-Iso groups verifies that nicotine alone and social pairings alone were sub-threshold parameters that failed to produce CPP.

Fos Protein Immunolabeling

The means of Fos-labeled cells for each group in each region for both experiments are shown in Table 1. For rats that were sacrificed following the CPP expression test in Experiment 1, ANOVAs of Fos protein expression revealed a main effect of Social Condition ($F(3,36) =$

4.15, $p < 0.05$) in the NAcC following the CPP expression test, where rats that were social-conditioned exhibited less Fos protein expression relative to groups that were isolate-conditioned, regardless of drug exposure (Table 1). A non-significant trend toward a main effect of nicotine was observed in the Cg1 ($F(3,36) = 3.64$, $p = 0.06$, where nicotine appeared to increase Fos expression relative to saline, regardless of social condition. No effects were observed in the Cg2, dmCPu, dlCPu, NAcSh, MeA, CeA, BlA, or VTA.

For rats that were sacrificed following the last US conditioning session in Experiment 2, both social condition and nicotine influenced Fos protein expression (see Figures 4-6 and Table 1). ANOVAs of Fos expression revealed a significant main effect of Social Condition in the dlCPu ($F(3,36) = 19.53$, $p < 0.001$), MeA ($F(3,36) = 4.71$, $p < 0.05$) CeA ($F(3,36) = 4.51$, $p < 0.05$), NAcC ($F(3,36) = 7.70$, $p < 0.01$), and NAcSh ($F(3,36) = 20.03$, $p < 0.001$), where social conditioning increased Fos expression following the last US exposure, relative to isolate-conditioning, regardless of drug exposure. Only a non-significant trend towards a main effect of Social Condition was observed in the Cg1 ($F(3,36) = 3.78$, $p = 0.06$). There was also a main effect of Drug in the Cg1 ($F(3,36) = 13.69$, $p < 0.001$), Cg2 ($F(3,36) = 9.56$, $p < 0.01$), dlCPu ($F(3,36) = 4.88$, $p < 0.05$), and the NAcC ($F(3,36) = 5.81$, $p < 0.05$), where nicotine-conditioned groups exhibited decreased Fos expression relative to saline-conditioned groups, regardless of social condition.

Significant Drug × Social Condition interactions were observed in rats from Experiment 2 (see Figure 6) in the BlA ($F(3,36) = 10.22$, $p < 0.01$) and VTA ($F(3, 34)= 9.65$, $p < 0.01$). In the BlA, subsequent post-hoc tests revealed decreased Fos expression in the Nic+Iso and Sal+Soc rats relative to the Sal+Iso rats (Newman-Keuls tests, $p < 0.05$). Additionally, the Nic+Iso rats exhibited less Fos expression in the BlA than the Nic+Soc rats (Newman-Keuls

tests, $p < 0.01$). Similarly in the VTA, post-hoc tests revealed decreased Fos protein expression in the Sal+Soc and Nic+Iso rats relative to Sal+Iso rats (Newman-Keuls tests, $p < 0.01$) and also to Nic+Soc rats (Newman-Keuls tests, $p < 0.05$)

Discussion

The findings from the present study demonstrated that only 2 exposures to nicotine, a social partner or their combination produced robust CPP in adolescent male rats when US conditioning sessions occurred in close temporal proximity (i.e., over 2 consecutive days); however, only the combination of nicotine paired with a social partner elicited robust CPP when the time between US conditioning sessions was extended (i.e., 2 days intervening). The robust conditioning observed in Experiment 1 was surprising because we previously found that the conditioning parameters used here were sub-threshold for establishing CPP with nicotine or a social partner alone (Thiel, et al., 2009); however, one key difference across studies was the conditioning apparatus used. The present study used conditioning chambers adapted for use with smaller rodents that differed in olfactory, tactile and visual cues from the chambers used in our previous study, and these changes likely altered the sensitivity for establishing CPP. CPP procedures are susceptible to ceiling effects, where CPP expression may appear equal even when the reward strength of the US varies as shown by using different conditioning parameters (Bardo & Bevins, 2000; Bevins, 2005; Cunningham, Ferree, & Howard, 2003; Peartree, Hood, et al., 2012; Thiel, et al., 2009). The extended time between US conditioning sessions in Experiment 2 compared to Experiment 1 likely produced weaker conditioning, such that we once again obtained sub-threshold parameters for expression of nicotine and social reward.

Our neurochemical findings revealed that Fos protein expression patterns varied considerably when animals were expressing CPP (Experiment 1) versus experiencing the US

(Experiment 2). Contrary to our predictions, the only effect observed following CPP expression testing was in the NAcC where rats that were socially-conditioned exhibited less Fos relative to their isolated counterparts, regardless of whether they received nicotine or saline. These findings were surprising since both nicotine and social rewards produced CPP when given alone and previous research has shown an increase in Fos upon exposure to environmental cues associated with rewarding and reinforcing stimuli (Neisewander et al., 2000; Pascual, Pastor, & Bernabeu, 2009; Schroeder, Binzak, & Kelley, 2001). The lack of increased Fos in the present study may have been due to a 'cancellation effect' since rats were exposed to both the CS+ and CS- environments during the CPP test. In any case, the decrease in Fos in the NAcC may have been due to violation of reward expectation. Previous studies have found that the reward circuitry, particularly the NAc, is heavily involved in processing incentive stimuli (Berridge & Robinson, 1998; Ikemoto & Panksepp, 1999), incentive learning (Berridge, 2007; Di Chiara, 2002), and reward prediction errors (i.e., expectation of reward is violated) (Schultz, 2006; Tobler, O'Doherty J, Dolan, & Schultz, 2006) including prediction errors associated with social reward (Behrens, Hunt, Woolrich, & Rushworth, 2008; Fehr & Camerer, 2007; Jones et al., 2011; Poore et al., 2012). Since exposure to conspecifics is a highly salient reward in adolescent rats, being alone in the previously social-paired side of the chamber on test day may have resulted in prediction-error effects leading to a decrease in Fos expression in the NAcC.

In contrast to the limited effects of environmental cues on Fos expression during the CPP test, Fos expression in response to the last US exposure in Experiment 2 was altered in all of the regions analyzed, with the exception of the dorsomedial caudate-putamen (dmCPu). Three distinct patterns emerged, where 1) social-conditioned rats exhibited *elevated* Fos in the dorsolateral CPu (dlCPu), the medial and central amygdala (MeA, CeA), and the nucleus

accumbens core and shell (NAcC and NAcSh) relative to isolated rats (Fig 4), 2) nicotine-conditioned rats exhibited *less* Fos in the anterior cingulate of the medial prefrontal cortex (Cg1, Cg2), dlCPu, and the NAcC relative to saline-conditioned rats (Fig 5), and 3) nicotine-conditioned and social-conditioned rats exhibited less Fos in the ventral tegmental area (VTA) and basolateral amygdala (BLA) than both saline-conditioned and nicotine + social-conditioned rats (Fig 6). Contrary to our hypothesis, these neurochemical interactions suggest that the synergistic interaction of nicotine and social rewards does not necessarily involve stronger activation of a common part of the corticolimbic circuitry.

The elevated Fos expression after social exposure in corticolimbic regions is consistent with previous reports demonstrating that these regions are involved in processing social information. For example, c-fos mRNA is increased in the dorsal and ventral striatum and the lateral amygdala after brief (i.e., 15 and 30-min) social exposure in juvenile rats (Gordon, Kollack-Walker, Akil, & Panksepp, 2002; van Kerkhof et al., 2014). In adolescent rats, a 60-minute social exposure induced Fos protein expression in the basolateral and central amygdala, but this effect was not present in adult rats, suggesting that changes in the amygdala may be age-dependent (Varlinskaya, Vogt, & Spear, 2013). The amygdala and striatum are likely involved in social play for non-human primates (Lewis & Barton, 2006). Similarly, the amygdala appears to be necessary for normal prosocial behavior in rodents (Daenen, Wolterink, Gerrits, & Van Ree, 2002a, 2002b; Panksepp, et al., 1984; Wolterink et al., 2001) and exhibits changes in *c-fos* expression after play behavior (Cheng, Taravosh-Lahn, & Delville, 2008).

Contrary to our predictions, we observed *less* Fos expression in the Cg1, Cg2, dlCPu, and NAcC in nicotine-conditioned rats relative to saline-conditioned rats in Experiment 2. These results are inconsistent with previous findings that have shown acute nicotine administration

increases Fos protein and mRNA expression in the cingulate cortex (Mathieu-Kia, Pages, & Besson, 1998; Salminen, Seppa, Gaddnas, & Ahtee, 1999; Schochet, Kelley, & Landry, 2005; Seppa, Salminen, Moed, & Ahtee, 2001), dorsal striatum (Salminen, et al., 1999; Schochet, et al., 2005; Seppa, et al., 2001), and ventral striatum, particularly NAcC (Mathieu-Kia, et al., 1998; Pascual, et al., 2009; Salminen, et al., 1999; Schilstrom, De Villiers, Malmerfelt, Svensson, & Nomikos, 2000; Schochet, et al., 2005; Seppa, et al., 2001). However, one study reported decreased *c-fos* and Erg1 in the frontal cortex, basolateral amygdala and the hippocampus of the mouse brain in response to a high dose of nicotine (1.0 mg/kg, i.p.) (Bachtell & Ryabinin, 2001). The reason for these discrepancies is unclear but may be due to the dose of nicotine used and/or age at the time of exposure. All but one of these studies utilized adult rats (Schochet, et al., 2005) and all of the reported studies administered a higher nicotine dose that was at least double (i.e., 0.21-0.5 mg/kg) the nicotine dose used in the present study, suggesting that Fos protein and mRNA may be sensitive to age and dose effects.

The *c-fos* gene is transiently expressed as part of intracellular signaling in response to a variety of stimuli and its induction diminishes with repeated exposure to a given stimulus (Nestler, 2001). Since we administered nicotine twice in the present study, it is possible that the repeated exposure diminished nicotine-induced *c-fos* induction. However, this explanation seems unlikely because the ability of pharmacological stimuli to induce c-fos after repeated administration usually recovers within a few days, and therefore we spaced the 2 exposures in this experiment 72 hours apart. Another possibility is that nicotinic acetylcholine receptors (NAChRs) underwent rapid desensitization after the second nicotine exposure causing less activation of intracellular signaling, resulting in low levels of Fos expression compared to saline controls (Giniatullin, Nistri, & Yakel, 2005; Picciotto, Addy, Mineur, & Brunzell, 2008). This

too seems unlikely however because we used a low dose of nicotine. Finally, social interaction can reduce expression of Fos as well as other immediate early genes induced by drugs of abuse. For example, when social conditioning competes with an already established preference for cocaine, then decreases in FosB, zif268, and ERG1 have been found in several regions including the accumbens, amygdala and VTA (El Rawas et al., 2012; Fritz, et al., 2011; Zernig, Kummer, & Prast, 2013). This is not likely the reason for the decrease in Fos by nicotine however since decreases were also observed in rats not exposed to a social partner. Despite the unexpected decreased in Fos expression after nicotine administration, these data appear to be orderly and the changes observed were region-specific rather than nonspecific across all brain regions; therefore, it is unlikely the changes observed are spurious.

Interestingly, the patterns of Fos activation in Experiment 2 were similar in the BlA and VTA, where both nicotine- and social-conditioned rats exhibited less Fos relative to control (i.e., Sal-Iso) rats, and surprisingly rats conditioned with both nicotine and social rewards exhibited similar levels of Fos compared to control rats. Social isolation is a robust stressor, which is known to activate the HPA-axis (Blanchard, McKittrick, & Blanchard, 2001; Serra, Pisu, Mostallino, Sanna, & Biggio, 2008) and the BlA (Hsu et al., 2013; Karst, Berger, Erdmann, Schutz, & Joels, 2010; Rainnie et al., 2004). Furthermore, the BlA has reciprocal connections to the VTA (see Oades & Halliday, 1987 for review). Thus, the Fos expression in these two regions in controls may be indicative of isolation-induced stress reactivity rather than serving as a neutral baseline for comparison as intended. Moreover, controls underwent the same procedure at the same time as rats that received a social and/or nicotine US. Therefore, hearing rats playing in adjacent chambers may have been stressful for the controls. Indeed adolescent rats are prosocial (Panksepp, 1981; P. K. Smith, 1982; Spear, 2000) and highly motivated to

seek-out and approach conspecifics (Humphreys & Einon, 1981; Thiel, et al., 2008; Thiel, et al., 2009; Trezza, Damsteegt, et al., 2009). In fact, social motivation increases the more socially-deprived a rat becomes (Douglas, et al., 2004; Van den Berg, et al., 1999); therefore, the controls were likely in a state of high social motivation during CS+ conditioning sessions. This may have produced frustration stress due to the inability to interact with a partner, resulting in increased Fos in the BlA and VTA. On the other hand, rats conditioned with both social and nicotine rewards were likely having a more intense rewarding experience relative to the rats conditioned with only one of these rewards, resulting in elevated Fos levels in the Nic+Soc group relative to Sal+Soc and Nic+Iso groups.

In conclusion, the results from Experiment 1 suggest that the nucleus accumbens core may be particularly sensitive to processes involved in incentive motivational effects of exposure to environmental stimuli previously associated with social rewards in adolescent male rats. Experiment 2 replicated our previous findings of a synergistic interaction between nicotine and social rewards in adolescent male rats (Thiel, et al., 2009). The patterns of Fos expression observed in Experiment 2 contrasted markedly with our prediction that the combination of social and nicotine rewards would produce more robust Fos expression or Fos expression in additional regions relative to either reward given alone. While we did find that social conditioning alone increased Fos expression in amygdala and striatal regions the degree of Fos expression was the same regardless of whether or not nicotine was also given. Surprisingly we found that the anterior cingulate cortex, dlCPu, and NAcC displayed less Fos expression in rats that underwent nicotine conditioning regardless of whether or not they were conditioned with a social partner present. These patterns suggest that social and nicotine exposure uniquely alter intracellular signaling within cortical and limbic regions. Interestingly, we found elevated Fos expression in

our nicotine social groups similar to that of our control group in the BlA and VTA, suggesting potential isolation stress effects in these regions in controls. Taken together, these data may be useful for examining neural functioning for both social and nicotine exposure in other rodent models of reward, reinforcement, stress and anxiety, since mesocorticolimbic structures are heavily involved in processing social and drug stimuli. The findings from the present study highlight the significance for understanding the influence of social context on nicotine effect.

CHAPTER 3

LIMITED PHYSICAL CONTACT THROUGH A MESH BARRIER IS SUFFICIENT FOR SOCIAL REWARD-CONDITIONED PLACE PREFERENCE IN ADOLESCENT MALE RATS

Social interaction is a hallmark feature of normal development during adolescence that enables appropriate social behavior in adulthood (Einon, et al., 1978; Panksepp, 1981; Spear, 2000). Play behaviors in particular are thought to be important for the transition into normal sexual behaviors (M.J. Meaney, Stewart, & Beatty, 1985; Moore, 1985) and the establishment of dominance hierarchies among adult rodents (Pellis & Pellis, 1991). Social interaction is a substantial natural reward for rodents. For instance, rats will learn to traverse a T-maze to gain access to another rat (Humphreys & Einon, 1981; Normansell & Panksepp, 1990; Werner & Anderson, 1976). In addition, conditioned place preference (CPP) studies reveal that both adolescent and adult rats will display robust approach towards, and spend more time in, an environment paired with access to another rat (Calcagnetti & Schechter, 1992; Douglas, et al., 2004; Thiel, et al., 2008; Van den Berg, et al., 1999) and a single re-exposure to a social partner in the associated environment will reinstate an extinguished preference for that environment (Trezza, Damsteegt, et al., 2009). We have observed synergistic interactions between social and drug rewards using the CPP paradigm (Thiel, et al., 2008; Thiel, et al., 2009) and such interactions may be involved in the vulnerability of adolescents to initiate drug use during this developmental period (Chassin, Presson, & Sherman, 1984; Leslie et al., 2004; O'Dell et al., 2006; Sussman, 2005).

Specific aspects underlying the rewarding effects of social encounters in rodents remain unclear. It has been suggested that the primary rewarding feature of a social context is the ability to engage in rough-and-tumble play behavior (i.e. play fighting) (Douglas, et al., 2004; Panksepp & Beatty, 1980; Panksepp, et al., 1984). For instance, social reinforcement is reduced when full physical contact is restricted or when the play drive of a social partner is pharmacologically inhibited with amphetamine, chlorpromazine, scopolamine or methylphenidate (Calcagnetti & Schechter, 1992; Humphreys & Einon, 1981; Trezza, Damsteegt, et al., 2009). In addition, rats will display conditioned place preference (CPP) for an environment associated with a playful rat partner over one associated with a scopolamine-induced non-playful rat, suggesting that relative reward strength of social encounters are graded in nature (Calcagnetti & Schechter, 1992). Even though scopolamine disrupts play behavior, other social behaviors persist in the non-altered playmate despite the partner's lack of response, such as dorsal contacts, social sniffing and crawl-overs (Deak & Panksepp, 2006; Pellis & McKenna, 1995), but the degree to which these behaviors are rewarding is not known.

The necessity of play behavior for establishing social reward-CPP is unclear and under some circumstances play behavior is insufficient for establishing CPP. For example, adolescent social reward is observed in socially experienced (e.g., socially-housed) rats that receive play pairings with other socially experienced rats, but not when the play pairings occur with a previously isolated partner (Douglas, et al., 2004). Socially experienced rats engage in play behaviors with both types of partners, but will avoid the socially deprived partner if given the opportunity (Varlinskaya, et al., 1999). Furthermore, we have shown that there is no relationship between the magnitude of social

reward-CPP and the amount of play behavior that occurs during conditioning (Thiel, et al., 2008). Also, under conditions in which nicotine or cocaine reduce play behavior, these drugs also enhance social reward-CPP (Thiel, et al., 2008; Thiel, et al., 2009).

This study directly tested the hypothesis that play behavior in adolescent rats is not necessary for social reward. We used a modified CPP apparatus that allowed for a rat to be placed behind a mesh screen (see Figure 7), which created opportunities for social encounters with limited physical contact but without rough-and-tumble play behavior. We included controls to examine physical and restricted contact to an inanimate play object (i.e., a tennis ball).

Methods

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). To avoid prolonged isolation and foster healthy play development, rats were pair-housed upon arrival until PND 26, at which point they were single-housed thereafter. Rats were housed in a climate-controlled facility with a 12-h light/dark cycle (lights on at 7 PM) with *ad libitum* access to food and water. Housing and care were conducted in accordance with the 1996 Guide for the Care and Use of Laboratory Rats (Clark, Gebhart, Gonder, Keeling, & Kohn, 1997). All experiments were conducted within a conservative estimate of rodent adolescence - PNDs 28-42 (Spear, 2000) - given that social reward peaks during this developmental period (Douglas, et al., 2004). Prior to baseline testing, animals were acclimated to handling for 5-12 days (see Figure 8 for specific timeline of each experiment). On each of these days, rats were handled for at least 2 min/day. Once the

rats were single-housed they remained isolated except when paired together during conditioning.

Apparatus

Conditioning took place in rectangular Plexiglas chambers (Figure 7). Each chamber contained a removable partition that separated the chamber into two equal-sized compartments, each measuring 35 × 24 × 31 cm high. One compartment had corn cob bedding beneath a wire 1 × 1 cm grid floor and alternating black and white vertical stripes on the walls. The other compartment had pine-scented bedding beneath a parallel bar floor (5 mm diameter) and alternating black and white horizontal stripes on the walls. The stripes on the walls of both compartments were 2 cm wide. Additional end compartments were created by inserting a divider that split the original compartment into a main compartment (27 × 24 × 31 cm high) and a small end compartment (8 × 24 × 31 cm high), such that during conditioning a conspecific or a ball could be placed in the end compartment as an unconditioned stimulus (US) (see Figure 7). The divider was made of clear Plexiglas except for 1 × 1 cm wire grid mesh (16.5 × 8 cm) on the bottom portion of the dividing wall. On the pre- and post-conditioning test days, the removable center partition of the apparatus was replaced by a similar partition that contained an opening in the center (28 × 6 cm), allowing the rats free-access to the bordering compartments simultaneously. To prevent the rats from escaping from the chamber while maintaining the ability to record their behavior via an overhanging video camera, a rectangular tower measuring 70 × 24 × 74 cm high of clear Plexiglas was used as an extension of the apparatus. Unpublished data from our laboratory established that the main compartments on either side of the center were equally preferred across adolescent and adult rats (i.e.,

the apparatus was unbiased). The conditioning room was dimly lit with two overhead lamps, each containing a 25 watt light bulb.

A camera (Panasonic WV-CP284, color CCTV, Suzhou, China) used to record testing sessions was mounted 101 cm above the center of the apparatus. A WinTV 350 personal video recorder (Hauppage, NJ, USA) captured live video and encoded it to MPEG streams. A modified version of TopScan Software (Clever Sys., Inc. Reston, VA, USA) used the orientation of an animal's body parts (e.g. nose, head, center of body, forepaws, base of tail, etc.) to identify behaviors that are specified by the user and recognized by the program. The software employed the whole position of the body to estimate other body parts (e.g. nose, forepaw, head etc.) when they were not in view in order to yield measures of time spent in each compartment.

