



Nicotine-induced plasma corticosterone is attenuated by social interactions in male and female adolescent rats

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ABSTRACT

Most smokers begin smoking during adolescence, a period during which social reward is highly influential. Initial exposure to nicotine can produce anxiogenic effects that may be influenced by social context. This study examined play behavior and plasma corticosterone following nicotine administration (0.6 mg/kg, s.c.) in both male and female adolescent (PND39) Sprague–Dawley rats in either isolate or social contexts. In blood samples collected immediately following the 15-min test session, nicotine increased plasma corticosterone relative to saline in both male and female isolate rats, but failed to do so in both males and females placed together in same-sex pairs. Nicotine also attenuated several indices of play behavior including nape attacks, pins and social contact. In isolate rats, nicotine selectively increased locomotor activity in females; however, when administered to social pairs, nicotine decreased locomotion in both sexes. These findings suggest that the presence of a social partner may decrease the initial negative, stress-activating effects of nicotine, perhaps leading to increased nicotine reward.

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1. Introduction

Despite the serious health consequences associated with smoking and considerable prevention efforts, tobacco use among adolescents is widespread (Gilpin et al., 1999; Johnston et al., 2008; Wang et al., 1999). Adolescence is the most common stage of life for initiating tobacco use (Breslau et al., 1993; Kandel and Logan, 1984; Taioli and Wynder, 1991), with most adult smokers reporting that they first began experimenting with tobacco between the ages of 13 and 15 (Olds and Thoms, 2001). Early onset of use also increases the risk for developing tobacco dependence in adulthood (DiFranza et al., 2006; Jefferis et al., 2003; Kandel and Chen, 2000; Nelson et al., 1995) and in contrast to adult onset, adolescents develop tobacco dependence at a faster rate and are less successful discontinuing use (Breslau and Peterson, 1996; Chen and Millar, 1998; Colby et al., 2000; Kandel and Chen, 2000). Therefore, understanding the neurobiological factors that lead to the onset and continued use of tobacco during adolescence is needed in order to improve prevention and intervention strategies.

Animal models have helped elucidate adolescent vulnerability to smoking by examining the effects of nicotine, a key psychoactive component in cigarettes. Adolescent rodents are more sensitive than

adults to the rewarding and reinforcing effects of nicotine as measured using the conditioned place preference (CPP; Belluzzi et al., 2004; Kota et al., 2007, 2008; Torres et al., 2008, 2009; Vastola et al., 2002) and self-administration (Adriani et al., 2002; Chen et al., 2007; Levin et al., 2007; Shram and Le, 2010) models, whereas they are less sensitive to the aversive effects (O'Dell et al., 2004, 2006; Shram et al., 2006; Wilmouth and Spear, 2004). Stimulant effects of nicotine are also age-dependent; acute nicotine increases locomotion in adolescents at postnatal days (PNDs) 27 and 28, but reduces locomotion in adulthood (Cao et al., 2007, 2010; Cruz et al., 2008). Furthermore, following chronic self-administration, nicotine increases locomotor activity in early adolescent (PNDs 24 and 25) mice, but reduces locomotor activity in late adolescent (PNDs 50 and 51) mice (Adriani et al., 2002). These factors likely increase vulnerability for developing nicotine dependence during adolescence.

The effects of nicotine are also gender-dependent. Female adolescent rats show enhanced motivation to self-administer nicotine (Chaudhri et al., 2005; Donny et al., 2000; Lynch, 2009), greater sensitivity to nicotine-induced CPP (Isiegas et al., 2009), enhanced sensitivity to locomotor effects of nicotine (Cao et al., 2010; Elliott et al., 2004; Kanyt et al., 1999), and decreased symptoms of withdrawal (Hamilton et al., 2010) relative to males. In humans, women become dependent at a faster rate, have more difficulty remaining abstinent, report greater abstinence-induced increases in negative affect, heightened withdrawal-related distress and increased urges to smoke to relieve withdrawal distress, and they are less responsive to

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nicotine replacement therapy for smoking cessation compared to men (Leventhal et al., 2007; Perkins et al., 1999; Xu et al., 2008).

Social reinforcement and affiliation with friends that smoke are prevalent reasons for initiation of smoking in adolescent teens (Baker et al., 2004; Jackson, 1997; Leatherdale et al., 2005; Madarasova Geckova et al., 2005; Skara and Sussman, 2003; Sussman, 2005). Social interaction is a basic need for physical and psychological health in humans and rodents, and in rodents social interaction during adolescence promotes the development of normal adult social behavior and cognitive functioning (Einson et al., 1978; Meaney and Stewart, 1979; Smith, 1982; Spinka et al., 2001; van den Berg et al., 1999; Vanderschuren et al., 1997). Social reinforcement has been demonstrated in rats using operant (Angermeier et al., 1959; Evans et al., 1994) and instrumental (Normansell and Panksepp, 1990; Werner and Anderson, 1976) conditioning. Rats also exhibit CPP for an environment paired with a conspecific (Calcagnetti and Schechter, 1992; Crowder and Hutto, 1992; Douglas et al., 2004; Trezza et al., 2009). Importantly, social reward interacts synergistically with nicotine to produce a more robust CPP (Thiel et al., 2009a).

Stress is a critical factor in nicotine use and dependence, and early life stress is a risk factor for initiation of smoking (Lloyd and Taylor, 2006; Sinha, 2008). Acute administration of nicotine activates the hypothalamic–pituitary–adrenal (HPA) axis resulting in increased plasma corticosterone (CORT) in both male and female adult (Balfour et al., 1975; Cam and Bassett, 1983; Cao et al., 2007; Cruz et al., 2008; Davis et al., 2005; Matta et al., 1998; Porcu et al., 2003) and adolescent (Cao et al., 2010; Cruz et al., 2005, 2008) rats, effects that are more pronounced in adult females compared to adult males (Cao et al., 2010; Rhodes et al., 2001, 2004). Paradoxically, in humans nicotine self-administration is associated with reduced symptoms of stress and anxiety (Morissette et al., 2007; Pomerleau and Pomerleau, 1990; Wesnes and Warburton, 1983), and chronic nicotine administration in adult rodents not only desensitizes nicotine-induced CORT, but also attenuates stressor-induced CORT (Faraday et al., 2005). Importantly, social interaction in adolescent rats attenuates novelty-induced CORT (Terranova et al., 1999), suggesting that the rewarding effects of sociality may enhance the initial rewarding effects of nicotine exposure (Thiel et al., 2009a) by attenuating nicotine-induced CORT.

