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Age, sex and early environment contribute to individual differences in nicotine/acetaldehyde-induced behavioral and endocrine responses in rats

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Abstract

Neonatal handling was used to evaluate the influence of early environment on responses to nicotine. Rats exposed as pups to daily short-term separation from the dam (H) were compared to non-handled (NH) controls. In experiment 1, prepubescent males and females, aged postnatal day (P) 30, were tested for the effect of nicotine/acetaldehyde (NicAc) on open field behavior and plasma corticosterone levels. NicAc induced increases in ambulatory activity and time spent in the center of the field in NH, but not H, males. Drug-induced increases in initial ambulatory activity, but not center time, were also seen in NH and H females. Handling, but not sex, contributed to group differences in plasma corticosterone levels. In experiment 2, NH and H rats were tested for acquisition of NicAc self-administration at three ages, P27–31, P34–38 and P90–94. Age and sex, but not handling, contributed to differences in performance of this task. Whereas males exhibited a decrease in responding with age, females did not. These findings demonstrate that neonatal handling may serve as an experimental model for individual differences in sensitivity to tobacco constituents. Furthermore, the current study indicates that stress reactivity, age and sex may play differential roles in initiating smoking behavior.

Keywords: Nicotine self-administration; Age; Sex differences; Postnatal handling; Corticosterone; Locomotion

1. Introduction

Adolescence is a critical period for smoking initiation (Eissenberg and Balster, 2000). Although most teenagers try smoking, longitudinal studies have shown that there is considerable inter-individual variability in the trajectories of subsequent adolescent smoking patterns (Stanton et al., 2004; Karp et al., 2005). Whereas a subset of adolescents progress to become regular tobacco users, the majority do not. In order to target early intervention efforts, it is important to understand what factors contribute to the rapid escalation of smoking in these adolescents. Although social and environmental factors are clearly important, biological factors including age, sex and temperament also play important roles. Those who initiate smoking during early adolescence are more likely to continue and have greater difficulty quitting than those who begin later (Breslau and Peterson, 1996). Whereas female smokers show lower sensitivity than male smokers to the discriminative stimulus effects of nicotine, women are more sensitive to mood changes after smoking and during abstinence, resulting in shorter or less frequent abstinence periods compared to their male counterparts (Perkins et al., 1999). In addition, nicotine has been shown to inhibit negative mood induced by moderate stress in females but to enhance it in males (File et al., 2001). Finally, although negative affect is highly associated with smoking in teenagers of both sexes, there are significant differences between males and females in how such factors interact to influence tobacco use (Whalen et al., 2001; Jamner et al., 2003).

Rodents provide a useful model for studying the biological factors that contribute to initiation of tobacco use, since there are fewer ethical constraints regarding drug treatment and experimental conditions can be more readily controlled than in

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clinical studies. Such studies clearly show that age and sex are important factors in the sensitivity to tobacco constituents. Adolescent rodents exhibit increased sensitivity to the rewarding effect of nicotine as evidenced in conditioned place preference (Belluzzi et al., 2004; Vastola et al., 2002), two-bottle choice tests (Adriani et al., 2002) and self-administration (Belluzzi et al., 2005; Levin et al., 2003). Exposure to nicotine during adolescence also induces numerous long-term cellular changes (Abreu-Villaca et al., 2003a,b; Slotkin et al., 2004) and increases nicotine self-administration during adulthood (Adriani et al., 2003). Sex differences in response to nicotine, which are well characterized in adulthood, begin to emerge during adolescence. In animal studies, female adolescents have been shown to consume more nicotine in two bottle choice tests (Klein et al., 2004), are more sensitive to the anxiolytic effect of nicotine during social stress (Cheeta et al., 2001) and to disruption of neuronal cell markers following chronic nicotine exposure (Abreu-Villaca et al., 2003b). Self-administration studies also indicate that adult female rats are more motivated to take nicotine, have increased total intake at lower doses (Donny et al., 2000) and respond more to nonpharmacological cues (Chaudhri et al., 2005).