Baseline Preference

On the first day of the procedure for both experiments, rats were transported to the conditioning room and were placed into the CPP apparatus where they had free access to both main compartments for 10 min to habituate them to the novel environment. The mesh dividers restricting access to the small end compartments were in place throughout the entire experiment. Initial baseline preference was assessed across the next 2 consecutive days by again allowing each rat free-access to the main compartments for 10 min each day. The starting compartment was counterbalanced across days and entry into a compartment was operationally defined as a rat's forepaws entering a compartment as determined by the software. Time spent in each compartment was averaged across the two baseline days to determine each rat's initial side preference. Rats that failed to demonstrate at least five compartment crossovers during either baseline test day were

excluded from the experiments due to inadequate environmental exploration; however, they were assigned as a physical play partner for experimental rats when initial preferences and body weights did not allow for pairing experimental rats together.

Conditioning and Testing

Rats were assigned to one of four groups (n =9-10/group) that received the following US exposure upon placement into their initially non-preferred side (i.e., conditioned stimulus, CS): 1) physical access to another rat in the same compartment with nothing behind the mesh (Rat/Phys); 2) restricted access to another rat behind the mesh divider (Rat/Mesh); 3) physical access to a tennis ball in the same compartment with nothing behind the mesh (Ball/Phys); or 4) restricted access to a tennis ball behind the mesh divider (Ball/Mesh). During separate sessions, all rats were placed alone in their initially preferred side without anything behind the mesh. Rats in the 'Ball' conditions (i.e., Ball/Phys and Ball/Mesh) were exposed to their own brand new tennis ball and that ball remained constant throughout conditioning to control for relative novelty. Rats in the Rat/Phys group were assigned to pairs that were matched for initial compartment preference and body weight within 10 g. All rat partners were unfamiliar with each other prior to conditioning, but remained constant throughout conditioning.

Conditioning sessions were conducted twice per day at the same time of day with each rat confined to one side of the CPP apparatus for 10 min during the morning session, and confined to the opposite side of the apparatus for 10 min during the afternoon session. At least 6 h intervened between morning and afternoon sessions. Previous research from our laboratory demonstrated that social reward-CPP is established regardless of whether a biased or unbiased design is used (Thiel, et al., 2008). Therefore,

we chose a biased CPP design [i.e., pairing the US with the initially non-preferred side of the apparatus (CS)] because this design allows for a greater range of preference change as well as observation of a preference *switch* (i.e., >50% time spent in initially non-preferred side on test day) indicative of a reward effect rather than a reduction of initial aversion to the CS. The starting side for the first conditioning session was counterbalanced such that half of the rats in each group were exposed first to their initially non-preferred side containing their respective US, and the other half were exposed to their initially preferred side with no stimulus (i.e., alone). Rats then received the opposite of these conditions during the afternoon session. Two separate experiments were conducted, with Experiment 1 employing two CS-US pairings and Experiment 2 employing eight CS-US pairings, both followed by a final test for CPP. The specific timeline for each experiment is summarized in Figure 8.

Crossovers during baseline and preference tests were counted from previously recorded video files by an observer blind to experimental conditions. As mentioned previously, a crossover was defined as entry of a rat's forepaws into one of the two compartments. During the first and last US-paired conditioning sessions, frequency and duration of contact with a partner rat or a tennis ball were scored for rats in the Rat/Phys and Ball/Phys groups and contacts with the mesh screen were scored for rats in the Rat/Mesh and Ball/Mesh groups using Observer 5.0 software (Noldus Information Technology BV, Wageningen, The Netherlands). This program allows for a frame by frame analysis of behavior. Contact was operationally defined as any part of the body with the exception of the tail touching either the object (i.e., ball or rat) or the mesh screen. Since contacts between rat partners were not independent, contact data from the

Rat/Phys group was scored and analyzed per pair. Thus for Experiment 1, the 'Phys' object contact behavioral analyses included n=6 pairs for the Rat/Phys group and n=10 for the Ball/Phys group. For Experiment 2, two rats from the Rat/Phys group and two rats from the Ball/Phys group were removed from the object contact behavioral analysis due to loss of video footage of either the first or last day resulting in n=4 pairs for the Rat/Phys group and n=7 for Ball/Phys group.

Data Analysis

CPP was operationally defined as a significant increase in time spent in the initially non-preferred side (i.e., US-paired side) on the post-conditioning test relative to the average of the pre-conditioning tests (i.e., baseline). Time spent in the initially non-preferred side from both experiments was analyzed using a mixed factor ANOVA with Day (baseline vs. test day) as a repeated measures factor and Object (ball vs. rat), Contact (mesh vs. physical) and number of pairings (2 vs. 8) as between subjects factors. In addition, we transformed the data to difference scores of time in the initially non-preferred side on the test day minus the baseline and analyzed the difference scores using ANOVA with Object, Contact and Number of pairings as between subjects factors. Significant interactions were further analyzed using smaller ANOVAs, tests of simple effects and/or paired-sample t-tests with a Bonferroni correction where appropriate (Keppel, 1991).

Crossovers were analyzed using mixed factors ANOVAs with Day (baseline vs. test day) as a repeated measures factor and Object and Contact as between subjects factors. The number of physical contacts (i.e., Rat/Phys contacts with partner rat and Ball/Phys contacts with tennis ball), duration of contacts and duration per contact were all

analyzed for each experiment using separate mixed factors ANOVAs with Day (first conditioning day vs. last conditioning day) as a repeated measures factors and Physical Object as a between subjects factor. The number of mesh screen contacts, duration of contact with the mesh screen, and the duration per contact were all analyzed using mixed factors ANOVAs with Day (first conditioning day vs. last conditioning day) as a repeated measures factor and Object behind the mesh as a between subjects factor. Significant interactions were further analyzed using tests of simple effects.

Results

Conditioned Place Preference

The CPP results of both experiments are shown in Figure 9. The ANOVA of time spent in the initially non-preferred side revealed main effects of Day ($F(1,70)=89.44$, $p<0.001$), Object ($F(1,70)=9.56$, $p<0.01$) and Number of pairings ($F(1,70)=8.51$, $p<0.01$) as well as Day × Object ($F(1,70)=8.68$, $p<0.01$), Day × Number of pairings ($F(1,70)=8.35$, $p<0.01$), Contact × Object × Number of pairings ($F(1,70)=3.79$, $p=0.05$), and Day × Object × Contact × Number of pairings interactions ($F(1,70)=3.85$, $p=0.05$). We analyzed the source of the 4-way interaction by conducting separate ANOVAs of the CPP data from each experiment. For Experiment 1 involving 2 CS-US pairings, the ANOVA of time spent in the initially non-preferred side revealed main effects of both Day ($F(1,36)=27.83$, $p<0.001$) and Object ($F(1,36)=5.29$, $p<0.05$) as well as an Object × Day interaction ($F(1,36)=5.60$, $p<0.05$). The significant Object × Day interaction was further analyzed using tests of simple effects of Object with the data collapsed across Contact conditions. These tests revealed that although there was no significant difference between baseline measures, rats spent significantly more time in the initially non-

preferred side on test day when rat was the US object relative to when ball was the object, $t(38)=2.38$, $p<0.05$. These findings suggest that only a rat and not a ball shifted preference when 2 CS-US pairings were given during conditioning. In addition to the ANOVA, we conducted Bonferroni *t*-tests comparing baseline to test for each group setting alpha at $p<0.0125$ for significance. These t-tests revealed that only the Rat/Phys group ($t(9)=7.68$, $p<0.001$) spent more time in the initially non-preferred side on test day relative to baseline, whereas there were no significant differences between test and baseline for any other group.

For Experiment 2 involving 8 CS-US pairings, the ANOVA of time spent in the initially non-preferred side revealed main effects of both Object ($F(1,34)=4.42$, $p<0.05$) and Day ($F(1,34)=61.07$, $p<0.001$), but no interactions. Thus, when the data were collapsed across the contact variable, rats conditioned with a social partner demonstrated greater preference shifts than rats conditioned with a ball. In addition, all rats in general demonstrated preference shifts toward their initially non-preferred compartment following eight days of conditioning. However, it is important to note that only the Ball/Phys, Rat/Mesh, and Rat/Phys groups exhibited a preference switch indicative of reward (i.e., >50% of the total test time in their initially non-preferred side during the post-conditioning test), whereas the Ball/Mesh control group still spent < 50% of the test time in their initially non-preferred side, which may reflect reduction of initial aversion rather than conditioned reward (see Figure 9B). The strong main effect of Day may have obscured the detection of potential group differences. Therefore, paired-sample *t*-tests with Bonferroni correction (i.e., alpha set at $p< 0.0125$ for significance) were conducted and revealed significant increases in the time spent in the initially non-preferred side on

the test day relative to baseline in the Rat/Mesh group ($t(9)=4.07$, $p<.01$), the Rat/Phys group ($t(9)=5.70$, $p<0.001$) and the Ball/Phys group ($t(8)=4.10$, $p<0.01$), but not the Ball/Mesh group.

An independent samples t-test revealed a significant difference between baseline values for Experiment 1 and 2, ($t(51.70)=5.7$, $p<0.001$), and therefore, we conducted additional analyses on differences scores calculated as time spent in the initially nonpreferred side during the test minus baseline (Figure 9C and D). The ANOVA of difference scores revealed significant main effects of Object ($F(1,70)=8.68$, $p<0.01$) and Number of pairings ($F(1,70)=8.35$, $p<0.01$), as well as an Object × Contact × Number of pairings interaction ($F(1,70)=3.85$, $p=0.05$). To further probe this interaction, separate ANOVAs were conducted for each experiment. For Experiment 1 involving 2 CS-US pairings, the ANOVA of difference scores revealed a significant main effect of Object ($F(1,36)=5.60$, $p<0.05$) where rats conditioned with another rat spent more time on the US-paired side compared to rats conditioned with a ball, regardless of type of contact. Furthermore, planned comparisons of difference scores between experiments of respective groups conditioned with 2 versus 8 pairings revealed a significant difference in the Ball/Phys group only ($t(17)=2.66$, $p<0.05$). Collectively, these findings indicate that the difference scores significantly increased after 8 pairings when rats received physical contact with a ball.

Crossovers on test day

Crossovers from one side of the chamber to the other on baseline and test days are shown in Table 2. The ANOVA of crossovers revealed a within subjects main effect of Day for both Experiment 1 ($F(1,34)=104.55$, $p<0.001$) and 2 ($F(1,36)=83.04$, $p<0.001$),

where all groups displayed significantly more crossovers on test day compared to baseline. Independent samples t-tests with Bonferroni correction (i.e., alpha set at $p < 0.025$ for significance) revealed significantly more baseline crossovers with 2 pairings compared to 8 ($t(72)=5.3$, $p<0.001$), but no difference in the number of crossovers on test day.

Behavior During Conditioning Sessions

For physical contact with a ball or a rat, number of contacts and time spent in contact with object are shown in Table 2 and time per contact is shown in Figure 10. The ANOVA for number of physical contacts revealed no significant effects for either experiment. However, time spent in contact yielded a significant main effect of Object in both Experiment 1 ($F(1,14)=703.46$, $p<0.001$) and 2 ($F(1,9)=551.63$, $p<0.001$), and a significant main effect of Day in Experiment 2 ($F(1,9)=8.02$, $p<0.05$) indicating that more time was spent in contact with the object when the object was a rat compared to a ball, and that regardless of object, contact time increased from day 1 to day 8. The ANOVA of time per contact for Experiment 1 revealed main effects of Day ($F(1,14)=7.06$, $p<0.05$) and Object ($F(1,14)=36.88$, $p<0.001$) indicating that time per contact increased from day one to day two and time per contact on both days was significantly higher when the physical object was a rat compared to a ball. The ANOVA of time per contact for Experiment 2 revealed significant main effects of Day ($F(1,9)=24.79$, $p<0.001$) and Object ($F(1,9)=104.79$, $p<0.001$) as well as a significant Day × Object interaction ($F(1,9)=14.78$, $p<0.01$). Tests of simple effects revealed that duration per contact with a rat increased from day 1 to day 8 ($t(3)=3.53$, $p<0.05$), but not

for a ball, and was significantly higher on both day 1 ($t(3.14)=6.50$, $p<0.01$) and day 8 ($t(3.16)=6.58$, $p<0.01$) compared time per contact with a ball.

For contact with the mesh screen in front of either a ball or a rat, number of contacts are shown in Figure 11 and time spent in contact and time per contact are shown in Table 2. For Experiment 1, the ANOVA of number of contacts with the mesh screen revealed a significant effect of Object ($F(1,18)=414.74$, $p<0.001$) indicating that regardless of day, rats contacted the mesh screen significantly more when a rat was the object behind the mesh compared to a ball. For Experiment 2, the ANOVA of number of mesh contacts revealed a significant main effect of Day ($F(1,17)=96.70$, $p<0.001$) and Object ($F(1,17)=31.62$, $p<0.001$) as well as an Object × Day interaction ($F(1,17)=7.80$, $p<0.05$). Tests of simple effects revealed that number of mesh contacts increased from day 1 to day 8 for both the rat ($t(9)=7.58$, $p<0.001$) and the ball behind the mesh screen ($t(8)=7.03$, $p<0.001$) and was significantly higher on both day 1 ($t(17)=5.73$, $p<0.001$) and day 8 ($t(17)=5.10$, $p<0.001$) when the object behind the mesh was a rat compared to a ball. The interaction indicates that the increase in number of mesh contacts from day 1 to day 8 was greater in the Rat/Mesh group relative to the Ball/Mesh group. The ANOVAs for time in contact with the mesh revealed a significant main effect of Object for both Experiment 1 ($F(1,18)=38.23$, $p<0.001$) and Experiment 2 ($F(1,17)=94.67$, $p<0.001$) indicating that rats spent more time contacting the mesh when the object behind it was another rat compared to a ball. A main effect of Day was also found in Experiment 2 ($F(1,17)=29.94$, $p<0.001$) indicating that the number of contacts increased on day 8 compared to day 1. The ANOVAs of time per contact yielded a significant effect of Day ($F(1,17)= 7.54$, $p<0.05$) for Experiment 2 indicating that time per contact with the mesh

screen decreases by day 8 compared to day 1. No significant effects were found for time per contact with mesh for Experiment 1.

Discussion

The results indicate that social reward-CPP can be obtained in adolescent male rats even when physical contact is limited and rough-and-tumble play is prevented. These findings provide conclusive evidence that rough-and-tumble play behavior is not necessary for a social encounter between adolescent male rats to be rewarding, and also provide evidence that full physical contact enhances the rewarding effects produced by a conspecific. We conclude that the unconditioned stimuli used in this study differ in reward magnitude with the following rank order from most to least rewarding: Rat/Phys > Rat/Mesh > Ball/Phys > Ball/Mesh.

Reward magnitude is in part inferred by the degree of preference shift; however, because there is often a ceiling effect where similar CPP is observed with stimuli that vary in reward magnitude (Bevins, 2005), the number of pairings needed to establish CPP is another measure indicative of reward magnitude. The more highly rewarding a US, the more rapidly CPP is established (Bardo, Rowlett, & Harris, 1995). In the present study, the physical presence of another rat was the only US strong enough to produce CPP after 2 CS-US pairings in Experiment 1, suggesting that it was the most rewarding US. There was also a significant Object x Day interaction in Experiment 1 in which the time spent in the US-paired side increased more relative to baseline when a rat was the US object than when a ball was the US object regardless of contact condition (physical vs. mesh), suggesting that in general the rat US is more rewarding than the ball US. After 8 CS-US pairings in Experiment 2, similar CPP was observed among the Rat/Phys, Rat/Mesh, and

Ball/Phys groups yet the Ball/Mesh group still failed to exhibit CPP. Collectively, these findings suggest that encountering another rat even if it is behind a mesh is more rewarding than the physical presence of a non-social play object, and that physical contact is needed to observe reward with the non-social play object and only after 8 pairings. This point is further bolstered by the Ball/Phys group displaying a significantly lower difference score with 2 pairings compared to 8. In contrast, the Rat/Phys group remained consistently high from 2 to 8 pairings, the Ball/Mesh group remained consistently low, and there was no significant change in the Rat/Mesh group's difference scores from 2 to 8 pairings likely because their scores were already somewhat elevated after 2 pairings. The difference between the Rat/Phys and Ball/Phys groups further suggests that social reward-CPP cannot be explained by the presence of another object within the conditioning environment.

Previous research has shown that isolated adolescent rats are highly sensitive to novel object-CPP (Douglas, Varlinskaya, & Spear, 2003), but because the ball was most novel initially when CPP was not observed in the Ball/Phys group, we do not think that the CPP observed in this group after eight pairings was a result of novelty. Furthermore, novelty-CPP is typically established with repeated access to different novel objects in one of two distinct environments or a choice between a familiar or novel environment (Bardo, Neisewander, & Pierce, 1989; Bevins & Bardo, 1999; Bevins et al., 2002; Wright & Conrad, 2005). We speculate when the US is an inanimate object, the rats may need to have full physical contact with it to find the experience rewarding in contrast to when the US is another rat.

Approach behaviors measured during the conditioning sessions further support differences in the reward value of a conspecific compared to an inanimate play object. In groups that had physical contact during conditioning, time per physical contact increased from day 1 to either day 2 or day 8, and after 8 sessions, the time per contact was greater for a rat than a ball (Figure 10). In the groups that did not have full physical contact with the play object, the most sensitive measure of approach behavior was the number of mesh contacts. With 2 CS-US pairings, there were more contacts with the mesh when rat was the object than when ball was the object regardless of day. With 8 CS-US pairings, again there were more mesh contacts when rat was the object and there were more mesh contacts on day 8 than on day 1, with the Rat/Mesh group exhibiting the highest rate of mesh contacts on day 8 (Fig. 11). The findings that these approach behaviors increase rather than decrease with repeated exposures is likely because rats habituate to other environmental cues but not to the object itself. The finding that approach measures were the highest after 8 pairings with a rat US is likely because the rat is more rewarding than the ball, perhaps due to reciprocation of interaction by the partner rat but not by the ball.

Our findings expand upon previous research that has examined the contribution of play to rewarding effects of a social encounter. For instance, Humphreys and Einon (1981) demonstrated that an adolescent rat will choose a conspecific that is able to engage in play over a restricted or unmotivated play partner in a T-maze. Our results are consistent with their study and extend the findings to the CPP model. In this model, a relationship between the amount of play behavior during conditioning and the magnitude of social reward-CPP has been found but may not be reliable (Douglas, et al., 2004; Thiel, et al., 2008). Calcagnetti and Schecter (1992) have shown that adolescent rats fail

to exhibit CPP if they are paired with a partner whose play drive is pharmacologically inactivated. Similarly, our findings suggest that in adolescent rats that are motivated to play, a rat that is restricted from playing provides a less rewarding stimulus than one that is able to play. Importantly, our results further suggest that a restricted rat (i.e., Rat/Mesh) is nonetheless rewarding, and therefore play is not necessary for social reward-CPP.

The present results are consistent with previous research suggesting that both adolescent and adult rodents find other elements of social encounters to be rewarding besides rough-and-tumble play. These other elements are influenced by social deprivation and the ability to engage in play. For instance, adult rats that have a choice between an opening in an apparatus facing another rat versus one that does not face another rat will spend more time investigating the social opening rather than non-social opening, which does not habituate over multiple trials (Deak, Arakawa, Bekkedal, & Panksepp, 2009). Similarly, we found that rats contact the mesh screen separating them from another rat more frequently than if it were separating them from a ball and contacts with screen in front of a rat US increase by the 8th trial, suggesting that approach behavior or investigation of a conspecific increases over time and persists beyond the novelty stage. In addition, shifts to social behaviors unrelated to play (i.e., crawling over, grooming and sniffing the social partner) are observed in adolescent rats when motivation to play is decreased by social experience such as group housing (Varlinskaya, et al., 1999) or through pharmacological inactivation of play behaviors (Deak & Panksepp, 2006; Pellis & McKenna, 1995). Furthermore, periods of isolation in adolescence elevate social motivation (Ikemoto & Panksepp, 1992; Panksepp & Beatty, 1980; Panksepp, et

al., 1984; Trezza, Damsteegt, et al., 2009; Varlinskaya & Spear, 2008; Varlinskaya, et al., 1999). Thus, it is possible that social motivation in the present study was high due to isolation housing during conditioning, thereby allowing for non-play social encounters to substitute for social reward typically derived from play behavior.

Barriers restricting physical access to a stimulus are frequently used to examine motivation for social investigation as well as social recognition in adolescent and adult rodents. In fact, rodents will inherently prefer a novel conspecific compared to a novel object in initial testing (Moy et al., 2004; Moy et al., 2007; Nadler et al., 2004), similar to our day 1 of conditioning where mesh screen contacts are higher with an initially unfamiliar partner behind the screen than with a novel ball. Mesh screens have also been used in experiments examining the effects of differential housing conditions on play behavior. Results from these studies suggest that rats living in duplex housing (i.e., separated by a mesh screen) demonstrate a 'play rebound' similar to fully isolated rats (Hole, 1991; Holloway & Suter, 2004; Panksepp, et al., 1984). This effect of play deprivation is attenuated when housing conditions allow for bodily contact, but not vigorous attributes of play behavior (i.e., chasing and pinning), indicating that the "need" for play can be attenuated with more mild forms of social contact (Panksepp, et al., 1984).

A potential concern in the present study is that animals that were given 2 pairings had less of a preference for their initially preferred side, and therefore higher baseline values of time spent in the initially non-preferred side, than animals given 8 pairings. Higher initial baseline values decrease sensitivity for detecting a significant increase in time spent in the US-paired side post-conditioning and this may have contributed to the

lack of CPP in the Ball/Phys and Rat/Mesh groups given only 2 pairings. One mitigating argument against this concern is that neither of these groups exhibited as much time spent in the US-paired side post-conditioning after 2 pairings as they did after 8 pairings, suggesting that the lack of effect with 2 pairings was not simply due to a higher baseline. Nonetheless, preference data were also analyzed after transformation to difference scores of test-baseline to minimize variability across cohorts. The variation in baselines across experiments likely reflects age differences between the cohorts of rats since those in Experiment 1 were tested for baseline preference on PND 35-36, whereas those in Experiment 2 were tested on PND 28-29.