Based on the above findings, we evaluated the influences of acute nicotine administration on plasma CORT and locomotor activity in male and female adolescent rats that were either alone or with a same-sex partner for 15 min post-injection. Play behaviors were also evaluated in social pairs.

2. Materials and methods

2.1. Animals

Separate cohorts of male ($n=40$) and female ($n=40$) Sprague–Dawley rats (Charles River, San Diego, CA) arrived at the Arizona State University animal vivarium on post-natal day (PND) 22 (i.e., 22 days old, 55–60 g). Rats were housed in same sex pairs in standard home cages (21.6×45.7×17.8 cm) in a climate-controlled facility with a 12-h reverse light–dark cycle (lights on at 7 pm) with ad libitum access to food and water. Rats remained pair-housed until PND 35, at which time they were singly housed for the remainder of each experiment. All testing was conducted during the animals' dark phase within a conservative estimate of rodent adolescence: PNDs 36–39 (Spear, 2000). Housing and care were conducted in accordance with the Guide for the Care and Use of Laboratory Rats (Institute of Laboratory Animal Resources on Life Sciences, National Research Council 1996).

2.2. Drugs

(–)Nicotine hydrogen tartrate salt (Sigma, St. Louis, MO) was dissolved in sterile saline (0.9%) and the pH was adjusted to approxi-

mately 7.2. All subcutaneous (s.c.) injections were administered at a volume of 1.0 ml/kg, and the dose (0.6 mg/kg) is reported as nicotine base. We utilized this nicotine dose because previous research has shown robust nicotine-induced CPP following systemic injections (Thiel et al., 2009a).

2.3. Behavioral procedures

Two experiments were conducted, one with male and one with female rats. Following arrival to the vivarium (i.e., PND 22), all rats were handled daily in order to minimize handling stress. Beginning on PND 36 each rat was individually habituated to their assigned test cage for 1 h on each of the 3 days prior to testing (i.e., PNDs 36–38). The test cage was located in a dimly lit room and was otherwise identical to the animals' home cage, including Sani Chip bedding (Harlan Laboratories, Inc., Madison, WI) covering the floor. Immediately prior to placement into his or her assigned test cage, each rat received a saline injection (1 ml/kg, s.c.) in order to habituate the rat to the injection process. Rats were randomly assigned to either an isolate test condition ($n=20$) or a social test condition (20 rats; $n=10$ pairs). On test day (i.e., PND 39) half of the rats under each of these test conditions received saline and the other half received nicotine (0.6 mg/kg, s.c.). All rats were then immediately placed into the test cage for a 15-min test session. Rats tested in social pairs both received the same drug pretreatment and were tested with the same partner that they were initially pair-housed with upon arrival to the vivarium (i.e., PNDs 22–35). Thus, the design of each experiment consisted of the following 4 groups: saline-isolated (SAL:ISO; $n=10$); saline-social (SAL:SOC; $n=10$ or 5 pairs for social behavior measures); nicotine-isolated (NIC:ISO; $n=10$); and nicotine-social (NIC:SOC; $n=10$ or 5 pairs). Testing occurred in cohorts of 6–7 rats each between 11 am and 3 pm, and group representation was counterbalanced across cohorts to control for normal daily CORT rhythms.

Locomotor activity and play behaviors were videotaped from cameras above the test cages and were later quantified by trained observers blind to group assignments using Observer 5.0 software (Noldus Information Technology BV, Wageningen, The Netherlands). This software allowed for a detailed frame by frame analysis of behavior. The incidence of the following behaviors was analyzed: *pins* defined as one rat lying in the supine posture (dorsal surface on the floor) with the other rat standing over/on him/her; *nape attacks* defined as one rat contacting/rubbing the other rat's neck; and *social contacts* measured as both the frequency and duration of time spent in any type of physical contact, including huddling, pins, nape attacks and social investigation (i.e., social sniffing, anogenital sniffing and allogrooming). Nape attacks and pinning were analyzed because nape attacks are thought to reflect initiation of play, and pinning correlates highly with several other more ambiguous play behaviors, suggesting that these measures offer efficient indices of play overall (Panksepp et al., 1984). Social contact was included in order to assess a possible social preference in rat pairs that received nicotine, which may suppress locomotor activity and other play behaviors. Horizontal locomotor activity was analyzed by observing the frequency of line crossing, which consisted of a line across the screen that separated the test chamber into two equal halves. Line crossing was defined as all 4 paws crossing from one side of the line to the other.

2.4. Plasma CORT assay

Immediately following testing, rats were decapitated and trunk blood was collected from each rat into heparin-coated tubes and stored on ice. Subsequently, blood samples were centrifuged for 10 min at 5000 rpm at 4 °C. Plasma was removed and stored at –80 °C until processing. Plasma CORT was measured using a validated commercial competitive enzyme immunoassay kit (ELISA; Assay Designs Inc., Ann Arbor, MI) according to the manufacturer's

protocol. Samples were diluted (1:50) in assay buffer and assayed in duplicate. Optical densities were measured at 405 nm using a microplate reader (Opsys MR, Dynex Technologies, Chantilly, VA) and CORT concentrations were interpolated from a standard curve using GraphPad Prism 5.0 (La Jolla, CA). Cross reactivity of the primary antibody with steroids other than CORT was minimal (i.e., progesterone (1.7%), testosterone (0.13%), aldosterone (0.18%) and pregnenolone (<0.03%)). The sensitivity of the assay was 26.99 pg/ml, and the mean intra-assay and inter-assay coefficients of variation were 7.6% and 9.7%, respectively. CORT levels are expressed as ng/ml.

2.5. Statistical analysis

Because the activity of a given rat in a social pair can influence the other, measures of locomotion of individual rats are not considered independent. Therefore, the average locomotion of each pair as well as locomotion of individual isolated rats were analyzed using separate two-way ANOVAs, with drug pretreatment (saline or nicotine) and social condition (isolate or social pair) as factors. A 2-way ANOVA was also used to analyze individual plasma CORT levels. Social play behaviors were totaled for a given pair (i.e., 1 value/pair) and analyzed using *t*-tests. ANOVAs were followed by Newman–Keuls post-hoc tests where appropriate; α was set at 0.05 for all statistical comparisons. Gender was not included as a factor in the analysis because the male and female experiments were conducted at different times. All data are expressed as mean \pm SEM.