The stress system, which regulates physiological response to the environment, has been highly implicated in addiction to many substances, including tobacco (Koob, 1999). There is a clear bi-directional interaction between nicotine and the hypothalamic-pituitary-adrenal (HPA) stress axis. Nicotine administration activates the HPA axis and increases glucocorticoid release in adult animals (Balfour et al., 1975; Cam and Bassett, 1983; Matta et al., 1998) and humans (Pomerleau and Pomerleau, 1990; Seyler et al., 1984). Glucocorticoid, in turn, modulates responsiveness to nicotine by mediating either drug tolerance or sensitization, depending on the test situation (Caggiula et al., 1998; Pauly et al., 1988). Whereas genetic predisposition influences the interaction between nicotine and the stress system (Pauly et al., 1990), the importance of gene × environment interactions is gaining attention (Cabib et al., 2000). High stress response to a novel environment has been associated with increased sensitivity of rodents to the actions of many addictive drugs (Piazza and Le Moal, 1996). Whereas natural variations occur in central and peripheral stress reactivity, such individual differences can be induced experimentally by manipulation of neonatal rearing conditions, such as brief or prolonged periods of early maternal separation (Kosten et al., 2000; Meaney et al., 2002; Pryce and Feldon, 2003). Such studies have shown that increases in stress reactivity, induced by early rearing conditions, increase sensitivity to psychostimulant drugs in adulthood (Matthews et al., 1999; Meaney et al., 2002; Flagel et al., 2003; Kosten et al., 2004). However, differential rearing models have not yet been used to determine whether intrinsic differences in stress reactivity are associated with increased response to tobacco constituents.

In the present study, we have tested the hypothesis that rats with differential stress reactivities induced by early rearing conditions will exhibit different behavioral and endocrine responses to the effects of tobacco constituents during adolescence. We have used a brief daily handling model, which has been shown to reduce subsequent HPA reactivity of male rat pups (Liu et al., 1997; Plotsky and Meaney, 1993) and alter their response to abused drugs (Brake et al., 2004; Kalinichev et al., 2002; Meaney et al., 2002). Recently, we have shown that acetaldehyde, one of the constituents of tobacco smoke, substantially increases nicotine self-administration during early adolescence (Belluzzi et al., 2005). Therefore, acetaldehyde was combined with nicotine (NicAc) in the present study in order to more closely mimic smoking.

2. Materials and methods

2.1. Animals

Animals were maintained in a temperature (21 ± 3 °C) and humidity (50%) controlled room on a 12-h light cycle (0600–1800) with unlimited access to food and water. All experiments were carried out in accordance with the Institutional Animal Care and Use Committee at the University of California, Irvine, and with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Thirty-two pregnant Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) were ordered in groups of two at gestational day (G) 13–15 and were single-housed for a week before giving birth to pups on G22. The first 24-h period after birth was designated as postnatal day (P) 0. Litters were culled to 8–12 and sex-balanced on P1. Litters were randomly assigned to one of two neonatal manipulation groups: handling (H) and non-handling (NH). Animals were weaned on P21 and 3–4 animals were housed by treatment group in single-sex rooms until surgical implantation of intravenous catheters, which was done 4–5 days prior to the initiation of behavioral tests. Animals were single housed after catheter implantation.

Two separate experiments were carried out. For Experiment 1, a male and female from each litter were assigned to each drug treatment group for a total of 6–7 animals per group. These animals, aged P30, were studied for locomotor and endocrine response to i.v. injection of NicAc. For Experiment 2, 1–2 males and 1–2 females from each litter were assigned to one of three age groups, prepubescent (P27), pubescent (P34) or adult (P90 or over). Duplicate animals were used from each litter because of the possible loss of catheter patency during the course of the experiment. A total of 22 animals aged P27, 37 aged P34 and 38 aged P90 or older were used in Experiment 2 for analysis of NicAc self-administration. Although the data shown include individual littermates, similar statistical findings were obtained when littermate values were averaged.