Age differences between cohorts may have also contributed to locomotor activity differences. Locomotor activity as measured by compartment crossovers during baseline testing was significantly lower for Experiment 2 compared to Experiment 1, probably because rats in Experiment 2 received baseline testing at an earlier PND than those in Experiment 1. Younger rats may have been more anxious during baseline testing, but we doubt that anxiety played a role during the test day because all groups displayed significantly more crossovers on test day compared to baseline and test day crossovers were not different between experiments.

Our lab is particularly interested in the reward strength of a rat behind a mesh screen because we aim to investigate the influence of this type of social context on acquisition of drug-self administration in adolescent rats. For the latter paradigm, it is necessary to keep the rats separate (i.e., behind a mesh barrier) so that they do not disrupt each other's drug infusion lines. Given that many adolescents initiate drug use in a social setting, it is important to integrate this factor into animal drug abuse paradigms to more

closely model social contributions to drug reward and reinforcement in humans. It has long been known that alcohol consumption in humans is more pleasurable when it takes place in a social context than when alone (Pliner & Cappell, 1974; R. C. Smith, et al., 1975). Similarly in rats, oral ethanol intake is facilitated by social interaction (Tomie, Burger, et al., 2004; Tomie, Uveges, Burger, Patterson-Buckendahl, & Pohorecky, 2004) and social context can influence sensitivity to alcohol and attenuate its aversive effects (Gauvin, et al., 1994; Varlinskaya, et al., 2001). We have observed a synergistic interaction between social reward and either cocaine or nicotine reward in adolescent rats (Thiel, et al., 2008; Thiel, et al., 2009), yet little is known about the influence of social context on intravenous drug self-administration. The results from the present study suggest that limited exposure of two rats separated by a mesh barrier is rewarding and should provide a valid model for examining effects of social interaction on acquisition, maintenance and extinction of intravenous drug self-administration.

In conclusion, our findings demonstrate that rough-and-tumble play is not necessary to establish social reward-CPP in adolescent male rats. Specifically, limited physical contact with another rat is rewarding but to a lesser degree than full physical contact with another rat. In addition, rats elicit more robust approach and contact behavior than an inanimate object during conditioning. The present results suggest that a mesh barrier between adolescent rats will be useful for examining social influences on other aspects of behavior, such as intravenous drug self-administration.

CHAPTER 4

SOCIAL CONTEXT AND ACQUISITION OF NICOTINE SELF-ADMINISTRATION IN MALE AND FEMALE RATS

Preclinical models of nicotine self-administration suggest that nicotine is a relatively weak reinforcer; however nicotine enhances reinforcing effects of nonpharmacological stimuli (Caggiula, et al., 2001; Chaudhri, Caggiula, Donny, Palmatier, et al., 2006; Palmatier, et al., 2006). An important feature of tobacco use in humans that has been largely overlooked in animal models is that initiation of use typically occurs in a social setting in which peer interaction serves to reinforce the behavior (Baker, et al., 2004; Geckova, et al., 2005; Sussman, 2005; West, et al., 1999). Peer interaction during drug administration appears to have an enhancing effect on drug reward and reinforcement (see Bardo, et al., 2013; and Neisewander, et al., 2012 for review). We have shown that the presence of a similarly-injected social partner can enhance nicotine conditioned place preference (CPP) in male adolescent rats (Thiel, et al., 2009) and attenuate the hypothalamic-pituitary-adrenal (HPA) axis stress response induced by initial exposure to nicotine in male and female adolescent rats (Pentkowski et al., 2011). Additionally, Chen and colleagues (2011) demonstrated that access to a social partner attenuates nicotine-induced taste aversion to a palatable olfactory gustatory cue paired with response-contingent intravenous (IV) nicotine infusions, suggesting social context attenuation of aversive nicotine effects.

Two other factors known to contribute to nicotine reinforcement are age and sex. Rodents are more sensitive to the rewarding, and less sensitive to the aversive, effects of nicotine during adolescence (Belluzzi, et al., 2004; Levin, et al., 2007; O'Dell, et al.,

2006; Vastola, et al., 2002) but see (Shram, Funk, Li, & Le, 2008). In fact, earlier onset of nicotine self-administration in rats leads to higher levels of intake, which persist into adulthood (Levin, et al., 2011). Sex differences involving drug abuse are well documented (see Carroll, et al., 2004; and Roth, et al., 2004 for review), but the involvement of sex and gonadal hormones on nicotine-related behaviors appears to be complicated by age as well as the drug paradigm utilized. Sex differences have been reported for nicotine-induced reward using CPP in both adolescent (Torres, et al., 2009) and adult rodents (Isiegas, et al., 2009; Pogun & Yararbas, 2009; Yararbas, et al., 2010); however, neither sex nor estrous cycle phase appears to influence nicotine self-administration in adults (Chaudhri, et al., 2005; Donny, et al., 2000; Feltenstein, et al., 2012). In adolescents, self-administration findings have been less consistent with either no sex difference (H. Chen, et al., 2011), enhancement in male (Levin, et al., 2011) or enhancement in female rats (Lynch, 2009), and inconsistent estrous cycle effects across these same studies. Additionally, other studies have failed to detect sex differences or estrous cycle effects on cue or stress-primed reinstatement of nicotine-seeking behavior (Feltenstein, et al., 2012) or nicotine-induced hyperlocomotion (Kuo, et al., 1999) in young adult rats.

The purpose of the present study was to directly test the effects of social context (i.e., presence of a conspecific) on acquisition of IV nicotine self-administration at a low (0.015 mg/kg) and intermediate dose (0.03 mg/kg) in male and female rats at the transition from adolescence to young adulthood. We custom-built operant chambers that were conjoined by a removable partition that was either solid to isolate the rats from contact with each other or contained a mesh window that allowed for limited social

contact during self-administration. Such limited social contact has been shown to be rewarding in CPP paradigms (Kummer et al., 2011; Peartree, Hood, et al., 2012). We avoided using procedures to facilitate acquisition of self-administration, such as food restriction, lever baiting, or response-contingent cues with intrinsic reinforcing value in order to avoid the confounding effects of these manipulations on acquisition. We hypothesized that social context facilitates acquisition of nicotine self-administration. We tested this hypothesis in both male and female rats given nicotine self-administration sessions (0.00, 0.015, 0.03 mg/kg, IV) in isolation or in same-sex pairs with limited social contact during the nicotine self-administration sessions**.**

Methods

Animals

Male and female Sprague Dawley rats (Charles River, San Diego, CA) arrived on post-natal day (PND) 27 for Experiment 1-3, with the exception of one cohort of males in experiment 1 that arrived on PND 22 and were pair-housed initially. All rats were placed into single housing on PND 27 for these experiments. For Experiment 4, male and female rats arrived on PND 37. To foster play development, rats were randomly pair-housed with a same sex partner (PND 47 for Experiments 1-3; PND 37 for Experiment 4) until surgery on PND 51, after which they were single-housed throughout the duration of the experiment. All rats were handled for 2 min/day until the start of self-administration. The colony room was climate-controlled with a 12-h reverse light/dark cycle. Rats had *ad libitum* access to food and water in their home cage. Housing, care and euthanasia were in accordance with the Guide for the Care and Use of Laboratory Animals (2011) and

National Institutes of Health standards; all procedures were approved by the IACUC at Arizona State University.

Drugs

(–)Nicotine hydrogen tartrate (Sigma, St. Louis, MO) was dissolved in saline, adjusted to a pH of 7.4±0.1, and then filtered through a 0.2 μm filter. Nicotine dose (0.015, 0.03 mg/kg) was given as the mg/kg free base concentration and was delivered IV at a volume of 0.1 ml. Saline (0.00 mg/kg) was filtered through a 0.2 μm filter and delivered IV at a volume of 0.1 ml.

Surgery

On PND 51, catheters were implanted intravenously as described by Pockros et al. (2011) under isoflurane (2-4%) anesthesia. To maintain catheter patency, a 0.1 ml IV solution of saline containing heparin sodium (70 USOU/ml; Baxter Healthcare Corporation, Deerfield, IL) and ticarcillin disodium (66.67 mg/ml: GlaxoSmithKline, Research Triangle Park, NC) was administered daily. Rats were given subcutaneous (S.C.) injections of buprenorphine analgesic (0.05 mg/kg, S.C.) immediately prior to surgery and an anti-inflammatory agent, meloxicam (1 mg/kg, S.C.) immediately after surgery. Catheter patency was confirmed immediately after the first and last self-administration sessions and as needed by infusing 0.05 ml methohexital sodium (16.67 mg/ml IV; Sigma), which produces anesthetic effects only when administered IV.

Apparatus

The apparatus and dimensions are detailed in Figure 12. Briefly, duplex operant conditioning chambers were constructed to create either an isolated (Iso) or social (Soc) conditioning context between 2 self-administration chambers. To produce Iso or Soc

environments, the adjoining wall contained a removable partition that was either solid, black Plexiglas or contained a wire mesh window, respectively. For Experiments 1-3, each chamber contained only 1, retractable lever that was used to control delivery of intravenous infusions of nicotine or saline (i.e., active lever). An additional, non-retractable lever was installed in each chamber for Experiment 4 that was present during the entire experiment. Responses on this lever had no programmed consequences (i.e., inactive lever) and were used as a control for inadvertent lever presses.

Specific Experiments

Experiment 1 examined nicotine (Nic; 0.015 mg/kg, IV) self-administration in male rats (N=42), Experiment 2 examined nicotine (Nic; 0.015 mg/kg, IV) self-administration in female rats (N=44), Experiment 3 examined saline (Sal; 0.00 mg/kg, IV) self-administration in male rats (N=18), and Experiment 4 examined saline (Sal; 0.00 mg/kg, IV) and nicotine self-administration at 2 doses (Nic; 0.015, Nic; 0.03 mg/kg, IV) in male (N=28 Sal; N=22 Nic 0.015; N=28 0.03 Nic) and female (N=22 Sal; N=32 Nic 0.015; N=32 0.03 Nic) rats.

Habituation Procedures

All rats underwent habituation sessions on PNDs 57-58 during which they were allowed to explore their respective conditioning chambers while attached to their infusion line; however, no drug was available during these habituation sessions. For Experiments 1-3, rats received 2, 30-min exposure sessions/day over 2 consecutive days. For one of the daily sessions, the partition between the 2 self-administration chambers was solid black Plexiglas and for the other session the partition contained a mesh window. For Experiment 4, rats received a 1-hour exposure session/day over 2 consecutive days. For

both of these habituation sessions, the partition between the 2 self-administration chambers was either solid black Plexiglas for the rats assigned to the Iso self-administration condition or contained a mesh window for the rats assigned to the Soc self-administration condition. For all experiments, the dyads of rats were paired with the same partner they had been pair-housed with previously.

Self-Administration Procedures

Experiments 1-3 were conducted using the same procedures. On PNDs 59 or 60, the dyads were randomly assigned to training conditions with either the solid partition (Iso) or the mesh partition (Soc) in place throughout acquisition training. Nine self-administration sessions occurred daily for 2 h at the same time of day and were conducted 6-7 days/week. Sessions began by connecting the rats to their infusion line followed by a 1-min habituation period after which the retractable active levers were presented. Completion of a fixed ratio 1 (FR1) schedule of reinforcement resulted in retraction of the active lever, followed 0.5 s later by a 0.1 ml infusion of nicotine (0.015 mg/kg, IV) or saline (0.00 mg/kg) over 1.2 s. The levers remained retracted for a 20 s timeout. No other response-contingent cue lights/tones were used nor were the rats food-restricted or lever-baited in order to avoid potential confounding effects of these stimuli on acquisition (Peartree et al. 2012b).

Experiment 4 employed similar procedures as Experiments 1-3, with the exceptions that an inactive control lever was added and that rats progressed from an FR1 to an FR3 reinforcement schedule across 20 self-administration session. Note also that male and female rats were included in the same experiment for analysis of sex differences. On PND 59, the same-sex dyads began self-administration sessions with

either their assigned solid partition (Iso) or the mesh partition (Soc) in place, and completion of a FR1 schedule of reinforcement resulted in retraction of the active lever followed 0.5 s later by a 0.1 ml infusion of either saline, 0.015 or 0.03 mg/kg nicotine, IV, delivered over 1.2 s. For sessions 4-20, a FR1schedule was used initially; however, the scheduled progressed from a FR1 to FR2 then FR3 schedule of reinforcement depending on the rats' performance. The schedule increases were programmed to occur after 5 reinforcers had been delivered on the current schedule within 1 h. Responses on the inactive lever had no programmed consequences. After the last self-administration session, three daily nicotine extinction sessions occurred using identical procedures as sessions 4-20, except that saline was substituted for both nicotine doses.

Rats with catheter failure were eliminated from analyses but remained in the study to maintain contextual conditions for the partner with a patent catheter. The final *n's*/group are as follows: Experiment 1: n=20 Soc and n=16 Iso males for 0.015 mg/kg, Nic, Experiment 2: n=19 Soc and n=15 Iso females for 0.015 mg/kg, Nic, Experiment 3: n=10 Soc and n=8 Iso males for 0.00 mg/kg, Sal, and Experiment 4: n=14 Soc and n=14 Iso males for 0.00 mg/kg, Sal, n=10 Soc and n=11 Iso females for 0.00 mg/kg, Sal, n=11 Soc and n=10 Iso males for 0.015 mg/kg, Nic, n=11 Soc and n=9 Iso females for 0.015 mg/kg, Nic, n=11 Soc and n=11 Iso males for 0.03 mg/kg, Nic, and n=12 Soc and n=8 Iso females for 0.03 mg/kg, Nic.

Time-Sampled Behavior Observations

Video recordings were made for one cohort of the male rats in Experiment 1 and the videos were later analyzed to determine whether there was a relationship between lever presses and locomotor activity for Soc male rats that displayed increased nicotine

intake during the first self-administration session. A given rat's location and activity was measured using a time-sampling procedure. A transparency splitting the chamber into 4 quadrants (see Fig. 12) was overlaid onto the computer display of recorded sessions. Quadrant 1 (Q1) contained both the lever and the adjoining wall to the neighboring chamber, which was either mesh or solid, quadrant 2 (Q2) contained the other half of the adjoining wall, and quadrants 3 (Q3) and 4 (Q4) were away from the adjoining wall. The time-sampling procedure included 4, 15-minute intervals with the first beginning once animals were placed into their chambers with levers presented, and subsequent intervals beginning 15 min after the end of each previous interval (i.e., alternating 15 min intervals of sampling vs. no sampling). This resulted in a total of 1 hour of behavioral analysis for each rat distributed across the 2-h session. Horizontal locomotor activity was measured as the number forepaw/head entries into each quadrant. Vertical activity was measured as the number of rears within each quadrant, defined as raising forepaws off the ground in a vertical motion. Additionally, the number of forepaw contacts with the adjoining wall in Q1 and Q2, as well as number of rears over the lever in Q1, were counted.

Estrous Cycle Monitoring

Female rats in Experiment 2 were monitored daily for estrous cycle phase beginning on PND 51 as detailed previously (Acosta et al. 2009; Becker et al. 2005; Goldman et al. 2007). Briefly, a sterile cotton applicator dipped in distilled water was gently inserted into the vaginal opening and removed after a circular motion along the vaginal walls to collect epithelial cells after every self-administration session. Cells were then transferred by rolling the entire circumference of the applicator onto the surface of labeled glass specimen slides. Assessment of vaginal cytology was conducted under

brightfield microscopy under 10× and 40× objective lenses (see Fig. 13). Proestrus was identified by the presence of predominantly nucleated epithelial cells, estrus by predominately cornified cells lacking nuclei, metestrus by similar proportions of cornified cells, leukocytes, and nucleated epithelial cells, and diestrus by predominately leukocytes (see Becker et al. 2005; Caligioni 2009; Goldman et al. 2007; and Marcondes et al. 2002 for review). Metestrus and diestrus data were combined for analyses. We observed vaginal cytology consistent with pseudo-pregnancy (i.e., more than 5 consecutive days in the met/diestrus phase) in 3 rats nearing the end of the self-administration training, therefore pseudo pregnancy was a factor in the analysis of estrous cycle phase effects. In order to maintain continuity with Experiment 2, females in Experiment 4 were also vaginally-swabbed after each self-administration session with a sterile cotton applicator. Since 'sex' was included as a factor in Experiment 4, we controlled for genital stimulation by also gently swabbing the males around the anogenital region after each self-administration session.

Data Analysis

For Experiments 1-3, total number of reinforcers obtained each day across the nine days of self-administration training were analyzed separately for each experiment using mixed factor ANOVAs with session (1-9) as a repeated measure and social condition (Iso vs. Soc) as a between subjects factor.

For Experiment 1, time-sampled behavioral analyses by quadrant on the first session were analyzed using independent sample t-tests and within subject differences across quadrants were analyzed by Wilcoxon signed-ranks tests and the alpha level was adjusted to correct for multiple comparison using Bonferroni correction.

For Experiment 2, the influence of estrous cycle phase on the number of reinforcers obtained during sessions 1, 8 and 9 were analyzed using separate ANOVAs with social condition (Iso vs. Soc) and estrous cycle phase (estrus, proestrus, met/diestrus, both with and without pseudo-pregnancy) as between subjects factors.

For Experiment 4, total number of reinforcers obtained on the first self-administration session were analyzed using ANOVA with dose (Sal, 0.015 Nic, vs. 0.03 Nic), sex (male vs. female) and social condition (Iso vs. Soc) as between subjects factors for rats that passed patency testing following the first day of self-administration. Since we observed an increase in intake for Soc vs. Iso males self-administering 0.015mg/kg Nic on the first session in Experiment 1, we predicted that Soc males would self-administer more Nic relative to Iso males on the first session at the 0.015mg/kg dose of nicotine. Therefore, number of reinforcers obtained on the first day for Iso vs. Soc males self-administering 0.015mg/kg Nic were analyzed using *a priori* independent samples t-tests. Total number of reinforcers obtained each day across the twenty days of self-administration training were analyzed using mixed factor ANOVAs with session (1-20) as a repeated measure and dose, sex, and social condition as between subjects factors for rats that remained patent throughout the entire experiment. Since reinforcement schedule requirements changed on session 4, two separate ANOVAs were also conducted for reinforcers obtained for sessions 1-3 and sessions 4-20 as repeated measures factors and dose, sex and social condition as between subjects factors. Active and Inactive lever presses were analyzed using 3 separate mixed factor ANOVAs with total lever presses (active vs. inactive) aggregated across sessions 1-20, sessions 1-3, and sessions 4-20 as separate repeated measures factors and dose, sex and social condition as between subjects

factors. Extinction sessions were analyzed using mixed factor ANOVAs with extinction session (1-3) as a repeated measure and dose, sex, and social condition as between subjects factors.

All significant interactions were further analyzed using tests of simple effects. In the case of 4-way interactions, additional simpler ANOVAs were conducted systematically removing factors to detect the source of each interaction. All data were analyzed using SPSS 21 (IBM, Somers, NY), graphed using Prism 5 (Graphpad Software, La Jolla, CA), and expressed as mean ± *SEM*.

Results

Experiments 1-3: Effects of Social Context on Self-Administration

Figure 14 illustrates the number of nicotine and saline reinforcers obtained across self-administration sessions for Experiments 1-3. In all three experiments, omnibus ANOVAs analyzing reinforcers/session revealed a main effect of Session: for Experiment 1 males $F(8,272) = 5.88$, $p<0.01$, for Experiment 2 females $F(8,256) = 2.78$, $p<0.01$, and for Experiment 3 males $F(1,8) = 9.88$, $p<0.0001$. A Session × Social Condition interaction was also found for males in Experiment 1 [$F(8,272) = 2.68$, $p<0.05$] and females in Experiment 2 [$F(8,256) = 2.36$, $p<0.05$], but not for males in Experiment 3. Subsequent tests of simple effects revealed that in males given access to nicotine in Experiment 1, nicotine intake was higher in the Soc group compared to the Iso group during the first session [$t(34) = 2.28$, $p<0.05$], suggesting social enhancement of nicotine intake initially. In females given access to nicotine in Experiment 2, nicotine intake was similar in the Soc and Iso groups initially; however, the Soc group's intake was lower than that of the Iso group during sessions 8 [$t(32) = 2.09$, $p<0.05$] and 9 [$t(32) = 1.99$,

p=0.05], suggesting that the increase in nicotine intake over time in Iso females is protected against by the social context. In males given access to saline in Experiment 3, the lack of a Social Condition main effect or Session × Social Condition interaction indicates there was no difference between Iso and Soc groups, suggesting that the presence of a social partner failed to alter saline intake. Both groups exhibited a decrease in intake across sessions regardless of social condition.