3. Results

3.1. Effects of nicotine and social interaction on plasma CORT

Fig. 1 illustrates plasma CORT across groups following the 15-min test session. Note that two samples from the male SAL:ISO group were lost during processing. The ANOVAs revealed a drug \times social condition interaction for both males [$F(1, 34) = 8.18, p < 0.01$] and females [$F(1, 36) = 4.61, p < 0.05$]. Regardless of gender, post-hoc Newman–Keuls indicated that nicotine pretreatment increased plasma CORT in isolated (NIC:ISO vs. SAL:ISO; $p < 0.05$ in each gender), but not socially paired (SAL:SOC vs. NIC:SOC), rats. In both sexes, the presence of a social partner alone failed to alter plasma CORT (SAL:ISO vs. SAL:SOC), but attenuated nicotine-induced plasma CORT (NIC:ISO vs. NIC:SOC; $p < 0.05$ in each gender). Changes in plasma CORT, as measured here, may reflect changes not only in hormone secretion,

but potentially also in metabolism, excretion and uptake by tissues, etc.

3.2. Effects of nicotine and social interaction on locomotor activity and social play behavior

Fig. 2 illustrates the effect of nicotine on locomotor activity during the 15-min test session. In males, the ANOVA indicated a drug \times social condition interaction [$F(1, 24) = 74.28, p < 0.001$]. In saline pretreated rats, the presence of a social partner increased locomotor activity (SAL:SOC vs. SAL:ISO); however nicotine pretreatment (NIC:SOC vs. SAL:SOC) prevented this stimulatory effect ($p < 0.05$ in each case). Nicotine alone failed to alter locomotion in isolated rats (NIC:ISO vs. SAL:ISO) and there was no effect of sociality on locomotion in nicotine pretreated rats (NIC:ISO vs. NIC:SOC). Similarly in females, the analysis revealed a drug \times social condition interaction [$F(1, 26) = 29.55, p < 0.001$]. The presence of a social partner increased locomotor activity in saline pretreated females (SAL:SOC vs. SAL:ISO) and nicotine pretreatment (NIC:SOC vs. SAL:SOC) blocked this stimulatory effect ($p < 0.05$ in each case). There was also no effect of sociality in nicotine pretreated rats (NIC:ISO and NIC:SOC); however, in contrast to males, nicotine alone (NIC:ISO vs. SAL:ISO) increased locomotor activity in isolated female rats ($p < 0.05$).

Fig. 3 illustrates the effect of nicotine on play behaviors during the 15-min test session. In males, nicotine pretreatment (NIC:SOC vs. SAL:SOC) reduced the number of pins [$t(8) = 15.51, p < 0.001$], nape attacks [$t(8) = 3.11, p < 0.05$] and social contacts [$t(8) = 11.10, p < 0.001$], and total contact duration [$t(8) = 15.35, p < 0.001$]. Similarly in females, nicotine (NIC:SOC vs. SAL:SOC) decreased the number of pins [$t(8) = 3.41, p < 0.01$], nape attacks [$t(8) = 3.72, p < 0.01$] and social contacts [$t(8) = 3.97, p < 0.005$], but failed to alter total contact duration; although there was a trend toward a decrease ($p = 0.15$).

4. Discussion

The present findings suggest that the presence of a same-sex social partner prevents the increase in plasma CORT that is detected after acute nicotine administration in isolated male and female adolescent (PND 39) rats. In contrast to Terranova et al. (1999), social interactions in the absence of nicotine had no effect on plasma CORT. This difference likely resulted from the extensive habituation to the testing environment in the present report. We have previously found that

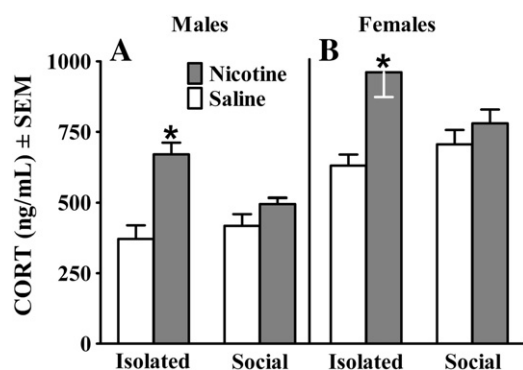


Fig. 1. Effects of nicotine (0.6 mg/kg/s.c.) and social interactions on plasma corticosterone (CORT) in male (A) and female (B) adolescent rats following a 15-min test session. Nicotine (gray bars) increased plasma CORT in both male and female isolated rats, but the presence of a same-sex social partner prevented this effect. Asterisk (*) represents an increase compared to all other groups (Newman–Keuls, $p < 0.05$). Experimental groups ($n = 10$ /group) included: saline-isolated (SAL:ISO); nicotine-isolated (NIC:ISO); saline-social (SAL:SOC); and nicotine-social (NIC:SOC).

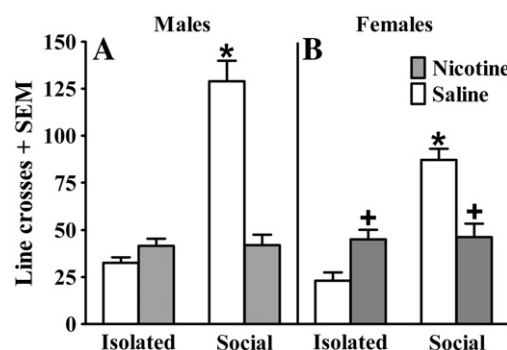


Fig. 2. Effects of nicotine (0.6 mg/kg/s.c.) and social interactions on locomotor activity in male (A) and female (B) adolescent rats during a 15-min test session. The presence of a social partner increased locomotor activity in both male and female rats, but nicotine (gray bars) pretreatment prevented this effect. In isolated rats, nicotine increased locomotion in females, but failed to alter locomotor activity in males. Asterisk (*) represents an increase compared to all other groups; plus (+) represents an increase compared to the SAL:ISO group (Newman–Keuls, $p < 0.05$). Experimental groups ($n = 10$ /group or 5 pairs/group) included: saline-isolated (SAL:ISO); nicotine-isolated (NIC:ISO); saline-social (SAL:SOC); and nicotine-social (NIC:SOC).