2.2. Neonatal manipulations

The current study used the handling paradigm described previously by Meaney (Liu et al., 1997). Handling, which was done once daily from P1–P14, involved the removal of the dam, and then the pups, from the home cage into separate cages lined with fresh bedding material. The pups were weighed and remained separated from the dam in a temperature-controlled chamber for a period of 15 min, after which they were returned to the home cage along with the dam. The pups were removed

together as an entire litter from the dam during the 15 min separation process. Handling was carried out between 9 AM and 12 PM each day. Non-handled control litters, ordered together with H groups, were not disturbed except for weekly cage changes until P14. After P14, both H and NH groups were subject to regular cage changes twice a week.

2.3. Surgical implantation of intravenous catheters

Catheters were constructed and surgery performed as described previously (Belluzzi et al., 2004). Animals were anesthetized with Equithesin (0.3 ml/100 g, i.p. for adult, 0.25 ml/100 g, i.p. for adolescent) and a chronic catheter was surgically implanted into the right external jugular vein. The polyethylene assembly was mounted on the animal's back and the catheter was passed subcutaneously from the animal's back to the jugular vein where the tubing was inserted. The wounds were closed with wound clips. Antiseptic ointment was applied to the wounds and the exposed cannula housing after each surgery to prevent infection, and a metal cap was attached to prevent damage to the cannula. The animals were kept in a warm cage for post-surgical observation until they emerged from anesthesia.

The catheters were flushed daily with 0.2 ml sterile heparinized saline solution (0.6 ml or 0.3 ml of 1000 U/ml heparin in 30 ml saline for adults or adolescents, respectively) to maintain catheter patency. On test days, heparinized saline was injected before and after the self-administration session. The injection tubing was heat-sealed near the top of the cannula and left on after injection in order to maintain a closed system and prevent clogging of the catheter. The exposed cannula was protected by a threaded aluminum standoff. At the end of the self-administration experiment, Propofol was injected (0.2 ml adult, 0.05–0.1 ml adolescent) through the catheter to test the patency of the i.v. catheter as indicated by rapid (5–10 s) anesthesia. Data were discarded from any animal not demonstrating rapid anesthesia.

2.4. Experiment 1. Open field locomotion and plasma corticosterone levels

For open field locomotion and plasma corticosterone measurements, both male and female rats were surgically implanted with intravenous catheters at P27 as described above. After 3 days of recovery, animals aged P30 were given two consecutive i.v. injections, a minute apart, with either saline–saline or a mixture of nicotine (30 μg/kg) plus acetaldehyde (16 μg/kg) (NicAc) in order to mimic initial drug exposure during self-administration. Animals were immediately placed in an open field locomotion box and monitored for 30 min. Locomotor activity was measured using 4 open-field activity systems (MED Associates, Inc., St. Albans, VT) measuring 43.2×43.2×30.5 cm. Subject location was recorded by 16 evenly spaced infrared beams located along two adjacent sides of the chamber. Center area was defined as the 28×28 cm square area occupying the center of the each chamber. Computer-assisted data acquisition was used to measure ambulatory counts and time spent in the center area.

After 30 min in the locomotion chamber, animals were decapitated and 2 ml samples of trunk blood were collected in