Experiment 1: Analysis of Locomotor Activity during the First Session in Males

Figure 15 illustrates time-sampled observations for both vertical and horizontal locomotor behavior presented as total number of adjoining wall contacts, quadrant entries, rears, and rears directly over the lever, as well as entries and rears by quadrant during the first self-administration session in Experiment 1 males. Independent samples *t*-tests revealed that Soc males made more contacts with the adjoining wall containing the mesh partition compared to the Iso males with a solid adjoining wall [$t(16) = 8.72$, $p<0.001$]. There were no significant differences between Soc and Iso males for total number of quadrant entries or rears, suggesting that locomotion did not differ between Iso and Soc males during the first session of nicotine self-administration. Additionally, there was no significant difference in number of rears directly over the lever between Iso and Soc rats, suggesting that increased nicotine intake in the Soc rats during this session was not merely due to inadvertent lever pressing as a result of increased proximity to the lever. Independent samples *t*-tests with Bonferroni correction indicated no differences between Soc and Iso rats' entries or rears by quadrant, although there were significantly more entries and rears in the adjoining side quadrants (i.e., Q1 and Q2) versus non-

adjoining side quadrants (Q3 and Q4) regardless of social condition (Wilcoxon Signed-Ranks tests, $p<0.05$).

Experiment 2: Effects of Estrous Cycle Phase on Self-Administration in Females

There were no significant effects of Estrous Cycle Phase on the Number of Reinforcers during session 1, 8 or 9, suggesting that estrous cycle did not alter nicotine intake in these sessions. Mean reinforcers/session (±SEM) are displayed in Table 3 for rats tested at different phases of the estrous cycle.

Experiment 4: Effects of Social Context, Sex and Dose on Self-Administration

The omnibus ANOVA of reinforcers obtained during the first session in Experiment 4, including all rats that were patent, revealed a Dose × Social Condition interaction [$F(2,139) = 4.37$, $p<0.05$; see Figure 16]. Subsequent *post-hoc* t-tests revealed that Sal intake was higher in the Soc rats relative to Iso rats on the first session [$t(34.23) = 2.32$, $p<0.05$]. Planned independent samples t-tests revealed that male Soc rats did self-administered significantly more nicotine at the 0.015mg/kg dose relative to their Iso counterparts [$p<0.05$], replicating the Social enhancement found in Experiment 1.

Figure 17 illustrates the number of nicotine and saline reinforcers obtained across 20 self-administration sessions for Experiment 4. The omnibus ANOVA of reinforcers/session for all 20 sessions revealed main effects of Session [$F(19,2280) = 11.17$, $p<0.001$], Dose [$F(2,120) = 4.07$, $p<0.05$], and Social Condition [$F(1,120) = 11.42$, $p<0.001$], and Sex × Social Condition [$F(1,120) = 8.13$, $p<0.01$], Session × Dose [$F(38,2280) = 2.61$, $p<0.001$], Session × Social Condition [$F(19,2280) = 2.05$, $p<0.01$], Session × Sex × Dose [$F(38,2280) = 2.41$, $p<0.001$], and Session × Sex × Dose × Social

Condition [$F(38,2280) = 1.59$, $p<0.05$] interactions. Since a significant 4-way interaction was found in our omnibus ANOVA, subsequent analyses using simpler ANOVAs were systematically conducted to detect the source of each interaction.

Figure 18 illustrates the Session × Social Condition and Sex × Social Condition interactions. Tests of simple effects of the Session × Social Condition interaction revealed that Iso rats obtained more reinforcers relative to Soc rats over sessions 4,7,11, 13-17 [t's(90.03-130) = 2.14-3.37, p's<0.05]. Tests of simple effects of the Sex × Social Condition interaction revealed that female Soc rats obtained fewer reinforcers than all other groups [t's(44.66-67) = 3.38-4.52, p's<0.01].

Figure 19 illustrates the Session × Dose and the Session × Sex × Dose interactions. Subsequent tests of simple effects of the Session × Dose interaction revealed that rats had lower Sal intake relative to 0.015mg/kg and 0.03mg/kg Nic on several of the later self-administration sessions: Sal vs. 0.015mg/kg Nic on sessions 13, 15, 18-20 [t's(52.83-88) = 2.31-2.66, p's<0.05] and Sal vs. 0.03mg/kg Nic on sessions 12, 13, 15-20 [t's(64.19-89) = 2.03-3.42, p's<0.05]. Subsequent Dose X Session ANOVAs conducted separately for males and females revealed a Session × Dose interaction for males [$F(38,1235) = 1.92$, $p<0.01$] as well as for females [$F(38,1045) = 3.06$, $p<0.001$]. Tests of simple effects revealed that male rats displayed increased intake at 0.015mg/kg and 0.03mg/kg Nic relative to Sal on multiple sessions: 0.015mg/kg Nic vs. Sal on sessions 13, 15, 19 [t's(23.91-47) = 2.00-2.38, p's<0.05] and 0.03mg/kg Nic vs Sal on sessions 3,19 [t's(48) = 2.21-2.52, p's<0.05]. Female rats displayed increased intake of 0.015mg/kg relative to 0.03mg/kg Nic [$t(33.30) = 2.29$, $p<0.05$] and relative to Sal [$t(32.03) = 2.06$, $p<0.05$] on the first session as well as increased intake for 0.03mg/kg

Nic relative to Sal on sessions 15, 16, 18, 20 [*t*'s(27.05-39) = 2.11-3.30, *p*'s<0.05]. To further analyze the Session × Dose × Sex interaction, subsequent ANOVAs at each dose revealed a Session × Sex interaction for 0.015mg/kg Nic [$F(19,741) = 1.76$, $p<0.05$] and 0.03mg/kg Nic [$F(19,760) = 2.22$, $p<0.01$], but not for Sal (note the data are not graphed to show this particular analysis). Tests of simple effects revealed that males self-administered more nicotine at the 0.03mg/kg dose relative to females on sessions 2 [$t(40) = 2.07$, $p<0.05$] and session 3 [$t(40) = 2.47$, $p<0.05$]. Though there was a significant Session × Sex interaction for 0.015mg/kg Nic, there were no significant simple effects, only marginally significant effects (*p*s = 0.059-0.10), where males appeared to self-administer more nicotine in later sessions relative to females.

Since reinforcement schedule requirements changed on session 4, separate ANOVAs were also conducted for reinforcers obtained for sessions 1-3 and sessions 4-20. The ANOVA analyzing sessions 1-3 revealed a significant main effect of Session [$F(2,240) = 21.18$, $p<0.001$], and significant Sex × Social Condition [$F(1,20) = 5.17$, $p<0.05$], Dose × Social Condition [$F(2,20) = 3.92$, $p<0.05$], and Sex × Dose × Social Condition [$F(2,20) = 3.48$, $p<0.05$] interactions. The ANOVA for sessions 4-20 revealed a significant main effect of Session [$F(16,1920) = 12.31$, $p<0.001$], Dose [$F(2,120) = 4.47$, $p<0.05$], Social Condition [$F(1,120) = 12.50$, $p<0.01$], and significant Sex × Social Condition [$F(1,120) = 7.12$, $p<0.01$], Session × Dose [$F(32,1920) = 2.95$, $p<0.001$], Session × Social Condition [$F(16,1920) = 1.68$, $p<0.05$], and Session × Sex × Dose [$F(32,1920) = 1.52$, $p<0.05$] interactions. Further interpretations of significant effects will be discussed using the ANOVA for reinforcers that incorporated all sessions (1-20) since

the ANOVAs with sessions 1-3 and 4-20 did not yield any novel information regarding our effects beyond the information that was obtained using the sessions 1-20 ANOVA.

Experiment 4: Effects of Social Context, Sex and Dose on Active and Inactive Lever Responding

To simplify presentation of lever presses, the difference between active and inactive lever presses is shown in Figure 20. The omnibus ANOVA with Lever (total active vs. inactive lever presses over sessions 1-20) as a within subjects factor and Sex, Dose and Social Condition as between subjects factors revealed a main effects of Lever [$F(1,120) = 35.84$, $p<0.001$], Dose [$F(2,120) = 3.39$, $p<0.05$], and Social Condition [$F(1,120) = 8.53$, $p<0.01$], as well as Sex × Social Condition [$F(1,120) = 4.48$, $p<0.05$], Lever × Social Condition [$F(1,120) = 10.52$, $p<0.01$], and Lever × Sex × Dose [$F(2,120) = 3.46$, $p<0.05$] interactions. Tests of simple effects revealed that active lever presses were significantly increased relative to inactive lever presses for Soc males self-administering 0.015mg/kg Nic [$t(10) = 3.13$, $p<0.05$], and Iso females self-administering 0.015mg/kg Nic [$t(8) = 2.68$, $p<0.05$] and 0.03mg/kg Nic [$t(7) = 3.04$, $p<0.05$], suggesting robust discrimination between the levers in these groups. The ANOVA with Lever presses over sessions 1-3 revealed no significant effects. The ANOVA with Lever presses over sessions 4-20 revealed a main effects of Lever [$F(1,120) = 36.34$, $p<0.001$], Dose [$F(2,120) = 3.52$, $p<0.05$], and Social Condition [$F(1,120) = 9.88$, $p<0.01$], as well as Sex × Social Condition [$F(1,120) = 4.09$, $p<0.05$], Lever × Social Condition [$F(1,120) = 9.75$, $p<0.01$], and Lever × Sex × Dose [$F(2,120) = 3.91$, $p<0.05$] interactions. Tests of simple effects revealed that active lever presses were increased relative to inactive lever presses for Soc males self-administering 0.015mg/kg Nic [$t(10) = 2.81$, $p<0.05$], and Iso

females self-administering 0.015mg/kg Nic [$t(8) = 2.54$, $p<0.05$] and 0.03mg/kg Nic [$t(7) = 3.15$, $p<0.05$]. Since the significant effects for sessions 1-20 and 4-20 were identical to the previous analyses, interpretations will be discussed using the ANOVA for lever presses that incorporated all 20 sessions.

Experiment 4: Effects of Social Context, Sex and Dose on Extinction Responding

The omnibus ANOVA of reinforcers/session during extinction revealed main effects of Session [$F(2,240) = 12.49$, $p<0.001$], Sex [$F(1,120) = 4.34$, $p<0.05$], Dose [$F(2,120) = 6.63$, $p<0.01$], and Social Condition [$F(1,120) = 4.32$, $p<0.05$] as well as a Session × Dose [$F(4,240) = 3.09$, $p<0.05$] interaction. The latter is illustrated in Figure 21. Tests of simple effects revealed that the 2 nicotine dosage groups exhibited higher response rates relative to saline controls on the first 2 days of extinction [Session 1: Sal vs. 0.015mg/kg Nic $t(58.81) = 3.26$, $p<0.01$ and Sal vs. 0.03mg/kg Nic $t(89) = 3.73$, $p<0.001$; Session 2: Sal vs. 0.015mg/kg Nic $t(62.72) = 2.55$, $p<0.05$ and 0.03mg/kg Nic $t(89) = 2.40$, $p<0.05$]. No significant differences were found among groups on session 3, suggesting that Nic rats extinguished their responding for nicotine by the third session.

Discussion

This study demonstrates that social context (i.e., presence of a conspecific behind a mesh barrier) differentially influences nicotine self-administration in male and female rats. We found 2 similar patterns across experiments: 1.) a transient social enhancement of low dose nicotine (0.015 mg/kg) intake on the first session of self-administration in male rats given access to nicotine in the presence of a same-sex partner compared to rats self-administering in isolation (Experiments 1 and 4) and 2.) a social partner suppressed nicotine intake during later sessions in female rats (Experiments 2 and 4), but had no

effect on saline intake in later sessions (Experiment 4). A large-scale, parametric study (Experiment 4) designed to further explore the relationship between sex, social condition and nicotine dose, failed to increase intake at an intermediate dose of nicotine (0.03 mg/kg) in social male rats on the first session. Unexpectedly, social facilitation of saline intake emerged during the first session (Experiment 4), appearing more robust in males. Findings from the parametric study revealed that isolated rats obtained more reinforcers regardless of dose (0.00 mg/kg, Sal; 0.015 mg/kg, Nic; 0.03 mg/kg, Nic), with isolated females primarily responsible for this effect. Effects of isolation also changed across sessions, growing more pronounced during later sessions. Not surprisingly, overall saline self-administration was significantly lower than that of both doses of nicotine in the later sessions, demonstrating that nicotine was serving as a reinforcer in this study.

Acquisition training took place at the age at which rats are transitioning from adolescence to adulthood since this is a developmental period during which humans commonly initiate smoking (Baker, et al., 2004; Geckova, et al., 2005; Sussman, 2005; West, et al., 1999). We had predicted that both males and females in this study would exhibit enhanced acquisition of nicotine self-administration in a social context and that the effect would be observed across both doses of nicotine based on three previous findings from our lab. First, we found that adolescent males exhibit enhanced nicotine-CPP when nicotine is experienced in a social context versus in isolation (Thiel, et al., 2009). Second, we have shown that limited social contact is rewarding among adolescent male rats interacting through a mesh divider similar to that used in the present study (Peartree, Hood, et al., 2012). Finally, we have shown that the nicotine-induced increase in the stress hormone corticosterone is attenuated in adolescent male and female rats

tested in a social context versus in isolation (Pentkowski, et al., 2011), suggesting that social context blunts nicotine-induced stress, perhaps resulting in enhanced nicotine reward. Contrary to our expectations, self-administration did not appear to be affected initially by social context in females whereas males exhibited increased responding at both the low dose of nicotine and saline in the social context relative to isolation. The latter suggests that males may have been more prone to interact with environmental stimuli in general when in a social context relative to when isolated, perhaps related to territorial behavior or overall excitement. As expected in Experiment 3, saline intake in males was unaffected by social context. Unexpectedly, social context elevated responding for saline on the first session in control rats in Experiment 4, suggesting that the social facilitation effect on low dose nicotine intake we observed during the first session for males in Experiment 1, was not drug-specific. The lack of drug-specificity in our experiments contrasts with previous studies reporting social facilitation of nicotine and cocaine self-administration compared to saline reinforcement (H. Chen, et al., 2011; M. A. Smith, 2012) and d-amphetamine self-administration compared to sucrose reinforcement (Gipson, et al., 2011). However, unpublished data from our lab suggests that stimulants, like cocaine, are more reliably self-administered than nicotine, which may account for the discrepancies regarding drug-specificity.

We did not obtain any evidence of Estrous cycle influences on the behavior of females in Experiment 1, though we were likely underpowered to detect such an effect. Estrous cycle effects on nicotine self-administration are not well understood and are likely complex given that some studies have failed to find an effect in adolescent (Levin, et al., 2011) or adult female rats (Donny, et al., 2000), whereas Lynch and colleagues

(2009) found enhanced nicotine intake on a progressive ratio schedule of reinforcement during estrus in female adolescent rats. The discrepancies may involve age and/or the schedule of reinforcement used. Research with other models has failed to find estrous cycle phase effects, including studies using nicotine-induced place preference (Torres, et al., 2009), cue- or stress-primed reinstatement of nicotine-seeking behavior (Feltenstein, et al., 2012) or nicotine-induced hyperlocomotion (Kuo, et al., 1999). Taken together, it seems estrous cycle phase does not exert a strong effect on nicotine self-administration, although there may be subtle influences.

Since social facilitation dissipated by the second session and failed to occur when nicotine dose was increased (0.03 mg/kg), social facilitation of nicotine intake may be sensitive to habituation and dose. Similarly, Gipson and colleagues (2011) found that social facilitation of d-amphetamine self-administration was transient as well as dose-dependent in adult male rats, where the presence of a social partner failed to enhance intake upon the second exposure and only occurred at the highest dose tested. Some would argue that the social enhancement effect we observed solely in Experiment 1 was a result of inadvertent lever pressing and/or increased locomotor activity within the self-administration chamber. However, we measured time-sampled locomotor behavior and activity near the active lever (i.e., entries into the quadrant of the chamber containing the lever and rears above the lever) and found no differences between isolated and social males, nor were there any differences in measures of locomotor activity in any of the other quadrants of the chamber. Therefore, it seems unlikely that inadvertent lever pressing due to alterations in locomotor behavior resulted in increased lever pressing in social males in Experiment 1. Regardless of social condition, males in Experiment 1

made more entries into the quadrants with the adjoining wall than the other quadrants. However, the only measure that varied between social and isolated males was an increase in contacts with the adjoining wall of the chamber, which was expected due to the opportunity to interact with a social partner.

Contrary to our predictions, the present study failed to demonstrate an *overall* social facilitation of nicotine intake when measured across 20 sessions. In fact, self-administration in isolation robustly enhanced intake with repeated sessions. This effect was more apparent during later sessions and was largely driven by females. Our findings are contrary to several recent studies examining the effects of a social partner on stimulant self-administration. For example, the presence of a social partner behind a barrier within the operant chamber during self-administration sessions has been shown to enhance responding for IV cocaine, d-amphetamine, and nicotine (H. Chen, et al., 2011; Gipson, et al., 2011; M. A. Smith, 2012). However, it is possible that decreased reinforcement in social rats in the present study may be a result of reward competition within the self-administration chamber (i.e., social interaction with a partner and responding on a lever for drug self-administration). Our findings that social rats took less nicotine, yet spent more time contacting the adjoining wall of the apparatus compared to isolated rats in Experiment 1 is consistent with this explanation. Perhaps engaging in social investigation interferes with lever pressing either by distracting the rats or directly competing with nicotine reward. Indeed, previous studies have shown that drug and social rewards compete (Carroll, et al., 2004; Fritz, et al., 2011; Hecht, Spear, & Spear, 1999; Seip & Morrell, 2007). Recently, Fritz and colleagues (2011) found that opportunity for social interaction can compete with established cocaine CPP in adult male

rats. Collectively, our findings suggest that social context does not enhance, but may actually exert a protective influence on maintenance of nicotine self-administration in rats that have simultaneous access to nicotine.

Not surprisingly, overall nicotine intake was greater in both nicotine groups relative to saline. This dose effect emerged in later sessions, presumably when rats had established stable self-administration responding. However, in contrast to our previous findings in same-aged male rats (Peartree, Sanabria, et al., 2012), there was not an overall difference in intake between the low and intermediate nicotine doses in the present study, likely due to the aggregate fluctuations across sex. Though we failed to observe *overall* sex differences on nicotine reinforcement, there were several dose-dependent effects that were contingent on both sex and self-administration session. Specifically, females self-administered significantly more 0.03 mg/kg nicotine, while males self-administered significantly more 0.015 mg/kg nicotine, relative to saline during later sessions. Additionally, we found that at the 0.015 mg/kg dose, males self-administered more nicotine than females during later sessions and at the 0.03 mg/kg dose, females self-administered more nicotine than males during earlier sessions. As expected, there was no difference between males and females for saline self-administration. With the addition of a non-reinforced lever in the chamber in Experiment 4, we detected preferential responding on the reinforced (active) versus non-reinforced (inactive) lever for the low dose of nicotine in social males and for both the low and intermediate doses of nicotine in isolated females, but not for saline, suggesting that only these 3 groups were responding discriminately for nicotine. These effects were robust during later sessions, likely due to increased demand on the progressive schedule of reinforcement after session 4 (FR1 ->

FR2 -> FR3). The addition of extinction sessions in Experiment 4 allows for further interpretation for the ability of nicotine to serve as a reinforcer. Similar to previous reports of nicotine extinction (LeSage, Burroughs, Dufek, Keyler, & Pentel, 2004; Liu et al., 2006; O'Dell et al., 2007), we observed a serial decrease in reinforcement rates for rats previously trained with nicotine when saline was substituted in place of nicotine. By the third extinction session, nicotine-trained rats had decreased to minimal responding, like that observed in rats trained to self-administer saline, bolstering the point that nicotine served as a reinforcer during previous self-administration sessions.

In summary, the present findings suggest that social factors exert strong and sex-dependent influences on self-administration. Specifically, social interaction within the self-administration environment initially facilitates intake in male rats at a low nicotine dose, yet suppresses acquisition and maintenance of nicotine intake in and female rats transitioning from adolescence to adulthood. Since initiation of nicotine use in humans typically occurs in a social setting (Baker, et al., 2004; Geckova, et al., 2005; Sussman, 2005; West, et al., 1999), the use of social context during acquisition of nicotine self-administration is important and under-utilized in preclinical studies using animal models. To our knowledge, this is the first study to examine spontaneous acquisition of nicotine self-administration in a social context where both rats have simultaneous access to nicotine in the absence of appetitive response-contingent cues. The present findings underscore the impact of social context on nicotine intake, which is particularly important given that most pre-clinical nicotine research has neglected to consider social context as an influential factor. Future research aimed at understanding the neural mechanisms that

underlie social influences on nicotine self-administration may have important implications for developing treatments for nicotine dependence.

CHAPTER 5

CONCLUDING REMARKS

The purpose of this dissertation was to investigate social influences on nicotine-related behaviors and neural signaling. Specifically, we examined the effect of social interaction combined with nicotine on immediate early gene expression within the mesocorticolimbic circuity (Chapter 2), social reward as a function of the degree of physical access to a social partner (Chapter 3), and social influences on nicotine self-administration using limited physical access (Chapter 4). Investigating the modulatory role that social interaction plays during the initial drug experience is crucial for understanding neural processes involved in the development of nicotine addiction since the period between adolescence and early adulthood is considered a window of increased vulnerability for initiation of smoking coupled with enhanced sensitivity to social cues. Therefore, we focused on the adolescent to young adult age range in our rodent models of nicotine reward and reinforcement for this dissertation. We hypothesized that a social context would enhance nicotine reward and reinforcement, as well as the accompanying neural signaling.