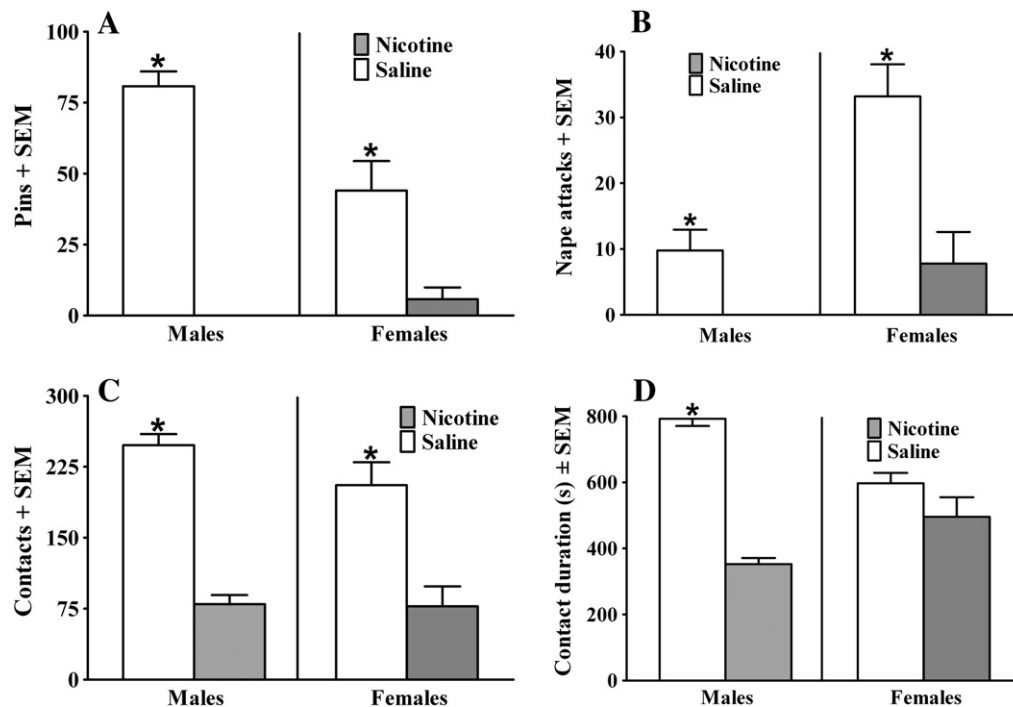


Fig. 3. Effects of nicotine (0.6 mg/kg/s.c.) on play behaviors, including the number of pins (A), nape attacks (B) and contacts (C), and the duration (s) of contact (D), in male (left bars) and female (right bars) adolescent rats during a 15-min test session. Nicotine (gray bars) administration decreased play behaviors and social contact in both male and female rats. Asterisk (*) represents an increase compared to nicotine treated rats (Newman-Keuls, $p < 0.05$). Experimental groups ($n = 10$ /group) included: saline-social (SAL:SOC); and nicotine-social (NIC:SOC).

nicotine and social rewards interact synergistically (Thiel et al., 2009a), and the present findings suggest that this interaction is accompanied by a reduction in nicotine-induced plasma CORT rather than an enhancement. Thus, during adolescence, a period of increased vulnerability for initiation of smoking in teens, peer social interactions may reduce stressful effects of nicotine thereby enhancing the rewarding effects of nicotine. This pattern of effects may depend on individuals experiencing a history of social deprivation given that social housing and environmental enrichment can reduce the rewarding effects of stimulants experienced in isolation (Schenk et al., 1986; Zakharova et al., 2009). Collectively, these results add to a growing literature that emphasizes the critical role of social and environmental factors in influencing the actions of nicotine and other psychostimulant drugs (Campbell and Carroll, 2000; Stairs and Bardo, 2009; Thiel et al., 2008, 2009a,b).

The lack of a nicotine-induced increase in plasma CORT in the paired adolescent rats was not due to increased play or other social activities and was not gender dependent. In both male and female social pairs, nicotine administration reduced several play behaviors, including the number of pins, nape attacks and social contacts, and attenuated locomotor activity. These findings extend upon previous research demonstrating that in both male and female adolescent rats the presence of a same-sex social partner blunts novelty-induced plasma CORT (Terranova et al., 1999), a phenomenon termed social buffering (for review see Kikusui et al., 2006). These social influences on nicotine effects may involve opioid, endocannabinoid, dopamine, and/or oxytocin systems (Kikusui et al., 2006; Trezza and Vanderschuren, 2008a,b). For example, in rodents stimulation of opioid receptors (Beatty and Costello, 1982; Panksepp et al., 1985; Siegel and Jensen, 1986; Siegel et al., 1985; Vanderschuren et al., 1995a,b), particularly in the nucleus accumbens (Kraebel et al., 2002), enhances social reward. In rodents oxytocin promotes affiliative behavior and social recognition (Insel, 1992; Lim and Young, 2006; Storm and Tecott, 2005), and in humans oxytocin produces prosocial behaviors by enhancing trust (Kosfeld et al., 2005) and reducing fear (Kirsch et al., 2005). Importantly, oxytocin attenuates basal and stress-induced activation of the HPA axis

in both male and female adult rats (Neumann et al., 2000; Windle et al., 1997), and increases dopamine release in the nucleus accumbens (Melis et al., 2007). Thus, peers may facilitate continued smoking via blunting nicotine-induced HPA axis activation, while at the same time increasing dopamine in the nucleus accumbens during the initial nicotine experience. In support of this assertion, low doses of nicotine (0.01–0.1 mg/kg, i.p.) produce anxiolytic-like effects in rodents (Costall et al., 1989; File et al., 1998; Vale and Green, 1996), while higher doses (0.5–1.0 mg/kg, i.p.) similar to that used in the present study are anxiogenic (File et al., 1998; Ouagazzal et al., 1999). Furthermore, anxiolytic effects of nicotine have been implicated in nicotine dependence, with dependent humans self-reporting reduced stress and anxiolytic-like effects following tobacco use (Gilbert et al., 1989; Pomerleau, 1986; Pomerleau and Pomerleau, 1990; Wesnes and Warburton, 1983).

Previous research has indicated that the acute effects of nicotine on plasma CORT and locomotor activity are age-, dose-, gender-, and time-dependent (Cheeta et al., 2001; Elliott et al., 2004; Faraday et al., 1999). The present study found that females were more sensitive to the stimulant effects of nicotine and appeared to have higher basal and nicotine-induced CORT levels than males. However, a caveat in the present study is that males and females were run in different experiments, precluding a statistical analysis of sex differences per se. Despite this limitation, higher plasma CORT in females relative to males across all experimental groups is consistent with previous research showing that adolescent females have higher basal and novelty-induced plasma CORT compared to males (Terranova et al., 1999). Furthermore, adult females have higher basal, stress- and nicotine-induced plasma CORT and adrenocorticotrophic hormone compared to males (Faraday et al., 2005; Rhodes et al., 2001, 2004; Rhodes and Rubin, 1999). This heightened HPA axis responsivity may represent one mechanism underlying the increased vulnerability for developing nicotine dependence in females, and may account for enhanced sensitivity to the reinforcing (Lynch, 2009), rewarding (Isiegas et al., 2009) and stimulant (Cao et al., 2010; Elliott et al., 2004; Kanyt et al., 1999) effects of nicotine in female rodents relative to males.

