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## 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.

### Keywords

Nicotine; Corticosterone; Sociality; Sex differences; Stress; Play behavior

## 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, 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 Thombs, 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

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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.*, 2008, Kota *et al.*, 2007, Torres *et al.*, 2009, Torres *et al.*, 2008, 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, O'Dell *et al.*, 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 (PND) 27 and 28, but reduces locomotion in adulthood (Cao *et al.*, 2010, Cao *et al.*, 2007, 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 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 (Einon *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, Cruz *et al.*, 2008) rats, effects that are more pronounced in adult females compared to adult males (Cao *et al.*, 2010, Rhodes *et al.*, 2004, Rhodes *et al.*, 2001). Paradoxically, in humans nicotine self-administration is associated with reduced symptoms of stress and anxiety (Morissette *et al.*, 2007, Pomerleau and Pomerleau, 1990, Wesnes K, 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 postnatal 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 p.m.) 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 approximately 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 hr 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 video taped 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 5,000 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 were 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;  $\alpha$  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

Figure 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

Figure 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 ( $p<0.05$ ).

Figure 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 and colleagues (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 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, Thiel *et al.*, 2009a, Thiel *et al.*, 2009b).

The lack of a nicotine-induced increase in plasma CORT in the paired adolescent rats was not due to increased play or other social activity 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.*, 2004, Rhodes *et al.*, 2001, 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 seems 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.*, 2010, Cao *et al.*, 2007). 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.*, 2010, Cao *et al.*, 2007), 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 subsides (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.

### Research Highlights

- Nicotine reduces play behaviors including nape attacks, pins and social contact.
- Nicotine increases CORT in both male and female isolate rats.
- The presence of a same-sex social partner prevents nicotine-induced CORT.

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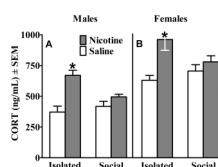
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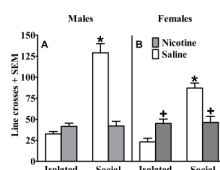
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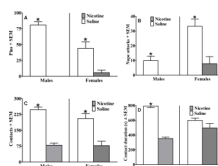
**Figure 1.**

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 (left bars) and female (right bars) 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).



**Figure 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).



**Figure 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).