The results of the first study supported my hypothesis nicotine reward is enhanced by social stimuli in adolescent male rats (Chapter 2). However, the lack of Fos expression elicited by environmental stimuli associated with both nicotine and social rewards was surprising given that others have shown that exposure to environmental cues associated with rewarding stimuli induces Fos expression within the regions examined (Gil, Nguyen, McDonald, & Albers, 2013; Neisewander, et al., 2000; Pascual, et al., 2009; Schroeder, et al., 2001). A critical difference between the present study and

previous work is that adolescent rats were given free-access to both sides of the conditioning apparatus on test day, and it stands to reason that enhanced signaling from CS+ exposure may have been countered by inhibition of signaling associated with CS- exposure (Biesdorf et al., 2015; Nakahara, Itoh, Kawagoe, Takikawa, & Hikosaka, 2004). More specifically, the social-induced reduction of Fos expression in the NAcC may reflect prediction error due to the absence of a social partner in the chamber on test day (Behrens, et al., 2008; Fehr & Camerer, 2007; Jones, et al., 2011; Poore, et al., 2012). A potential future direction would be to confine the rats to their US-paired side on test day to control for exposure solely to US-associated environmental stimuli. Furthermore, examining another plasticity-associated gene, such as Arc, would perhaps yield more sensitive results relative to Fos protein since Arc has been found to be upregulated in response to nicotine-associated cues in rats (Schiltz, Kelley, & Landry, 2005).

The findings that nicotine-treated rats exhibited *less* Fos expression in the Cg1, Cg2, dlCPu, and NAcC relative to saline-treated rats in chapter two are inconsistent with previous findings following acute nicotine administration (Mathieu-Kia, et al., 1998; Pascual, et al., 2009; Salminen, et al., 1999; Schilstrom, et al., 2000; Schochet, et al., 2005; Seppa, et al., 2001). However, dose and age appear to be important considerations for interpreting our discrepant findings. Most studies examined expression in adult animals and all of the studies used at least twice the nicotine dose relative to our experiment. In addition, Fos protein is not constitutively expressed (Chao & Nestler, 2004; Nestler, 2001), so Fos expression is not typically interpreted as a decrease from a set baseline. Therefore, we may gain more insight into the patterns produced by nicotine and social rewards by examining the immediate early gene zif268. Given that zif268

protein is constitutively expressed (Zangenehpour & Chaudhuri, 2002), we would gain the ability to measure increases and decreases in protein relative to our control group. Another benefit of examining zif268 is that induction of protein expression peaks during the same period of time as Fos (i.e., 90 min) (Zangenehpour & Chaudhuri, 2002), therefore it would be a good candidate for future immunohistochemical processing on the remaining tissue collected from Chapter 2 where rats were sacrificed 90 min after stimulus exposure.

The exact molecular mechanisms for our social-induced increase in Fos expression (Chapter 2) is unknown since Fos is a broad measure of increased activity of several intracellular signaling cascades. Rewarding aspects of social interaction have typically been attributed to regulation by dopaminergic projections from the VTA to the nucleus accumbens (Beatty, Dodge, Dodge, White, & Panksepp, 1982; Niesink & Van Ree, 1989; Panksepp, et al., 1984); however, several other neurochemical systems have been implicated in processing information regarding prosocial interactions (see Siviy & Panksepp, 2011 for review), including endogenous opioids (Panksepp, 1981; Vanderschuren, Niesink, Spruijt, & Van Ree, 1995), cannabinoids (Trezza, Cuomo, & Vanderschuren, 2008; Trezza & Vanderschuren, 2008, 2009), other monoamines (Beatty, et al., 1982; Normansell & Panksepp, 1985; Vanderschuren et al., 2008), and the cholinergic system (Panksepp, et al., 1984; Thiel, et al., 2009; Trezza, Baarendse, & Vanderschuren, 2009). Taken together, the underlying neural mechanisms of social interaction-reward mechanisms appear to be highly complicated due, in part, to recruiting many brain regions with a diverse range of neurochemical substrates. The next steps in

elucidating the neural mechanisms responsible for our effects include identifying and thoroughly testing specific neurotransmitter systems in the regions of interest.

My hypothesis that social context facilitates nicotine reinforcement was supported in chapter four where we found that young-adult male rats self-administered more low dose nicotine in the presence of a social partner. However, the social enhancement was transient and did not occur at an intermediate nicotine dose. Although it is possible that experiencing enhanced reward upon the first drug exposure may lead to greater risk for developing dependence with continued use, we did not obtain evidence of such an effect in the later self-administration sessions. We may have lacked the sensitivity to detect differences in hedonic value of nicotine beyond what we can observe with low-demand partial reinforcement schedules (i.e., FR1-FR3). Perhaps increasing the schedule of reinforcement by incorporating a more challenging progressive ratio schedule would allow us to make further inferences regarding increased sensitivity and motivation for nicotine reinforcement. In addition, the initial social facilitation of nicotine self-administration did not extend to female rats, but rather seem to produce a suppressive effect. Given that we only tested adolescent male rats in our initial CPP studies (Chapters 2 & 3), it is unknown whether social enhancement of nicotine CPP is present in female rats. Second, variations in not only sex, but also age, across our experiments may contribute to our lack of social enhancement in nicotine reinforcement in females. An important future direction for this line of research would be to explore social influences on both nicotine and limited physical access CPP in females. It is possible that females would fail to exhibit CPP in either case and this would help explain our self-administration effects.

In an effort to more closely model smoking, another future direction for our laboratory would be to examine other non-nicotine ingredients in cigarettes and tobacco products (Palmatier, et al., 2006). There are over 5,000 known constituents found in tobacco smoke that have not been extensively studied in animal models (Center for Disease Control, 2015). Recently, several laboratories have started to examine the influence of major ingredients found in cigarettes on nicotine reinforcement in rodent models. Additives such as acetaldehyde (Belluzzi, Wang, & Leslie, 2005), alkaloids (Clemens, Caille, Stinus, & Cador, 2009; Harris et al., 2015), and monoamine oxidase (MAO) inhibitors (Arnold, Loughlin, Belluzzi, & Leslie, 2014; Hall et al., 2014; Lotfipour et al., 2011; Villegier, Lotfipour, McQuown, Belluzzi, & Leslie, 2007) enhance nicotine self-administration and nicotine-like behaviors in rodent models. Similarly, cigarette smoke sustains stable self-administration responding in rats (Costello et al., 2014). Taken together, these recent findings suggest that nicotine is not the sole reinforcing ingredient in tobacco products. Therefore, incorporating these non-nicotine additives in animal models of smoking may more closely model tobacco addiction in humans.

Social influences have been largely ignored in pre-clinical models of substance abuse. My dissertation has emphasized that social influences are significant determinants of drug-abuse related behaviors, highlighting the importance of incorporating social factors into drug-abuse paradigms in the future. Identifying the unique social contributions to human substance abuse is complex and animal models cannot completely capture the variability in human behavior; however, integrating social variables into pre-

clinical research methods may have translational implications for developing prevention and intervention strategies among youth at risk for developing substance use disorders.

Table 1 Mean (±SEM) of Fos-positive nuclei for each region for Experiments 1 and 2.

	Brain Region[b]									
Experiment 1 Group[a]	**Cg1**	**Cg2**	**dmCPu**	**dlCPu**	**NAcC**	**NAcSh**	**MeA**	**CeA**	**BlA**	**VTA**
Sal+Iso	95 ± 10.1	115 ± 13.9	17 ± 4.3	3 ± 0.8	73 ± 7.6	38 ± 3.7	62 ± 4.8	33 ± 3.7	67 ± 5.7	7 ± 1.6
Sal+Soc	92 ± 6.3	91 ± 8.8	16 ± 2.4	4 ± 1.1	67 ± 3.9*	33 ± 2.6	56 ± 3.7	33 ± 2.8	52 ± 2.2	8 ± 1.2
Nic+Iso	106 ± 13.2	99 ± 8.9	21 ± 4.2	5 ± 1.1	72 ± 9.1	32 ± 4.1	59 ± 3.0	37 ± 3.6	64 ± 5.4	7 ± 2.5
Nic+Soc	119 ± 8.0	99 ± 7.5	17 ± 2.9	4 ± 0.7	52 ± 3.7*	36 ± 2.9	64 ± 4.0	34 ± 3.2	64 ± 4.7	8 ± 1.0
Experiment 2 Group[a]	**Cg1**	**Cg2**	**dmCPu**	**dlCPu**	**NAcC**	**NAcSh**	**MeA**	**CeA**	**BlA**	**VTA**
Sal+Iso	92 ± 10.5	87 ± 16.0	19 ± 4.0	3 ± 0.6	36 ± 4.6	25 ± 3.1	13 ± 1.8	10 ± 2.0	28 ± 4.0	6 ± 0.6
Sal+Soc	102 ± 7.4	98 ± 13.1	26 ± 4.7	10 ± 1.7*	52 ± 6.1*	37 ± 2.5*	13 ± 1.8*	14 ± 2.3*	20 ± 2.3#	4 ± 0.5#†
Nic+Iso	55 ± 8.2+	54 ± 8.0+	12 ± 2.8	1 ± 0.3+	23 ± 5.2+	20 ± 3.1	9 ± 1.0	11 ± 2.0	15 ± 1.9#†	4 ± 0.8#†
Nic+Soc	77 ± 6.3+	62 ± 5.3+	17 ± 3.9	5 ± 1.6*+	38 ± 6.2*+	34 ± 2.5*	17 ± 2.5*	17 ± 2.7*	24 ± 2.5	6 ± 0.8

[a] Saline (Sal), Nicotine (Nic), Isolation (Iso), and Social partner (Soc).
[b] Abbreviations are described in the Methods (*Fos immunolabeling analysis*) section and Fig. 2 caption.
Asterisk (*) indicates a main effect of Social Condition, $p < 0.05$
Plus sign (+) indicates a main effect of Drug, $p < 0.05$
Dagger (†) indicates a decrease relative to Nic+Soc group (ps < 0.05, *post-hoc* Newman-Keuls following a Drug x Social Condition interaction)
Pound sign (#) represents a decrease relative to Sal+Iso negative controls (ps < 0.05, *post-hoc* Newman-Keuls following a Drug x Social Condition interaction).

Table 2 Behaviors measured during baseline, conditioning and test days

		Ball		Rat	
		Mesh	**Physical**	**Mesh**	**Physical**
Crossovers					
2 pairings	Baseline	30.0 ± 2.0	24.6 ± 1.9	30.0 ± 3.2	25.6 ± 1.4
	Test	45.2 ± 4.4*	41.2 ± 5.5*	53.9 ± 3.9*	47.1 ± 5.9*
8 pairings	Baseline	17.8 ± 1.4†	19.7 ± 2.2†	22.1 ± 2.0†	17.6 ± 2.6†
	Test	42.9 ± 7.0*	47.0 ± 4.9*	40.2 ± 4.5*	41.3 ± 1.7*
Number of physical contacts					
2 pairings	Day 1	—	46.8 ± 4.5	—	59.8 ± 6.9
	Day 2	—	47.3 ± 2.8	—	54.0 ± 8.7
8 pairings	Day 1	—	45.9 ± 4.5	—	53.3 ± 6.2
	Day 8	—	44.7 ± 6.7	—	37.4 ± 3.5
Time in physical contact					
2 pairings	Day 1	—	81.9 ± 9.2	—	464.5 ± 9.2+
	Day 2	—	113.8 ± 17.7	—	479.3 ± 15.5+
8 pairings	Day 1	—	61.5 ± 10.3	—	452.3 ± 13.8+
	Day 8	—	97.2 ± 23.2*	—	562.3 ± 11.9*+
Time in contact with mesh					
2 pairings	Day 1	124.0 ± 15.6	—	243.4 ± 21.3+	—
	Day 2	102.5 ± 14.0	—	254.8 ± 20.0+	—
8 pairings	Day 1	97.7 ± 10.8	—	285.4 ± 18.6+	—
	Day 8	184.1 ± 14.5*	—	333.6 ± 14.2*+	—
Time/contact with mesh					
2 pairings	Day 1	6.4 ± 0.6	—	6.4 ± 0.7	—
	Day 2	5.9 ± 0.5	—	6.8 ± 0.5	—
8 pairings	Day 1	6.9 ± 0.7	—	8.4 ± 0.7	—
	Day 8	6.4 ± 0.6*	—	5.8 ± 0.8*	—

Asterisk (*) indicates a main effect of Day; *Plus sign* (+) indicates a main effect of Object; Dagger (†) indicates difference from 2 pairings, Bonferroni t-test, $p<0.025$.

Table 3 Reinforcers obtained (±SEM) during sessions 1, 8 and 9 by female rats (*n* in parentheses) self-administering nicotine under isolated or social conditions at different phases of the estrous cycle in Experiment 2.

Cycle phase	Self-administration session					
	Isolated			Social		
	1	8	9	1	8	9
Estrus	9.2 ± 3.7 (6)	12.7 ± 7.9 (3)	7.7 ± 3.0 (7)	10.0 ± 2.7 (4)	6.5 ± 3.5 (2)	9.0 ± 2.3 (4)
Diestrus/metestrus	8.0 ± 3.1 (7)	12.6 ± 8.2 (5)	13.0 ± 5.0 (6)	8.5 ± 2.2 (12)	3.2 ± 0.8 (12)	4.6 ± 1.5 (11)
Proestrus	8.0 ± 3.0 (2)	10.0 ± 5.1 (7)	15.5 ± 15.5 (2)	15.3 ± 4.4 (3)	6.2 ± 2.1 (5)	3.3 ± 1.0 (4)

[1]Pseudopregnant females are represented in the Diestrus/metestrus phase.
No significant differences were found for estrous cycle on nicotine reinforcement.

a.

	Post-natal day and conditions/procedures																		
EXP 1	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
	Arrival, single-housed, handled												Habituation and baseline			Rest day	CS+ CS- Alt	CS+ CS- Alt	CPP Test & Perfused (N=40)
EXP 2	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
	Arrival, single-housed, handled										Habituation and baseline			Rest day	CS+	CS- Alt	CS- Alt	CS+ Perfused (N=40)	CPP Test (N=40)

b.

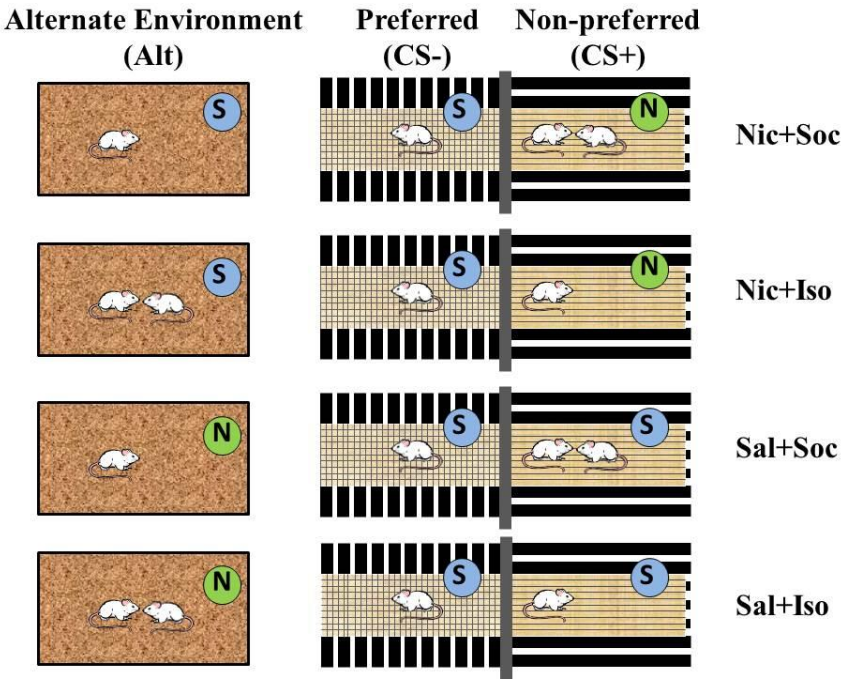
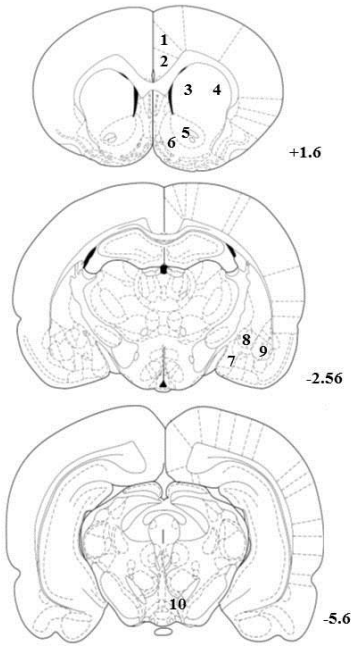


Figure 1 **a.** Timeline of the procedures across post-natal days (PNDs) for Experiments 1 and 2. Rats were given 2 conditioning sessions with their assigned unconditioned stimulus in their initially non-preferred side of the conditioned place preference (CPP) apparatus (CS+), 2 conditioning sessions in the absence of their unconditioned stimulus on their initially preferred side (CS-), and 2 conditioning sessions in the alternate environment (Alt) with exposure to unconditioned stimuli (US) that they had not received during CS+ sessions in order to equate US exposure across all groups (see b.). **b.** Illustration of US exposures in the alternate environment, initially preferred, and initially non-preferred sides of the apparatus. Baseline preference tests showed that roughly half of the rats preferred the horizontal-striped side and half preferred the vertical-striped side. US conditions included either nicotine (N; Nic) or saline (S; Sal) either while isolated (Iso) or with an age-, sex- and weight-matched social partner (Soc). Rats were deeply anesthetized, underwent perfusion, and brains were harvested for Fos protein immunohistochemistry.

a.



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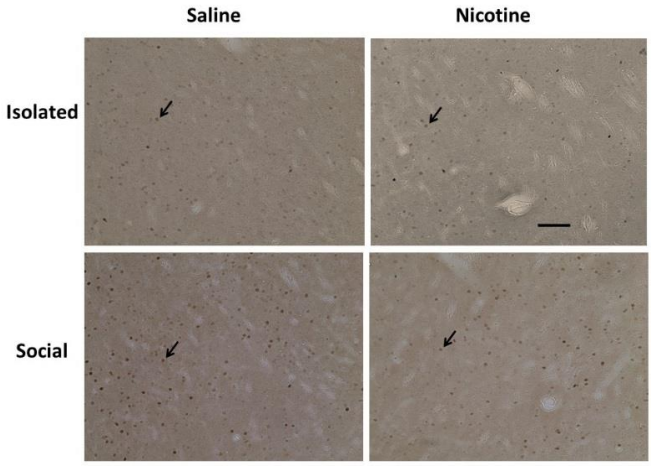
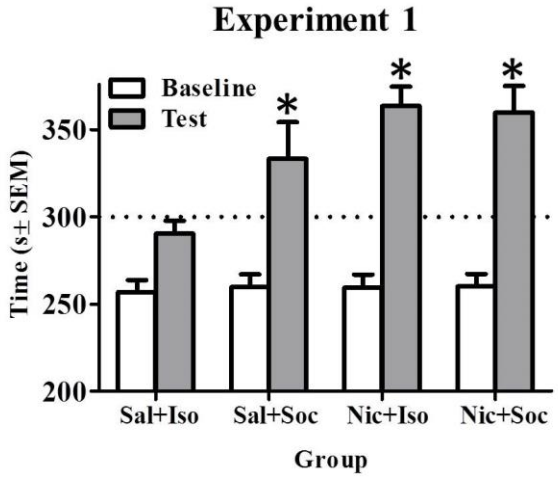


Figure 2 **a.** Schematic representation of coronal sections of the rat brain taken at +1.6, -2.56, and -5.6 mm from Bregma (Paxinos and Watson, 1998). Numbers in the sections represent the regions analyzed for Fos as follows: (1) Cg1 region of the anterior cingulate cortex (Cg1); (2) Cg2 region of the anterior cingulate cortex (Cg2); (3) dorsal medial caudate-putamen (dmCPu); (4) dorsal lateral caudate-putamen (dlCPu); (5) nucleus accumbens core (NAcC); (6) nucleus accumbens shell (NAcSh); (7) medial amygdala (MeA); (8) central amygdala (CeA); (9) basolateral amygdala (BlA); (10) ventral tegmental area (VTA). **a.** Representative photomicrographs from Experiment 2 showing coronal sections at 20× magnification in the nucleus accumbens core (NAcC). Examples of Fos protein labeling are shown by black arrows in representative rats that were sacrificed following the last US exposure, which is indicated by the row and column labels, such that all groups are represented. Scale bar is equal to 100 μm and all sample areas are equal to 0.26 mm^2.

a.



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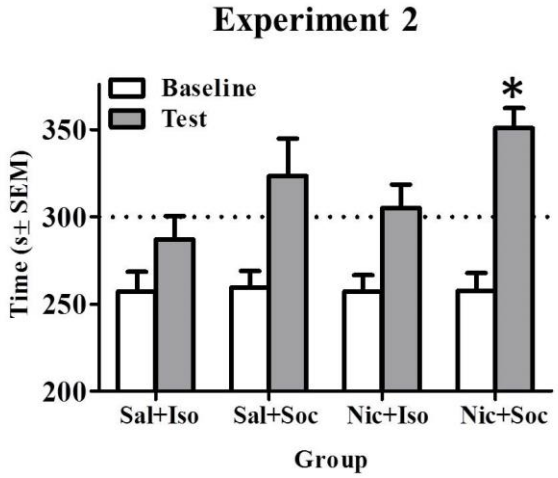


Figure 3 Nicotine (0.1 mg/kg S.C.) and/or social reward-CPP in Experiments 1 (a) and 2 (b) shown as time (mean s±SEM) spent in the US-paired side pre-conditioning (i.e., Baseline, white bars) vs. post-conditioning (i.e., Test, gray bars) across groups. The dotted line represents 50% of the total test time (i.e., 300 s). In both experiments, rats exhibited an increase in time spent on the US-paired side on Test day relative to Baseline regardless of group (main effect of Day, $p < 0.001$). However in Experiment 1, only the Sal+Soc (n=10), Nic+Iso (n=10) and Nic+Soc (n=11) groups exhibited a preference switch (i.e., > 50% of the total test time in their initially non-preferred side during the post-conditioning test), suggesting that these USs were rewarding. In Experiment 2, only the Sal+Soc (n=10), and Nic+Soc (n=10) groups spent > 50% of the total test time in their initially non-preferred side during the post-conditioning test. In both experiments, the Sal+Iso (n=9) controls spent < 50% of the total test time in their initially non-preferred side, suggesting reduction of initial aversion rather than conditioned reward. *Represents a significant increase relative to Sal+Iso controls (*a priori* planned comparisons, ps <0.01).