The present findings that nicotine increased locomotion in isolated adolescent females but not males seem discrepant with previous findings that in males a lower dose of nicotine (0.4 mg/kg/s.c.) enhanced both locomotion and plasma CORT (Cruz et al., 2005, 2008), and that intravenous nicotine (30 µg/kg i.v., twice at 1-min interval) increased locomotion without affecting plasma CORT (Cao et al., 2007, 2010). In addition to drug dose and route of administration, these apparent discrepancies may have occurred due to other differences in experimental parameters such as injection timing, animal age and differences in the rats' light–dark cycles. The previous reports examined the effects of nicotine on locomotion on PNDs 27 and 28 (Cao et al., 2007, 2010), or 37 (Cruz et al., 2005, 2008) across 30 min sessions during the rats' light cycle, while testing in the present study was 15 min in duration and occurred on PND 39 during the rats' dark cycle. Furthermore, Cao et al. (2007, 2010) tested animals in novel environments, while the present report and the Cruz et al. (2005, 2008) studies habituated animals to the testing environments. Despite these differences in experimental protocols, our results are consistent with prior findings showing a transition from locomotor stimulant effects of nicotine in early adolescence (PNDs 27, 28 and 37) to depressant effects in adulthood. Furthermore, our findings coupled with results from Cao et al. (2010) suggest that in males, PNDs 38 and 39 represent a pre-transition point of the shift in the ambulatory response to nicotine from stimulation to inhibition. Collectively, these results suggest that adolescence consists of different stages (i.e., early, mid and late adolescence), during which there may be large differences in drug response (Cao et al., 2010; McCormick and Mathews, 2007, for review see Spear, 2000).

Nicotine effects on social behavior are likely biphasic, with an initial suppression followed by an increase. The present study measured play behavior immediately following nicotine administration in order to examine the effects of social context on nicotine-induced plasma CORT. Consistent with previous findings, nicotine decreased play behavior despite enhancing social-CPP (Thiel et al., 2009a). However, Trezza et al. (2009) found an increase in social behaviors when nicotine was administered 10 min prior to testing, a time point when the locomotor-altering effects of nicotine in adolescents subside (Cao et al., 2010). Another possible reason for the difference between our findings and those of Trezza et al. (2009) is that we used a substantially higher nicotine dose (0.6 mg/kg compared to 0.1 mg/kg) that may have initially suppressed play behavior. In support of this assertion, Rhodes et al. (2004) reported that higher doses of nicotine (0.5 mg/kg) produce initial sedation that dissipates after 30 min. Thus, in the present report high-dose nicotine (0.6 mg/kg) administered immediately before testing may have produced initial sedation that suppressed play behavior, while lower nicotine doses administered at earlier time points enhance sociality.

5. Conclusions

In summary, the present results confirm that acute administration of nicotine increased plasma CORT in both male and female isolated adolescent rats; however, the presence of a same sex social partner may have abolished nicotine-induced plasma CORT. Regardless of gender, nicotine administration reduced several indices of play and produced gender dependent effects on locomotor activity. We speculate that the initial aversive effects of nicotine, including elevated levels of plasma CORT, are alleviated by social interactions, and that stress reduction may represent one mechanism by which peer tobacco use increases the vulnerability for smoking in adolescent teens (Jackson, 1997; Leatherdale et al., 2005; Skara and Sussman, 2003; Sussman, 2005). These data highlight the need for targeting stress and incorporating positive social interactions in research aimed at both prevention and cessation of smoking in adolescents.

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References

- Adriani W, Macri S, Pacifici R, Laviola G. Peculiar vulnerability to nicotine oral self-administration in mice during early adolescence. *Neuropsychopharmacology* 2002;27:212–24.
- Angermeier WF, Schaul LT, James WT. Social conditioning in rats. *J Comp Physiol Psychol* 1959;52:370–2.
- Baker TB, Brandon TH, Chassin L. Motivational influences on cigarette smoking. *Annu Rev Psychol* 2004;55:463–91.
- Balfour DJ, Khullar AK, Longden A. Effects of nicotine on plasma corticosterone and brain amines in stressed and unstressed rats. *Pharmacol Biochem Behav* 1975;3:179–84.
- Beatty WW, Costello KB. Naloxone and play fighting in juvenile rats. *Pharmacol Biochem Behav* 1982;17:905–7.
- Belluzzi JD, Lee AG, Oliff HS, Leslie FM. Age-dependent effects of nicotine on locomotor activity and conditioned place preference in rats. *Psychopharmacology (Berl)* 2004;174:389–95.
- Breslau N, Peterson EL. Smoking cessation in young adults: age at initiation of cigarette smoking and other suspected influences. *Am J Public Health* 1996;86:214–20.
- Breslau N, Fenn N, Peterson EL. Early smoking initiation and nicotine dependence in a cohort of young adults. *Drug Alcohol Depend* 1993;33:129–37.
- Calcagnetti DJ, Schechter MD. Place conditioning reveals the rewarding aspect of social interaction in juvenile rats. *Physiol Behav* 1992;51:667–72.
- Cam GR, Bassett JR. The effect of acute nicotine administration on plasma levels of the thyroid hormones and corticosterone in the rat. *Pharmacol Biochem Behav* 1983;19:559–61.
- Campbell UC, Carroll ME. Acquisition of drug self-administration: environmental and pharmacological interventions. *Exp Clin Psychopharmacol* 2000;8:312–25.
- Cao J, Belluzzi JD, Loughlin SE, Keyler DE, Pentel PR, Leslie FM. Acetaldehyde, a major constituent of tobacco smoke, enhances behavioral, endocrine, and neuronal responses to nicotine in adolescent and adult rats. *Neuropsychopharmacology* 2007;32:2025–35.
- Cao J, Belluzzi JD, Loughlin SE, Dao JM, Chen Y, Leslie FM. Locomotor and stress responses to nicotine differ in adolescent and adult rats. *Pharmacol Biochem Behav* 2010;96:82–90.
- Chaudhri N, Caggiula AR, Donny EC, Booth S, Gharib MA, Craven LA, et al. Sex differences in the contribution of nicotine and nonpharmacological stimuli to nicotine self-administration in rats. *Psychopharmacology (Berl)* 2005;180:258–66.
- Cheeta S, Irvine EE, Tucci S, Sandhu J, File SE. In adolescence, female rats are more sensitive to the anxiolytic effect of nicotine than are male rats. *Neuropsychopharmacology* 2001;25:601–7.
- Chen J, Millar WJ. Age of smoking initiation: implications for quitting. *Health Rep* 1998;9:39–46. [(Eng): 39–48(Fre)].
- Chen H, Matta SG, Sharp BM. Acquisition of nicotine self-administration in adolescent rats given prolonged access to the drug. *Neuropsychopharmacology* 2007;32:700–9.
- Colby SM, Tiffany ST, Shiffman S, Niaura RS. Are adolescent smokers dependent on nicotine? A review of the evidence. *Drug Alcohol Depend* 2000;59(Suppl. 1):S83–95.
- Costall B, Kelly ME, Naylor RJ, Onaivi ES. The actions of nicotine and cocaine in a mouse model of anxiety. *Pharmacol Biochem Behav* 1989;33:197–203.
- Crowder WF, Hutto Jr CW. Operant place conditioning measures examined using two nondrug reinforcers. *Pharmacol Biochem Behav* 1992;41:817–24.
- Cruz FC, Delucia R, Planeta CS. Differential behavioral and neuroendocrine effects of repeated nicotine in adolescent and adult rats. *Pharmacol Biochem Behav* 2005;80:411–7.
- Cruz FC, Delucia R, Planeta CS. Effects of chronic stress on nicotine-induced locomotor activity and corticosterone release in adult and adolescent rats. *Addict Biol* 2008;13:63–9.
- Davis KW, Cepeda-Benito A, Harraid JH, Wellman PJ. Plasma corticosterone in the rat in response to nicotine and saline injections in a context previously paired or unpaired with nicotine. *Psychopharmacology (Berl)* 2005;180:466–72.
- DiFranza JR, Wellman RJ, Sargent JD, Weitzman M, Hipple BJ, Winickoff JP. Tobacco promotion and the initiation of tobacco use: assessing the evidence for causality. *Pediatrics* 2006;117:e1237–48.
- Donny EC, Caggiula AR, Rowell PP, Gharib MA, Maldovan V, Booth S, et al. Nicotine self-administration in rats: estrous cycle effects, sex differences and nicotinic receptor binding. *Psychopharmacology (Berl)* 2000;151:392–405.
- Douglas LA, Varlinskaya EI, Spear LP. Rewarding properties of social interactions in adolescent and adult male and female rats: impact of social versus isolate housing of subjects and partners. *Dev Psychobiol* 2004;45:153–62.
- Eimon DF, Morgan MJ, Kibbler CC. Brief periods of socialization and later behavior in the rat. *Dev Psychobiol* 1978;11:213–25.
- Elliott BM, Faraday MM, Phillips JM, Grunberg NE. Effects of nicotine on elevated plus maze and locomotor activity in male and female adolescent and adult rats. *Pharmacol Biochem Behav* 2004;77:21–8.

- Evans MJ, Duvel A, Funk ML, Lehman B, Sparrow J, Watson NT, et al. Social reinforcement of operant behavior in rats: a methodological note. *J Exp Anal Behav* 1994;62:149–56.
- Faraday MM, Scheufele PM, Rahman MA, Grunberg NE. Effects of chronic nicotine administration on locomotion depend on rat sex and housing condition. *Nicotine Tob Res* 1999;1:143–51.
- Faraday MM, Blakeman KH, Grunberg NE. Strain and sex alter effects of stress and nicotine on feeding, body weight, and HPA axis hormones. *Pharmacol Biochem Behav* 2005;80:577–89.
- File SE, Kenny PJ, Ouagazzal AM. Bimodal modulation by nicotine of anxiety in the social interaction test: role of the dorsal hippocampus. *Behav Neurosci* 1998;112:1423–9.
- Gilbert DG, Robinson JH, Chamberlin CL, Spielberger CD. Effects of smoking/nicotine on anxiety, heart rate, and lateralization of EEG during a stressful movie. *Psychophysiology* 1989;26:311–20.
- Gilpin EA, Choi WS, Berry C, Pierce JP. How many adolescents start smoking each day in the United States? *J Adolesc Health* 1999;25:248–55.
- Hamilton KR, Perry ME, Berger SS, Grunberg NE. Behavioral effects of nicotine withdrawal differ by genetic strain in male and female adolescent rats. *Nicotine Tob Res* 2010;12:1236–45.
- Insel TR. Oxytocin – a neuropeptide for affiliation: evidence from behavioral, receptor autoradiographic, and comparative studies. *Psychoneuroendocrinology* 1992;17:3–35.
- Isiegas C, Mague SD, Blendy JA. Sex differences in response to nicotine in C57Bl/6:129SvEv mice. *Nicotine Tob Res* 2009;11:851–8.
- Jackson C. Initial and experimental stages of tobacco and alcohol use during late childhood: relation to peer, parent, and personal risk factors. *Addict Behav* 1997;22:685–98.
- Jefferis B, Graham H, Manor O, Power C. Cigarette consumption and socio-economic circumstances in adolescence as predictors of adult smoking. *Addiction* 2003;98:1765–72.
- Johnston LD, O'Malley PM, Bachman JG, Schulenberg JE. National results on adolescent drug use: overview of key findings. Bethesda: National Institute on Drug Abuse; 2008.
- Kandel DB, Chen K. Extent of smoking and nicotine dependence in the United States: 1991–1993. *Nicotine Tob Res* 2000;2:263–74.
- Kandel DB, Logan JA. Patterns of drug use from adolescence to young adulthood: I. Periods of risk for initiation, continued use, and discontinuation. *Am J Public Health* 1984;74:660–6.
- Kanyt L, Stoleran IP, Chandler CJ, Saigusa T, Pogun S. Influence of sex and female hormones on nicotine-induced changes in locomotor activity in rats. *Pharmacol Biochem Behav* 1999;62:179–87.
- Kikusui T, Winslow JT, Mori Y. Social buffering: relief from stress and anxiety. *Philos Trans R Soc Lond B Biol Sci* 2006;361:2215–28.
- Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddhanti S, et al. Oxytocin modulates neural circuitry for social cognition and fear in humans. *J Neurosci* 2005;25:11489–93.
- Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E. Oxytocin increases trust in humans. *Nature* 2005;435:673–6.
- Kota D, Martin BR, Robinson SE, Damaj MI. Nicotine dependence and reward differ between adolescent and adult male mice. *J Pharmacol Exp Ther* 2007;322:399–407.
- Kota D, Martin BR, Damaj MI. Age-dependent differences in nicotine reward and withdrawal in female mice. *Psychopharmacology (Berl)* 2008;198:201–10.
- Kraebel KS, Brasser SM, Campbell JO, Spear LP, Spear NE. Developmental differences in temporal patterns and potentiation of isolation-induced ultrasonic vocalizations: influence of temperature variables. *Dev Psychobiol* 2002;40:147–59.
- Leatherdale ST, McDonald PW, Cameron R, Brown KS. A multilevel analysis examining the relationship between social influences for smoking and smoking onset. *Am J Health Behav* 2005;29:520–30.
- Leventhal AM, Waters AJ, Boyd S, Moolchan ET, Lerman C, Pickworth WB. Gender differences in acute tobacco withdrawal: effects on subjective, cognitive, and physiological measures. *Exp Clin Psychopharmacol* 2007;15:21–36.
- Levin ED, Lawrence SS, Petro A, Horton K, Rezvani AH, Seidler FJ, et al. Adolescent vs. adult-onset nicotine self-administration in male rats: duration of effect and differential nicotinic receptor correlates. *Neurotoxicol Teratol* 2007;29:458–65.
- Lim MM, Young LJ. Neuropeptidergic regulation of affiliative behavior and social bonding in animals. *Horm Behav* 2006;50:506–17.
- Lloyd DA, Taylor J. Lifetime cumulative adversity, mental health and the risk of becoming a smoker. *Health (London)* 2006;10:95–112.
- Lynch WJ. Sex and ovarian hormones influence vulnerability and motivation for nicotine during adolescence in rats. *Pharmacol Biochem Behav* 2009;94:43–50.
- Madarasova Geckova A, Stewart R, van Dijk JP, Orosova O, Groothoff JW, Post D. Influence of socio-economic status, parents and peers on smoking behaviour of adolescents. *Eur Addict Res* 2005;11:204–9.
- Matta SG, Fu Y, Valentine JD, Sharp BM. Response of the hypothalamo-pituitary-adrenal axis to nicotine. *Psychoneuroendocrinology* 1998;23:103–13.
- McCormick CM, Mathews IZ. HPA function in adolescence: role of sex hormones in its regulation and the enduring consequences of exposure to stressors. *Pharmacol Biochem Behav* 2007;86:220–33.
- Meaney MJ, Stewart J. Environmental-factors influencing the affiliative behavior of male and female rats (*Rattus norvegicus*). *Anim Learn Behav* 1979;7:397–405.
- Melis MR, Melis T, Cocco C, Succu S, Sanna F, Pillolla G, et al. Oxytocin injected into the ventral tegmental area induces penile erection and increases extracellular dopamine in the nucleus accumbens and paraventricular nucleus of the hypothalamus of male rats. *Eur J Neurosci* 2007;26:1026–35.
- Morisette SB, Tull MT, Gulliver SB, Kamholz BW, Zimering RT. Anxiety, anxiety disorders, tobacco use, and nicotine: a critical review of interrelationships. *Psychol Bull* 2007;133:245–72.