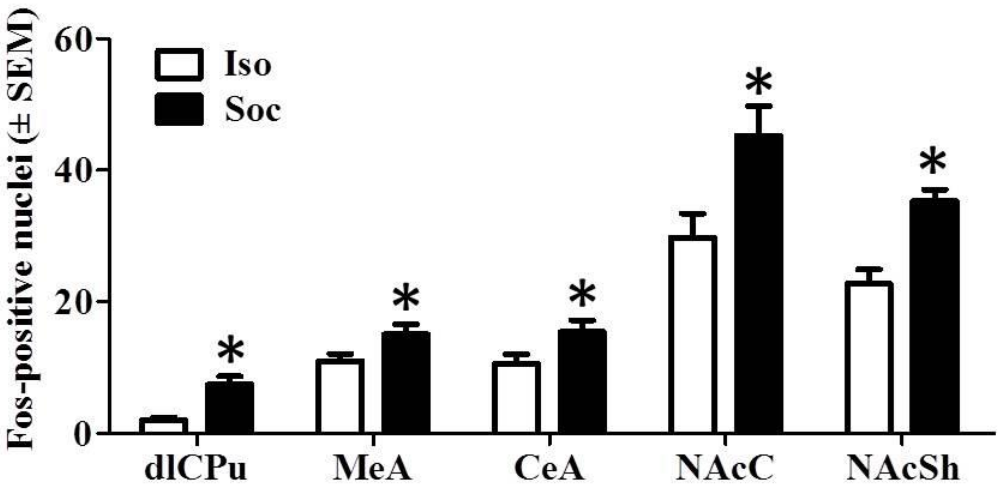


Figure 4 Number of Fos-positive nuclei/0.26 mm^2 ± SEM in regions where rats were sacrificed 90-min after the last US-conditioning session in the CS+ side of the apparatus alone (Iso) or with a social partner (Soc) in Experiment 2. Means shown are collapsed across Drug condition. *Asterisk* (*) represents a main effect of Social Condition, where social pairings increased Fos expression relative to isolation (ps < 0.05, ANOVA).

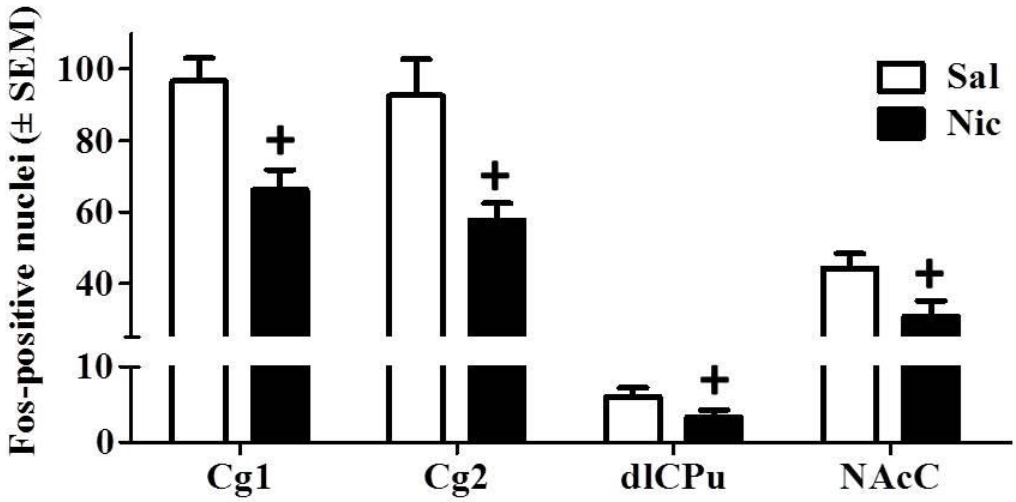


Figure 5 Number of Fos-positive nuclei/0.26 mm^2 ± SEM in regions where rats were sacrificed 90-min after the last US-conditioning session in the CS+ side of the apparatus with either saline (Sal) or nicotine (Nic) in Experiment 2. Means shown are collapsed across Social Condition. *Plus sign* (+) represents a main effect of Drug, where nicotine decreased Fos expression relative to saline (ps < 0.05, ANOVA).

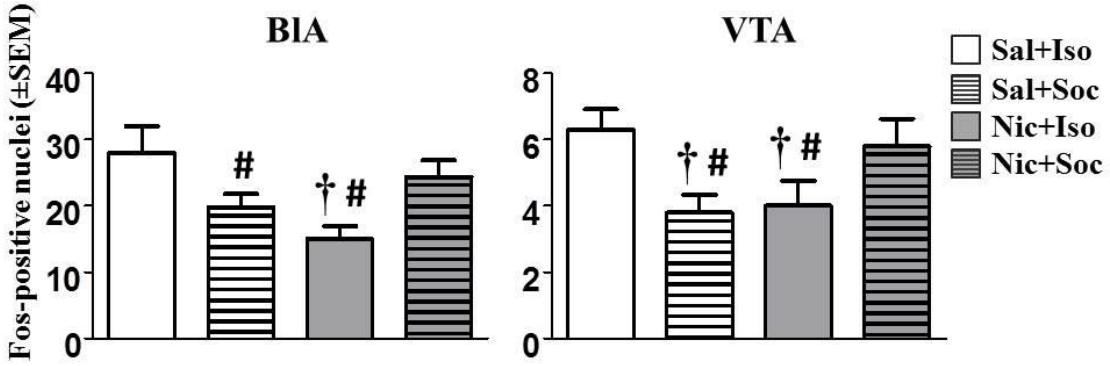
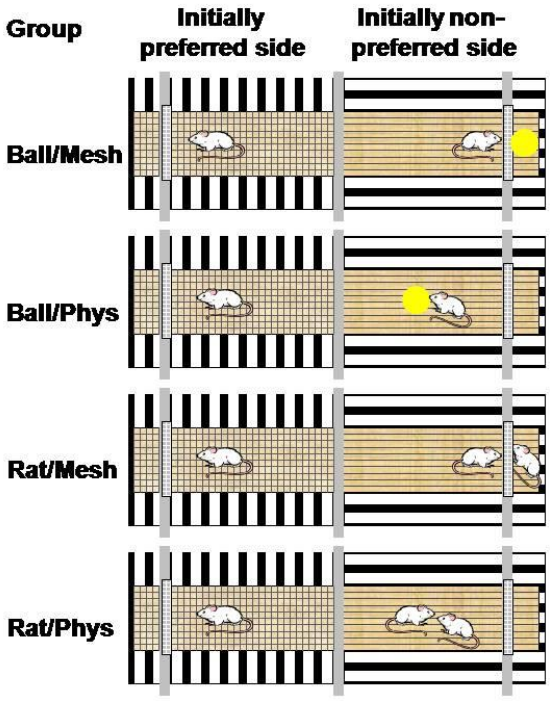


Figure 6 Number of Fos-positive nuclei/0.26 mm^2 ± SEM in regions where rats were sacrificed 90-min after the last US-conditioning session in the CS+ side of the apparatus with either saline (Sal) or nicotine (Nic) either alone (Iso) or with a social partner (Soc) in Experiment 2. *Pound sign* (#) represents a decrease relative to Sal+Iso negative controls (ps < 0.05, *post-hoc* Newman-Keuls). *Dagger* (†) represents a decrease relative to Nic+Soc group (ps ≤ 0.05, *post-hoc* Newman-Keuls).

a.



b.

Figure 7 Conditioning procedure (a) and apparatus (b). Two conditioning sessions took place daily for 10 min each separated by a 6-h interval. Baseline preference was determined and roughly half of the rats preferred the horizontal-striped side and half preferred the vertical-striped side. One session took place in the initially preferred side of the apparatus, during which the rat was alone. The other session took place in the initially nonpreferred side, during which the rat received exposure to the US. US conditions included either limited contact through the mesh barrier or full physical contact with either a tennis ball (Ball/Mesh and Ball/Phys, respectively) or a rat (Rat/Mesh or Rat/Phys, respectively). The photograph illustrates one side of the conditioning apparatus with a rat behind the mesh barrier.

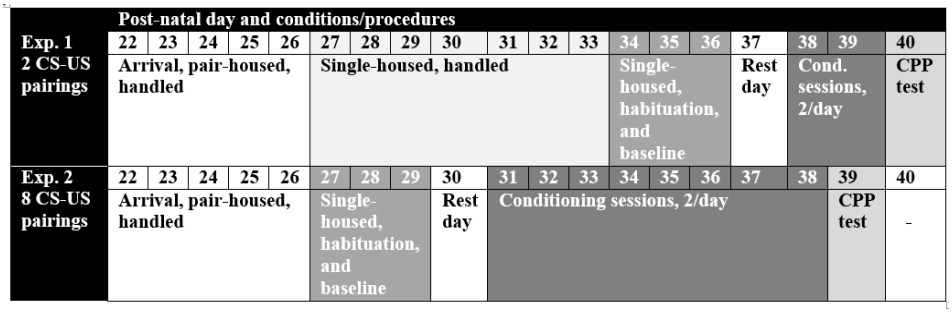


Figure 8 Timeline of the procedures across post-natal days (PNDs) for Experiments 1 and 2.

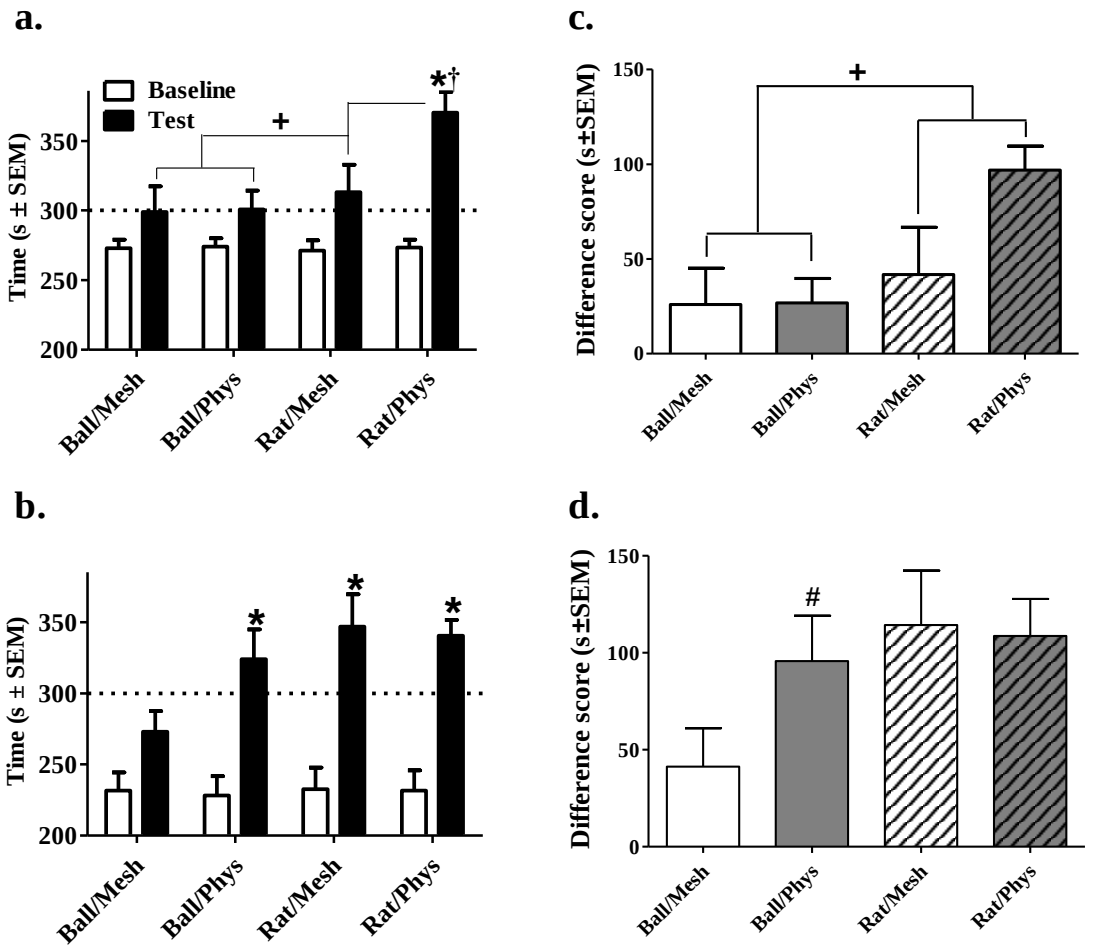


Figure 9 Object- (i.e., rat or ball) and Contact- (i.e., physical or mesh) dependent CPP after 2 or 8 CS-US pairings (a and b, respectively) expressed as the mean number of seconds ± SEM in the stimulus-paired side pre-conditioning (*i.e., Baseline, white bars*) vs. post-conditioning (*i.e., Test, black bars*). The *dotted line* represents 50% of the total test time (i.e., 300 seconds). Although all groups given 2 CS-US pairings exhibited an increase in time spent on the US-paired side on test day relative to baseline (main effect of day, $p<0.01$), the increase was greater when the object was a rat, and greatest in the Rat/Phys group. The only group that failed to display CPP with 8 CS-US pairings was the Ball/Mesh group. Preference data is also represented as difference scores of time spent in the US-paired side on test – baseline days (mean s ± SEM) after either 2 or 8 CS-US pairings (c and d, respectively) for the ball object (*i.e., solid bars*) or rat object (*i.e., striped bars*) with either physical contact (*i.e., gray bars*) or the object behind a mesh screen (*i.e., white bars*). *Plus sign* (+) indicates a main effect of Object, $p<0.05$; *Dagger* (†) indicates difference from all other groups, $p<0.05$; *Asterisk* (*) indicates an increase in the amount of time spent in the stimulus-paired side on Test day relative to Baseline, Bonferroni t-test, $p<0.0125$; *Pound sign* (#) indicates a difference from respective group given 2 CS-US pairings, test of simple effects, $p<0.05$.

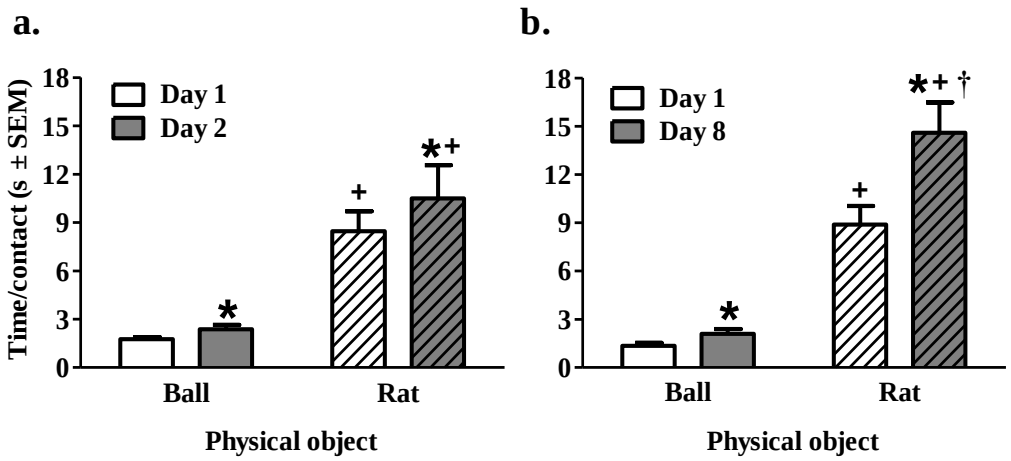


Figure 10 Time per contact for rats that received physical contact with a ball (Ball/Phys; *solid bars*) or a rat (Rat/Phys; *striped bars*) shown for the first (*i.e., white bars*) and last (*i.e., gray bars*) day of conditioning in Experiment 1 (a) and 2 (b). *Asterisk* (*) indicates a main effect of day where day 2 or day 8 is greater than day 1, $p<0.05$. *Plus sign* (+) indicates a main effect of Object where a rat is greater than a ball, $p<0.001$. *Dagger* (†) represents a greater increase from Day 1 to Day 8 relative to that of the Ball condition, tests of simple effects, $p<0.05$.

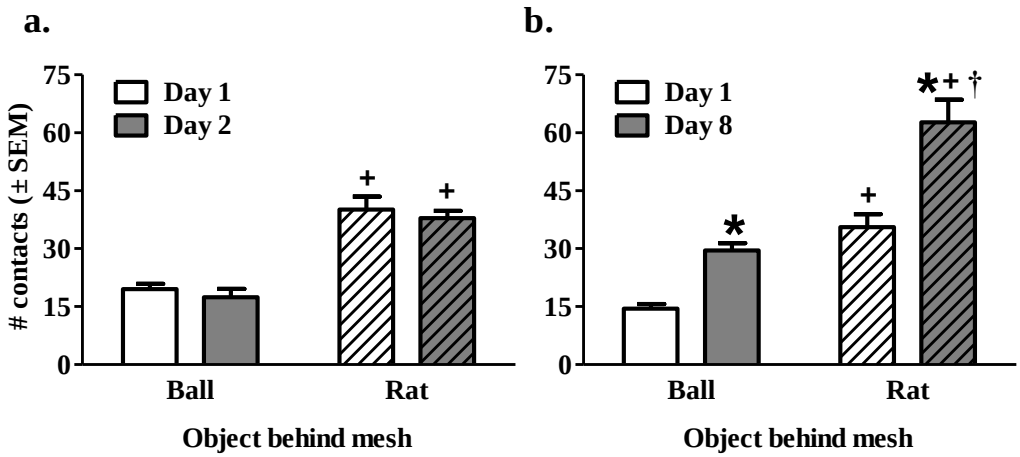
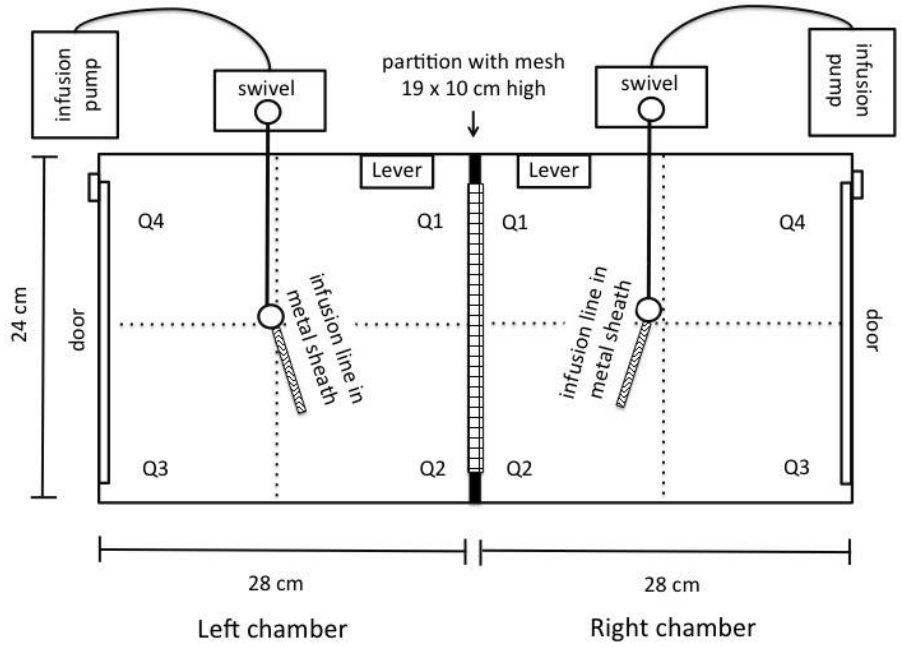


Figure 11 Number of contact with the mesh screen in front of a tennis ball (Ball/Mesh; *solid bars*) or rat (Rat/Mesh *striped bars*) on the first (*i.e., white bars*) and last (*i.e., gray bars*) day of conditioning in Experiment 1 (**a**) and 2 (**b**). *Asterisk* (*) indicates a main effect of day where day 8 is greater than day 1, $p<0.001$. *Plus sign* (+) indicates a main effect of object where a rat is greater than a ball, $p<0.001$. *Dagger* (†) represents a greater increase from Day 1 to Day 8 relative to that of the Ball condition, tests of simple effects, $p<0.05$.

a.



b.