- Nelson DE, Giovino GA, Shopland DR, Mowery PD, Mills SL, Eriksen MP. Trends in cigarette smoking among US adolescents, 1974 through 1991. *Am J Public Health* 1995;85:34–40.
- Neumann ID, Wigger A, Torner L, Holsboer F, Landgraf R. Brain oxytocin inhibits basal and stress-induced activity of the hypothalamo-pituitary-adrenal axis in male and female rats: partial action within the paraventricular nucleus. *J Neuroendocrinol* 2000;12:235–43.
- Normansell L, Panksepp J. Effects of morphine and naloxone on play-rewarded spatial discrimination in juvenile rats. *Dev Psychobiol* 1990;23:75–83.
- O'Dell LE, Bruijnzeel AW, Ghosland S, Markou A, Koob GF. Nicotine withdrawal in adolescent and adult rats. *Ann N Y Acad Sci* 2004;1021:167–74.
- O'Dell LE, Bruijnzeel AW, Smith RT, Parsons LH, Merves ML, Goldberger BA, et al. Diminished nicotine withdrawal in adolescent rats: implications for vulnerability to addiction. *Psychopharmacology (Berl)* 2006;186:612–9.
- Olds RS, Thoms DL. The relationship of adolescent perceptions of peer norms and parent involvement to cigarette and alcohol use. *J Sch Health* 2001;71:223–8.
- Ouagazzal AM, Kenny PJ, File SE. Modulation of behaviour on trials 1 and 2 in the elevated plus-maze test of anxiety after systemic and hippocampal administration of nicotine. *Psychopharmacology (Berl)* 1999;144:54–60.
- Panksepp J, Siviý S, Normansell L. The psychobiology of play: theoretical and methodological perspectives. *Neurosci Biobehav Rev* 1984;8:465–92.
- Panksepp J, Jalowiec J, DeEskinazi FG, Bishop P. Opiates and play dominance in juvenile rats. *Behav Neurosci* 1985;99:441–53.
- Perkins KA, Donny E, Caggiula AR. Sex differences in nicotine effects and self-administration: review of human and animal evidence. *Nicotine Tob Res* 1999;1:301–15.
- Pomerleau OF. Nicotine as a psychoactive drug: anxiety and pain reduction. *Psychopharmacol Bull* 1986;22:865–9.
- Pomerleau OF, Pomerleau CS. Behavioural studies in humans: anxiety, stress and smoking. *Ciba Found Symp* 1990;152:225–35. [discussion 35–9].
- Porcu P, Sogliano C, Cinus M, Purdy RH, Biggio G, Concas A. Nicotine-induced changes in cerebrocortical neuroactive steroids and plasma corticosterone concentrations in the rat. *Pharmacol Biochem Behav* 2003;74:683–90.
- Rhodes ME, Rubin RT. Functional sex differences ('sexual diergism') of central nervous system cholinergic systems, vasopressin, and hypothalamic-pituitary-adrenal axis activity in mammals: a selective review. *Brain Res Brain Res Rev* 1999;30:135–52.
- Rhodes ME, O'Toole SM, Czambel RK, Rubin RT. Male-female differences in rat hypothalamic-pituitary-adrenal axis responses to nicotine stimulation. *Brain Res Bull* 2001;54:681–8.
- Rhodes ME, Kennell JS, Belz EE, Czambel RK, Rubin RT. Rat estrous cycle influences the sexual diergism of HPA axis stimulation by nicotine. *Brain Res Bull* 2004;64:205–13.
- Schenk S, Hunt T, Malovechko R, Robertson A, Klukowski G, Amit Z. Differential effects of isolation housing on the conditioned place preference produced by cocaine and amphetamine. *Pharmacol Biochem Behav* 1986;24:1793–6.
- Shram MJ, Le AD. Adolescent male Wistar rats are more responsive than adult rats to the conditioned rewarding effects of intravenously administered nicotine in the place conditioning procedure. *Behav Brain Res* 2010;206:240–4.
- Shram MJ, Funk D, Li Z, Le AD. Periadolescent and adult rats respond differently in tests measuring the rewarding and aversive effects of nicotine. *Psychopharmacology (Berl)* 2006;186:201–8.
- Siegel MA, Jensen RA. The effects of naloxone and cage size on social play and activity in isolated young rats. *Behav Neural Biol* 1986;45:155–68.
- Siegel MA, Jensen RA, Panksepp J. The prolonged effects of naloxone on play behavior and feeding in the rat. *Behav Neural Biol* 1985;44:509–14.
- Sinha R. Chronic stress, drug use, and vulnerability to addiction. *Ann N Y Acad Sci* 2008;1141:105–30.
- Skara S, Sussman S. A review of 25 long-term adolescent tobacco and other drug use prevention program evaluations. *Prev Med* 2003;37:451–74.
- Smith PK. Does play matter – functional and evolutionary aspects of animal and human play. *Behav Brain Sci* 1982;5:139–55.
- Spear LP. The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev* 2000;24:417–63.
- Spinka M, Newberry RC, Bekoff M. Mammalian play: training for the unexpected. *Q Rev Biol* 2001;76:141–68.
- Stairs DJ, Bardo MT. Neurobehavioral effects of environmental enrichment and drug abuse vulnerability. *Pharmacol Biochem Behav* 2009;92:377–82.
- Storm EE, Tecott LH. Social circuits: peptidergic regulation of mammalian social behavior. *Neuron* 2005;47:483–6.
- Sussman S. Risk factors for and prevention of tobacco use. *Pediatr Blood Cancer* 2005;44:614–9.
- Taioli E, Wynder EL. Effect of the age at which smoking begins on frequency of smoking in adulthood. *N Engl J Med* 1991;325:968–9.
- Terranova ML, Cirulli F, Laviola G. Behavioral and hormonal effects of partner familiarity in periadolescent rat pairs upon novelty exposure. *Psychoneuroendocrinology* 1999;24:639–56.
- Thiel KJ, Okun AC, Neisewander JL. Social reward-conditioned place preference: a model revealing an interaction between cocaine and social context rewards in rats. *Drug Alcohol Depend* 2008;96:202–12.
- Thiel KJ, Sanabria F, Neisewander JL. Synergistic interaction between nicotine and social rewards in adolescent male rats. *Psychopharmacology* 2009a;204:391–402.
- Thiel KJ, Sanabria F, Pentkowski NS, Neisewander JL. Anti-craving effects of environmental enrichment. *Int J Neuropsychopharmacol* 2009b;12:1151–6.
- Torres OV, Tejeda HA, Natividad LA, O'Dell LE. Enhanced vulnerability to the rewarding effects of nicotine during the adolescent period of development. *Pharmacol Biochem Behav* 2008;90:658–63.

- Torres OV, Natividad LA, Tejeda HA, Van Weelden SA, O'Dell LE. Female rats display dose-dependent differences to the rewarding and aversive effects of nicotine in an age-, hormone-, and sex-dependent manner. *Psychopharmacology (Berl)* 2009;206:303–12.
- Trezza V, Vanderschuren LJ. Bidirectional cannabinoid modulation of social behavior in adolescent rats. *Psychopharmacology (Berl)* 2008a;197:217–27.
- Trezza V, Vanderschuren LJ. Cannabinoid and opioid modulation of social play behavior in adolescent rats: differential behavioral mechanisms. *Eur Neuropsychopharmacol* 2008b;18:519–30.
- Trezza V, Damsteegt R, Vanderschuren LJ. Conditioned place preference induced by social play behavior: parametrics, extinction, reinstatement and disruption by methylphenidate. *Eur Neuropsychopharmacol* 2009;19:659–69.
- Vale AL, Green S. Effects of chlordiazepoxide, nicotine and D-amphetamine in the rat potentiated startle model of anxiety. *Behav Pharmacol* 1996;7:138–43.
- van den Berg CL, Hol T, Van Ree JM, Spruijt BM, Everts H, Koolhaas JM. Play is indispensable for an adequate development of coping with social challenges in the rat. *Dev Psychobiol* 1999;34:129–38.
- Vanderschuren LJ, Niesink RJ, Spruijt BM, Van Ree JM. Effects of morphine on different aspects of social play in juvenile rats. *Psychopharmacology (Berl)* 1995a;117:225–31.
- Vanderschuren LJ, Niesink RJ, Spruijt BM, Van Ree JM. Mu- and kappa-opioid receptor-mediated opioid effects on social play in juvenile rats. *Eur J Pharmacol* 1995b;276:257–66.
- Vanderschuren LJ, Niesink RJ, Van Ree JM. The neurobiology of social play behavior in rats. *Neurosci Biobehav Rev* 1997;21:309–26.
- Vastola BJ, Douglas LA, Varlinskaya EI, Spear LP. Nicotine-induced conditioned place preference in adolescent and adult rats. *Physiol Behav* 2002;77:107–14.
- Wang MQ, Fitzhugh EC, Green BL, Turner LW, Eddy JM, Westerfield RC. Prospective social-psychological factors of adolescent smoking progression. *J Adolesc Health* 1999;24:2–9.
- Werner CM, Anderson DF. Opportunity for interaction as reinforcement in a T-maze. *Pers Soc Psychol Bull* 1976;2:166–9.
- Wesnes K, Warburton D. Stress and drugs. In: Hockey G, editor. *Stress and fatigue in human performance*. New York: Wiley; 1983. p. 203–43.
- Wilmouth CE, Spear LP. Adolescent and adult rats' aversion to flavors previously paired with nicotine. *Ann N Y Acad Sci* 2004;1021:462–4.
- Windle RJ, Shanks N, Lightman SL, Ingram CD. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. *Endocrinology* 1997;138:2829–34.
- Xu J, Azizian A, Monterosso J, Domier CP, Brody AL, Fong TW, et al. Gender effects on mood and cigarette craving during early abstinence and resumption of smoking. *Nicotine Tob Res* 2008;10:1653–61.
- Zakharova E, Miller J, Unterwald E, Wade D, Izenwasser S. Social and physical environment alter cocaine conditioned place preference and dopaminergic markers in adolescent male rats. *Neuroscience* 2009;163:890–7.