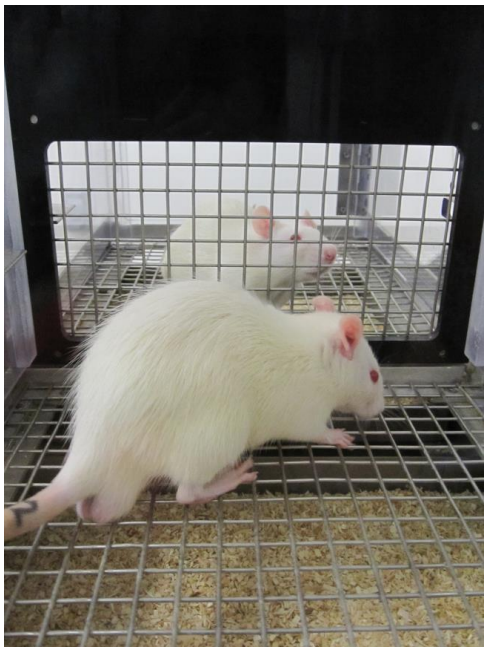


Figure 12 Arial configuration (a) and a side profile picture (b) of the self-administration apparatus with conjoined chambers that were separated by a partition. Rats were connected to infusion lines surrounded by a flexible metal sheath and then placed into the neighboring chambers either with a solid black Plexiglas partition in place isolating the rats during the session (Iso; not shown) or with a black Plexiglas partition containing a wire mesh section that allowed for visual and some tactile social cues, and stronger

olfactory and auditory social cues during sessions (Soc; shown in B). Each chamber contained a retractable lever (active lever; i.e., reinforced lever) located 2.5 cm from the dividing partition wall and 7.5 cm above the floor. Experiment 4 included the addition of a non-retractable inactive lever (i.e., non-reinforced control lever; not pictured) on the wall opposite the active lever located 2.5 cm from the dividing partition wall and 7.5 cm above the floor. A camera sensitive to low levels of light (Panasonic WV-CP284, color CCTV, Suzhou, China) was used to record self-administration sessions and was mounted 60 cm above the center of the apparatus. A WinTV 350 personal video recorder (Hauppage, NJ, USA) captured live video and encoded it into MPEG streams for later analysis. Later videos were analyzed for entries into the 4 quadrants (Q1-Q4) demarcated by lines drawn on the display.

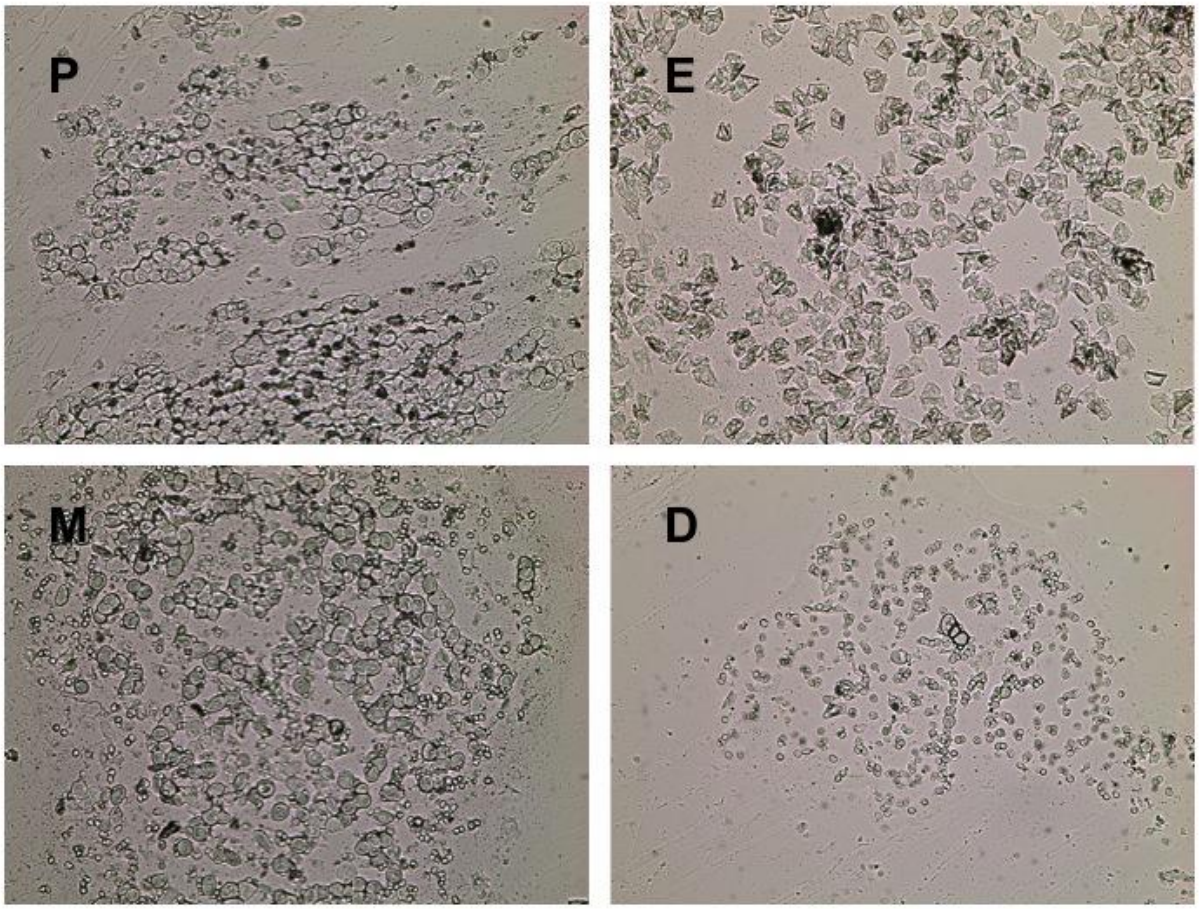


Figure 13 Representative photomicrographs (10x) of unstained vaginal smears taken at: proestrus (P), with predominantly nucleated epithelial cells; estrus (E), with cornified cells lacking nuclei; metestrus (M), with similar proportions of leukocytes, cornified and nucleated epithelial cells; and diestrus (D), with primarily leukocytes

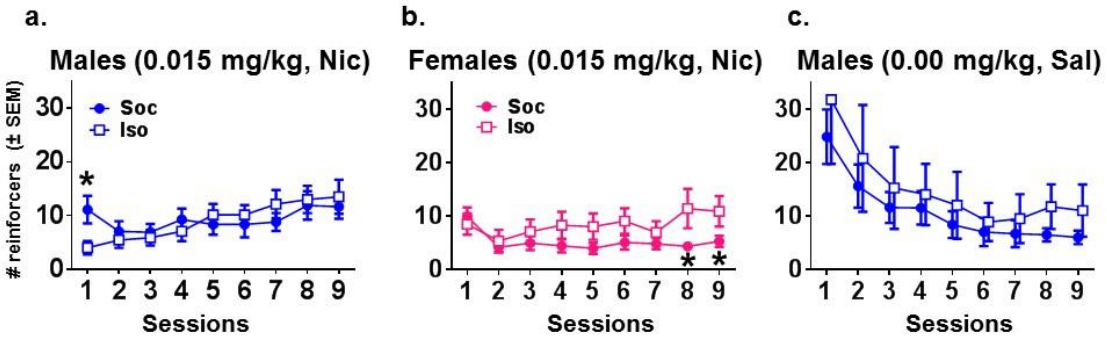


Figure 14 Mean reinforcers obtained (±SEM) in Experiments 1-3 across acquisition sessions conducted with male (a; Experiment 1; blue symbols) and female (b; Experiment 2; pink symbols) rats given nicotine (0.015 mg/kg, IV; Nic) or male (c: Experiment 3; blue symbols) rats given access to saline (0.00 mg/kg; Sal) on a FR1 schedule of reinforcement while isolated (Iso: open squares) or while allowed limited social contact through a mesh barrier (Soc: closed circles). Asterisk (*) represents a difference from Iso, test of simple effects, $p<0.05$

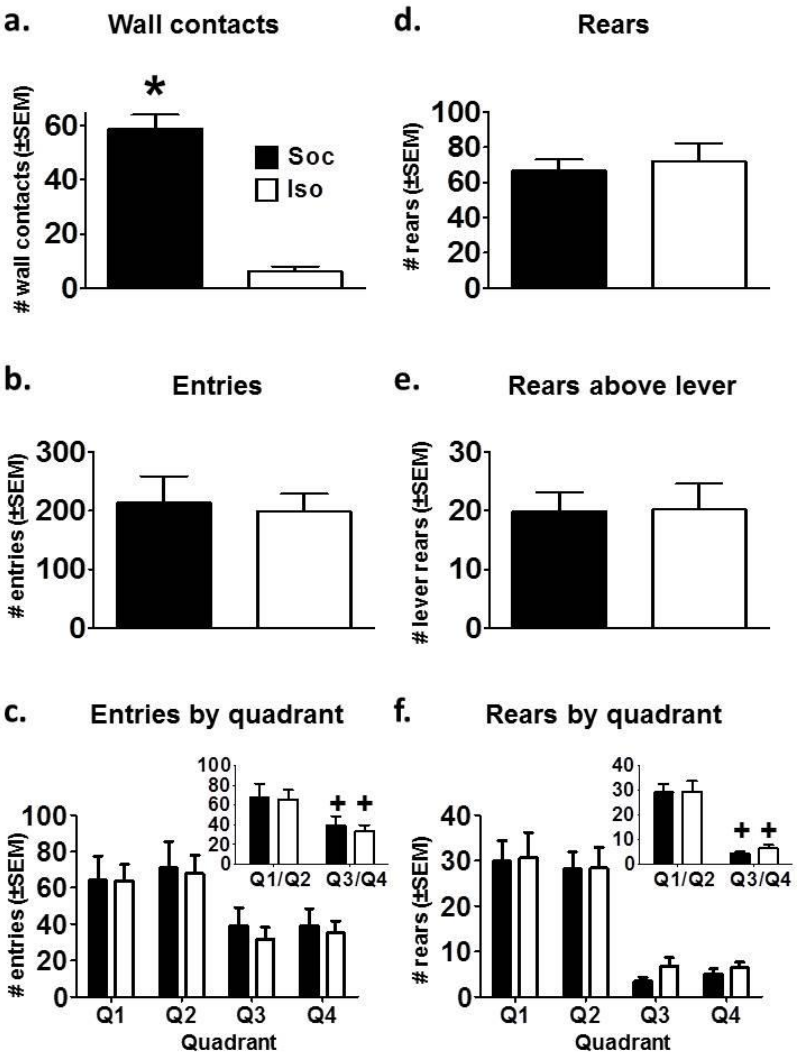


Figure 15 Mean (±SEM) time-sampled incidences of wall contacts in Q1 and Q2 (a), total forepaw entries into all quadrants (b), forepaw entries by quadrant (c), total rears in all quadrants (d), total rears directly over the lever in Q1 (e), and number of rears by quadrant (f) for male rats that were either isolated (Iso, white bars) or given limited social contact through a mesh barrier (Soc, black bars) during the first nicotine self-administration session in Experiment 1. Insets depict the average of Q1 and Q2 versus the average of Q3 and Q4 (i.e., main effect of quadrant). Asterisk (*) represents an increase compared to the Iso group, independent samples *t*-test, $p<0.001$ and Plus sign (+) represents a decrease from Q1/Q2, Wilcoxon, $p<0.05$

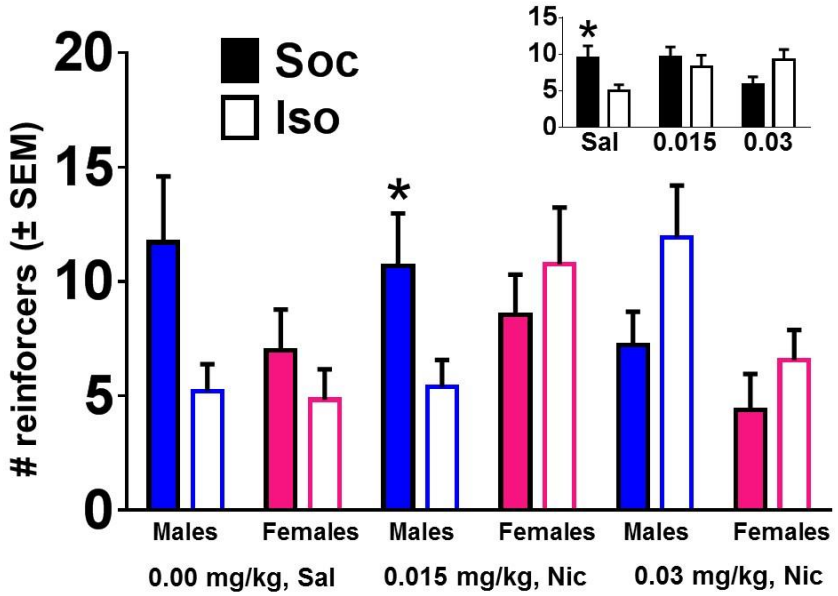


Figure 16 Mean reinforcers obtained (±SEM) on the first session of Experiment 4 conducted with male (blue bars) and female (pink bars) rats given saline (0.00 mg/kg, Sal) or nicotine (0.015, 0.03 mg/kg, IV; Nic) on a FR1 schedule of reinforcement while isolated (Iso: open bars) or while allowed limited social contact through a mesh barrier (Soc: solid bars). Asterisk (*) represents a difference from Iso, test of simple effects, $p<0.05$

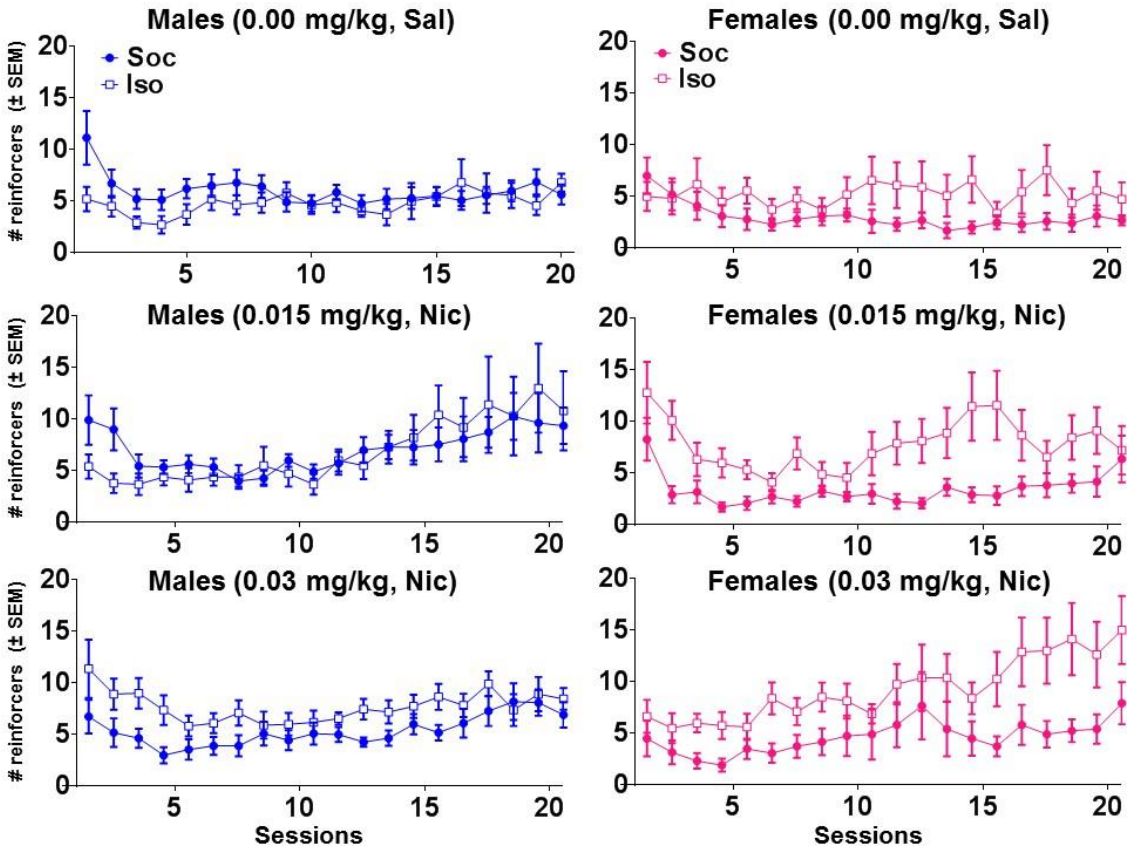


Figure 17 Mean reinforcers obtained (±SEM) in Experiment 4 across acquisition sessions conducted with male (blue symbols) and female (pink symbols) rats given saline (0.00 mg/kg, Sal) or nicotine (0.015, 0.03 mg/kg, IV; Nic) on a FR1 (sessions 1-3) or a progressive (sessions 4-20) schedule of reinforcement while isolated (Iso: open squares) or while allowed limited social contact through a mesh barrier (Soc: closed circles). A 4-way Session × Sex × Dose × Social Condition interaction was detected, ANOVA $p<0.05$. *Post-hoc* findings are shown in Figures 18 and 19.

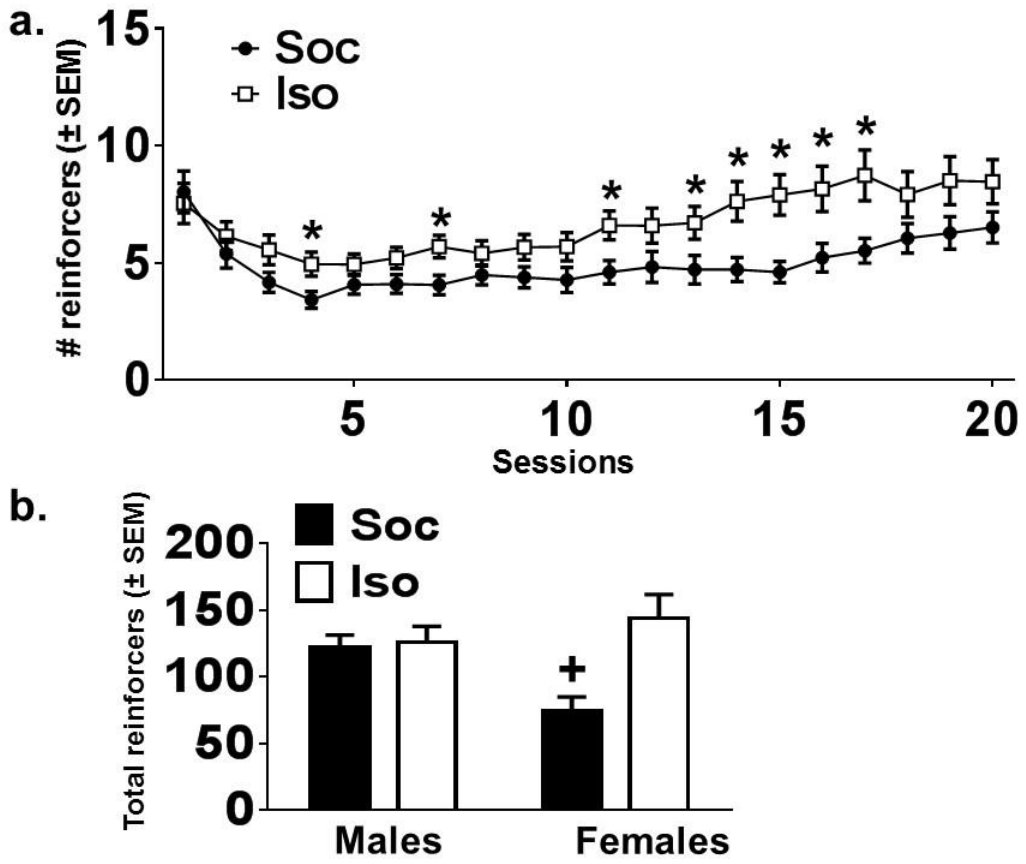


Figure 18 Mean reinforcers obtained (±SEM) in Experiment 4 across acquisition sessions (a) collapsed across Sex and Dose, total reinforcers obtained (±SEM) (b) collapsed across Session and Dose represented for isolated (Iso: open squares/bars) Social (Soc: closed circles/bars) rats in Experiment 4. Asterisk (*) represents a difference from Soc, test of simple effects, *ps*<0.05. Plus sign (+) represent a difference from all other groups, test of simple effects, *p*<0.05.

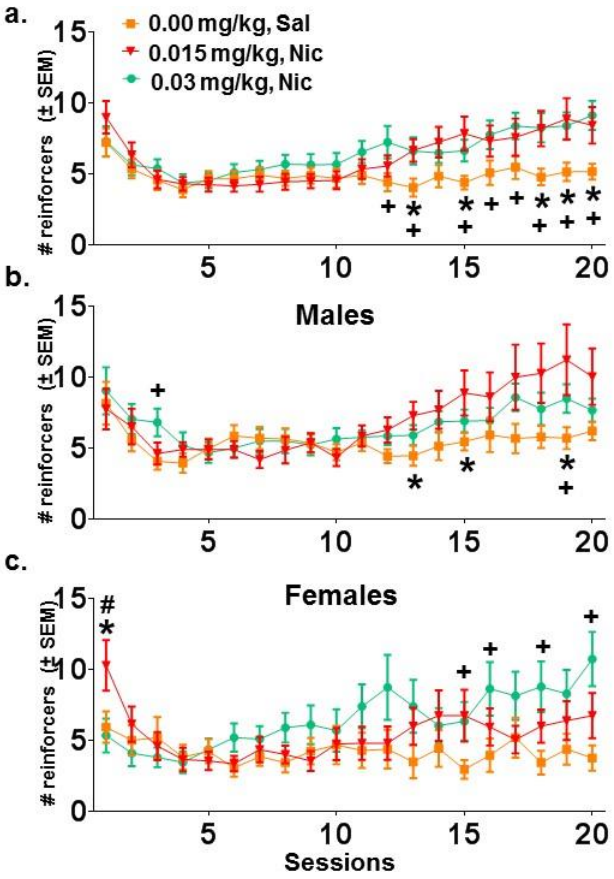


Figure 19 Mean reinforcers obtained (±SEM) across acquisition sessions in Experiment 4 (a) collapsed across Sex and Social Condition, and collapsed across Social Condition for males (b) and females (c) given Saline (0.00 mg/kg, Sal: orange squares) or nicotine (0.015 mg/kg, Nic: red triangles; 0.03 mg/kg, Nic: green circles). Asterisk (*) represents a difference between 0.015 mg/kg, Nic and 0.00 mg/kg (Sal), test of simple effects, *ps*<0.05. Plus sign (+) represents a difference between 0.03 mg/kg, Nic and 0.00 mg/kg, test of simple effects, *ps*<0.05. Pound sign (#) represents a difference between 0.015 mg/kg, Nic and 0.03 mg/kg, Nic, test of simple effects, *p*<0.05.

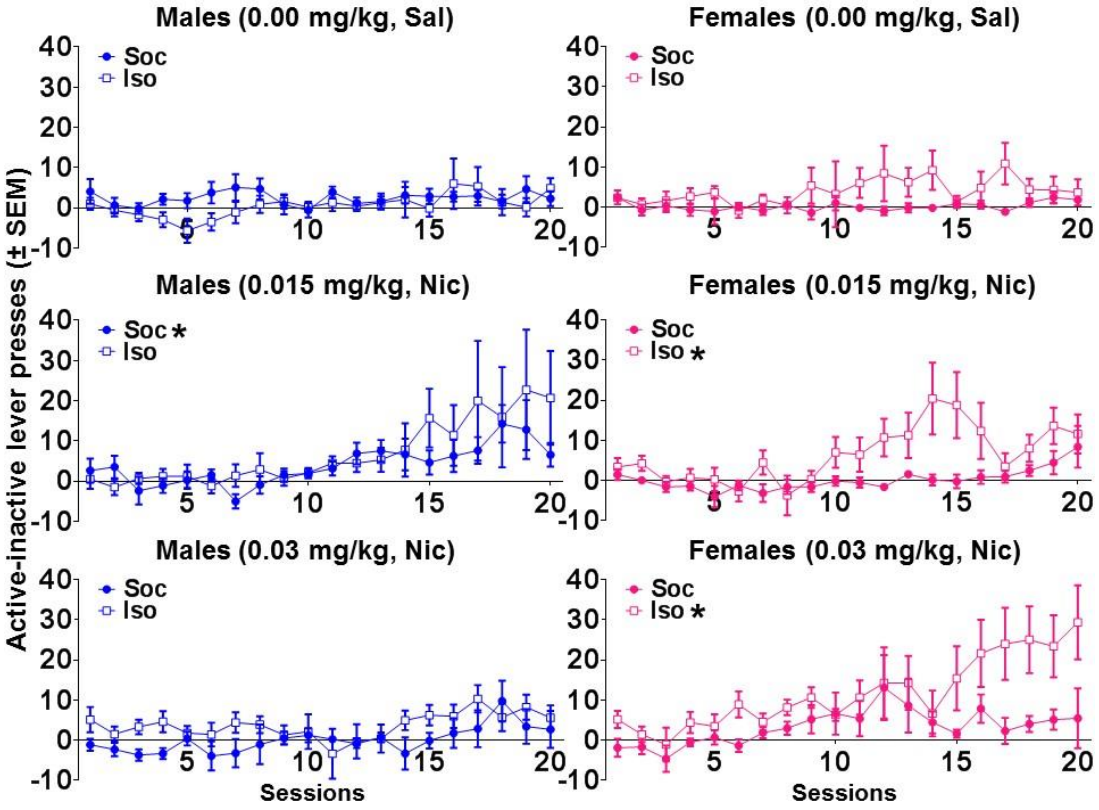


Figure 20 Mean difference for active minus inactive lever presses (±SEM) in Experiment 4 across acquisition sessions conducted with male (blue symbols) and female (pink symbols) rats given saline (0.00 mg/kg, Sal) or nicotine (0.015, 0.03 mg/kg, IV; Nic) on a FR1 (sessions 1-3) or a progressing FR1 to FR3 (sessions 4-20) schedule of reinforcement while isolated (Iso: open squares) or while allowed limited social contact through a mesh barrier (Soc: closed circles). Asterisk (*) represents groups that responded significantly more on the active vs. inactive lever collapsed across session; Soc males self-administering 0.015 mg/kg, Nic and Iso females self-administering 0.015 mg/kg and 0.03 mg/kg, Nic responded significantly more on the active vs. inactive lever, tests of simple effects, *ps*<0.05.

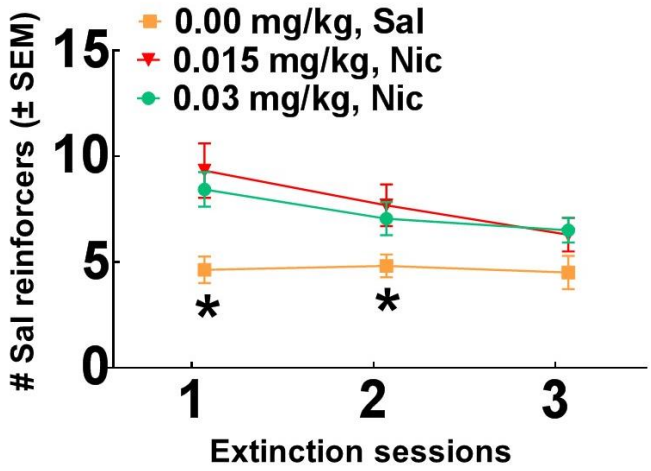


Figure 21 Mean Saline (Sal) reinforcers obtained (±SEM) in Experiment 4 across Extinction sessions collapsed across Sex and Social Condition for rats that were previously trained with Saline (0.00 mg/kg, Sal: orange squares) or nicotine (0.015 mg/kg, Nic: red triangles; 0.03 mg/kg, Nic: green circles). Asterisk (*) represents a decrease relative to 0.015 mg/kg, Nic and 0.03 mg/kg, Nic, test of simple effects, *ps*<0.05. Plus sign (+) represents a difference between 0.03 mg/kg, Nic and 0.00 mg/kg, Sal, test of simple effects, *ps*<0.05. Pound sign (#) represents a difference between 0.015 mg/kg, Nic and 0.03 mg/kg, Nic, test of simple effects, *ps*<0.05.

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APPENDIX A

CIRRICULUM VITAE

CURRICULUM VITAE

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FELLOWSHIPS AND AWARDS

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- NIDA Early Career Investigator Travel Award (2012), NIDA mini-conference/SfN
- NIDA Early Career Investigator Travel Award (2011), NIDA mini-conference/SfN
- NIDA Directors Travel Award (2011), College on Problems in Drug Dependence conference
- NIH Diversity Supplement Recipient (2010-2012)

University

- Faculty Women's Association Distinguished Graduate Student Award (2015)
- College of Liberal Arts and Sciences Graduate Excellence Award (2014)
- More Graduate Education @ Mountain State Alliances Pre-doctoral Program (2009-2014)
- Commendation for outstanding service, Arizona State Universty Faculty Women's Association (2012)
- Pre-Doctoral Diversity Fellowship: Arizona State Graduate College (2008-2009)

- Summa Cum Laude (2008)
- Academic Dean's Honor's List (2004-2008)

PROFESSIONAL EXPERIENCE

2008-current *Graduate Research Associate*, Department of Psychology, Arizona State University, Tempe.

2007-2008 *Undergraduate Research Assistant*, Department of Psychology, Arizona State University, Tempe.

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- College on Problems of Drug Dependence
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PUBLICATIONS

Kufahl, P.R., Peartree, N.A., Heintzelman, K.L., Chung, M., & Neisewander, J.L. 2015. Intravenous cocaine challenge induces c-fos under isoflurane anesthesia in rats with a history of repeated cocaine administration. *Brain Research.*1594:256-66.

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Peartree, N.A., Sanabria, F., Thiel, K.J., Weber, S.M., Cheung, T.H.C., & Neisewander, J.L. 2012. A new criterion for acquisition of nicotine self-administration in rats. *Drug & Alcohol Dependence*. 124(1-2):63-9.

Peartree, N.A., Hood, L.E., Thiel, K.J., Sanabria, F., & Neisewander, J.L. 2012. Limited physical contact through a mesh barrier is sufficient for social-reward conditioned place preference in adolescent male rats. *Physiology & Behavior*. 105(3):749-56.

Pentkowski, N.S., Painter, M.R., Thiel, K.J., Peartree, N.A., Cheung, T.H.C., Deviche, P., Adams, M., Alba, J., & Neisewander, J.L. 2011. Nicotine-induced plasma corticosterone is attenuated by social interactions in male and female adolescent rats. *Pharmacology Biochemistry & Behavior*. 100(1):1-7.

Thiel, K.J., Pentkowski, N.S., Peartree, N.A., Painter M.R., & Neisewander, J.L. 2010. Environmental living conditions introduced during forced abstinence alter cocaine-seeking behavior and Fos protein expression. *Neuroscience*. 171(4):1187-96.

Thiel, K.J., Engelhardt, B., Hood, L.E., Peartree, N.A., & Neisewander, J.L. 2010. The interactive effects of environmental enrichment and extinction interventions in attenuating cue-elicited cocaine-seeking behavior in rats. *Pharmacology Biochemistry & Behavior*. 97(3):595-602.

MEETING PRESENTATIONS AND ABSTRACTS

Peartree, N.A., Kufahl, P.R., Heintzelman, K.L., Chung, M., & Neisewander, J.L. (November 2013). Intravenous cocaine challenge induces *c-fos* under isoflurane anesthesia in rats with a history of repeated cocaine administration. **43rd Annual Society for Neuroscience Meeting Abstract**, San Diego, CA.

Peartree, N.A., Bastle, R.M., Williams, A.M., Goenaga, J., Chandler, K.N., Hood, L.E., & Neisewander, J.L. (June 2013). Fos expression after exposure to social and nicotine rewards or reward-conditioned environments in adolescent male rats. **22nd Annual International Behavioral Neuroscience Society Meeting Abstract**, Malahide, Ireland.

Peartree, N.A., Bastle, R.M., Williams, A.M., Chandler, K.N., Goenaga, J., Hood, L.E., & Neisewander, J.L. (April 2013). Brain activation after exposure to social and nicotine rewards in adolescent rats. **36thth Biennial Society for Research in Child Development Conference Symposium Presentation**, Seattle, WA.

Peartree, N.A., Williams, A.M., Hood, L.E., Chandler, K.N., Goenaga, J., Bastle, R.M., & Neisewander, J.L. (February 2013). Fos expression after exposure to social and nicotine rewards or reward-conditioned environments in adolescent male rats. **MGE@MSA 11th Annual Student Research Conference Abstract**, Tempe, AZ.

Peartree, N.A., Williams, A.M., Hood, L.E., Chandler, K.N., Goenaga, J., Bastle, R.M., & Neisewander, J.L. (October 2012). Fos expression after exposure to social and nicotine rewards or reward-conditioned environments in adolescent male rats. **42nd Annual Society for Neuroscience Meeting Abstract**, New Orleans, LA.

Chandler, K.N., Peartree, N.A., Goenaga, J., Whillock, C., Dado, N., Cheung, T.H.C., Bimonte-Nelson, H.A., & Neisewander, J.L. (October 2012). Sex differences in the effects of social context on acquisition of nicotine self-administration in adolescent rats. **42nd Annual Society for Neuroscience Meeting Abstract**, New Orleans, LA.

Peartree, N.A., Williams, A.M., Hood, L.E., Chandler, K.N., Goenaga, J., Bastle, R.M., & Neisewander, J.L. (October 2012). Fos expression after exposure to social and nicotine rewards or reward-conditioned environments in adolescent male rats. **Frontiers in Addiction Research NIDA mini-convention**, New Orleans, LA.

Chandler, K.N., Peartree, N.A., Goenaga, J., Whillock, C., Dado, N., Cheung, T.H.C., Bimonte-Nelson, H.A., & Neisewander, J.L. (October 2012). Sex differences in the effects of social context on acquisition of nicotine self-administration in adolescent rats. **3rd Annual Society for Social Neuroscience Meeting Abstract**, New Orleans, LA.

Peartree, N.A., Chandler, K.N., Goenaga, J., Dado, N., Whillock, C., & Neisewander, J.L. (June 2012). Social context enhances initial reinforcing effects of nicotine in male but

not female rats. **21st Annual International Behavioral Neuroscience Society Meeting Abstract**, Keauhou-Kona, Hawaii.

Peartree, N.A., Williams, A., Hood, L.E., Thiel, K.J., & Neisewander, J.L. (February 2012). Neural activation following exposure to social and nicotine reward-conditioned environments. **MGE@MSA 10th Annual Student Research Conference Abstract**, Tempe, AZ.

Peartree, N.A., Hood, L.E., Adams, M.A., Chandler, K.N., & Neisewander, J.L. (November 2011). Social context increases nicotine intake during initial opportunity for self-administration. **Frontiers in Addiction Research NIDA mini-convention**, Washington D.C.

Peartree, N.A., Hood, L.E., Adams, M.A., Chandler, K.N., & Neisewander, J.L. (November 2011). Social context increases nicotine intake during initial opportunity for self-administration. **41st Annual Society for Neuroscience Meeting Abstract**, Washington D.C.

Peartree, N.A., Pentkowski, N.S., Painter, M.R., Cheung, T.H.C., Thiel, K.J., & Neisewander, J.L. (June 2011). The influence of social interactions and nicotine on corticosterone and behavioral responses in female and male adolescent rats. **73rd Annual College on Problems of Drug Dependence Meeting Symposium Presentation**, Hollywood, FL.

Peartree, N.A., Williams, A., Hood, L.E., Thiel, K.J., & Neisewander, J.L. (February 2011). Fos expression after exposure to social and nicotine reward-conditioned environments. **MGE@MSA 9th Annual Student Research Conference Abstract**, Tempe, AZ.

Peartree, N.A., Williams, A., Hood, L.E., Thiel, K.J., & Neisewander, J.L. (November 2010). Fos expression after exposure to social and nicotine reward-conditioned environments. **40th Annual Society for Neuroscience Meeting Abstract**, San Diego, CA.

Neisewander, J.L., Thiel, K.J., Engelhardt, B., Hood, L.E., & Peartree, N.A. (November 2010). Extinction training enhances the beneficial effects of environmental enrichment in reducing cue-elicited cocaine-seeking in rats. **40th Annual Society for Neuroscience Meeting Abstract**, San Diego, CA.

Thiel, K.J., Pentkowski, N.S., Painter, M.R., Peartree, N.A., Mitroi, D., Crawford, C.A., & Neisewander, J.L. (November 2010). Environmental enrichment during abstinence from cocaine self-administration attenuates cocaine-seeking behavior and functional brain activation in rats. **40th Annual Society for Neuroscience Meeting Abstract**, San Diego, CA.

Elizabeth Engler-Chiurazzi, Candy Tsang, Alexandra Garcia, Sarah Mennenga, Madeline Andrews, Jazmin Acosta, Joshua Talboom, Brittany Blair Braden, Bryan Camp, Natalie Peartree, Cynthia Zay, Steven Holloway, Veronica Santos, Stephen Helms Tillery, Micheal McBeath & Heather Bimonte-Nelson. (November 2010). Generating new synapses at the first Arizona State University Brain Fair for Children: Teaching that "knowledge is power." **40th Annual Society for Neuroscience Meeting Abstract**, San Diego, CA.

Peartree, N.A., Hood, L.E., Thiel, K.J., Sanabria, F., & Neisewander, J. L. (June 2010). Social encounter with limited physical access produces conditioned place preference in

male adolescent rats. **19th Annual International Behavioral Neuroscience Society Meeting Abstract**, Villasimius, Sardinia, Italy.

Peartree, N.A., Sanabria, F., Hills, D., Painter M., Thiel, K.J., Weber, S., Hood, L.E., & Neisewander, J. L. (June 2010). Comparison of acquisition and maintenance of nicotine vs. cocaine self-administration using lever-retraction cues. **72nd Annual College on Problems of Drug Dependence Meeting Abstract**, Scottsdale, AZ.

Thiel, K.J., Engelhardt, B., Hood, L.E., Pentkowski, N.S., Peartree, N.A., Painter, M.R., & Neisewander, J.L. (June 2010). Preclinical examination of environmental enrichment as an anti-relapse strategy. **72nd Annual College on Problems of Drug Dependence Meeting Presentation**, Scottsdale, AZ.

Nolan, B.C., Peartree, N.A., Robertson, L.M., Weber, S.M., Barajas, M., Mach, R.H., Luedtke, R.R., & Neisewander, J.L. (June 2010). Attenuation of cocaine self-administration by novel dopamine D3 receptor ligands. **72nd Annual College on Problems of Drug Dependence Meeting Abstract**, Scottsdale, AZ.

Peartree, N.A., Sanabria, F., Hills, D., Painter M., Thiel, K.J., Weber, S., Hood, L.E., & Neisewander, J. L. (January 2010). Mathematical modeling of acquisition of nicotine self-administration in adolescent rats. **MGE@MSA 8th Annual Student Research Conference Abstract**, Tempe, AZ.

Peartree, N.A., Sababria, F., Hills, D., Painter M., Thiel, K.J., Weber, S., Hood, L.E., & Neisewander, J. L. (October 2009). Mathematical modeling of acquisition of nicotine self-administration in adolescent rats. **39th Annual Society for Neuroscience Meeting Abstract**, Chicago, IL.

Nolan, B.C., Peartree, N.A., Robertson, L.M., Weber, S.M., Barajas, M., Mach, R.H., Luedtke, R.R., & Neisewander, J.L. (October 2009). Attenuation of cocaine self-administration by novel dopamine D3 receptor ligands. **39th Annual Society for Neuroscience Meeting Abstract**, Chicago, IL.

COLLOQUIA AND INVITED PRESENTATIONS

"A tale of two studies: Peer influence on nicotine reward and reinforcement" (October 2013). Department of Psychology, Arizona State University, Tempe

"Social Buffering and Nicotine" (January 2013). Department of Psychology, Arizona State University, Tempe

"Multi-faceted role of social interaction in drug abuse:Are friends good or bad?" (October 2011). Department of Psychology, Arizona State University, Tempe

"Substance abuse and the brain" (April 2011). McClintock High School, Tempe

"Limited physical contact through a mesh barrier is sufficient for social-reward conditioned place preference in adolescent male rats" (February 2011). Department of Psychology, Arizona State University, Tempe

"Comparison of acquisition and maintenance of nicotine vs. cocaine self-administration using lever-retraction cues" (November 2009). Department of Psychology, Arizona State University, Tempe

"Nicotine self-administration in rodents" (May 2009). Department of Psychology, Arizona State University, Tempe

"Drug abuse in the animal model" (April 2009). Diversity Across Curriculum Organization, Arizona State University, Tempe

TEACHING AND TRAINING EXPERIENCE

Teaching Assistantships

2012 Teaching Assistant, Physiological Psychology, Arizona State University

2012 Teaching Assistant, Learning and Motivation Course, Arizona State University

2012 Teaching Assistant, Introduction to Psychology, Arizona State University

2011 Teaching Assistant, Psychopharmacology Course, Arizona State University

Undergraduate Student Research Training/Supervision

Arizona State University

Demi Hills	Mike Painter	Justin Wilson	Claudia Valles
Lauren Hood+	Nicole Fruth*	Lindsey Robertson	Heather Koch*
Angela Williams*	Claudia Valles	Ben Engelhardt*	Jose Alba ∆
Jared Deunsing•	Emile Saad•	Jonathan Griffin*	Kayla Chandler*+
Varun Patel*+	Julianna Goenaga*+	Colter Whillock	Nora Dado
Mandy Xu*	Breanne Menth	Samuel Brunwasser*	Kenneth Leslie
Hanna Molla^	Martin Dufwenberg*	Allegra Campagna*	Rachel Mendoza*

* *denotes Barrett honors college student*
• *denotes McClintock High School Peggy Payne Academy Student*
\+ *denotes SOLUR recipient*
∆ *NIH diversity supplement recipient*
^ *denotes PREP program student*

Technician Research Training/Supervision

Arizona State University

Suzanne Weber, B.S	Matthew Adams, B.S.
Lindsey Robertson, B.A.	John Paul Bonadonna, B.S.

Graduate Student/Postdoctoral Fellow Research Training/Supervision

Arizona State University

Peter Kufhal, Ph.D.	Brian C. Nolan, Ph.D.	Nathan Pentkowski, Ph.D.
Lara Pockros, MA	Ryan Bastle, BA	Timothy Cheung, Ph.D.

PROFESSIONAL ACTIVITIES

Committee Service

2013-2016 Committee Member, Program Committee, International Behavioral Neuroscience Society

2013-2014 Committee Member, Local Organizing Committee, International Behavioral Neuroscience Society

2012-2013 Committee Member, Animal Use/Ethics Committee & Membership Committee, International Behavioral Neuroscience Society

2012 Committee Member, Honors Thesis for Kayla Chandler, Arizona State University

2012 Committee Member, Honors Thesis for Julianna Goenaga, Arizona State University

2010 Committee Member, Honors Thesis for Heather Koch, Arizona State University

2009 Committee Member, Honors Thesis for Angela Williams, Arizona State University

Departmental Service

2009-2012 Exam Proctor, Arizona State University

Community Service

2014 Student Scholars Summer Collegiate Program

2012 Brain Awareness Week, Arizona State University

2011 Brain Awareness Week, Arizona State University Brain Fair

2011 Substance Abuse & the Brain, a presentation to students at McClintock High School

2010 Brain Awareness Week, Arizona State University Brain Fair

2009 Brain Awareness Week, Kyrene del Norte Elementary School

Scientific Service

2012 Reviewer for journal article submission in *Behavioral Brain Research*

2011 Reviewer for journal article submission in *Behavioral Brain Research*