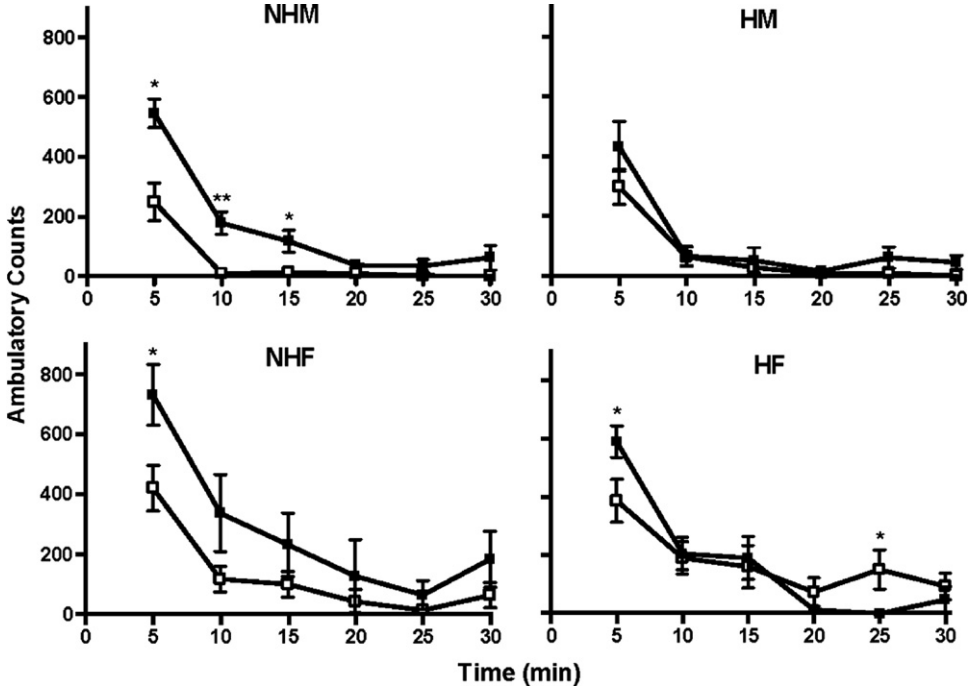

Fig. 1. Ambulatory counts of Non-Handled (NH) and Handled (H) male (M) and female (F) adolescent rats after i.v. injections of a mixture of nicotine (30 μg/kg) plus acetaldehyde (16 μg/kg) (NicAc). Animals received two consecutive i.v. injections of either saline (open square) or NicAc (closed square) and were placed in an open field for 30 min. Data shown are mean±SEM, $n=6$–7/group. $^{**}p<0.01$, $^{*}p<0.05$ vs. saline.

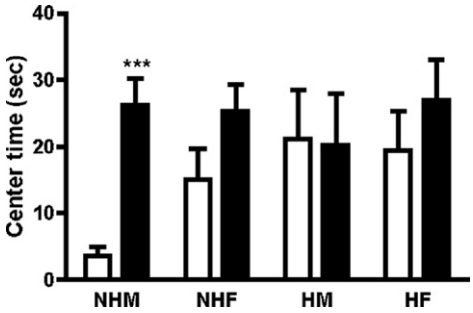

Fig. 2. Time spent in the center area of the open field following NicAc or saline injections in Non-handled (NH) and Handled (H) male (M) and female (F) adolescent rats. Animals received two consecutive i.v. injections of either saline (open bar) or NicAc (closed bar) and were placed in an open field for 30 min. Time spent in the center area during the initial 5-min period is shown for each group. NicAc significantly increased center time in NH males only. Data shown are mean+SEM, $n=6–7$/group. ***$p<0.001$ vs. saline.

the presence of 1.48 mg/ml of EDTA. After chilling on ice, blood samples were centrifuged at 2200 rpm at 4 °C for 30 min. Aliquots of plasma supernatant were stored at −20 °C prior to analysis of hormone concentrations in duplicate samples using a commercial RIA kit (ICN, Costa Mesa, CA), as described previously (Park et al., 2003). A standard curve was generated with a series of known corticosterone samples using commercial software (Prism, GraphPad Software, San Diego, California USA) and the plasma hormonal concentrations in the unknown samples were interpolated from the curve.

2.5. Experiment 2. Drug self-administration

Initial acquisition of NicAc self-administration was measured during three postnatal periods: prepubescent (P27–31), pubescent (P34–38) and adult (P90 or older). Three to five days after surgery, rats were tested in self-administration chambers with two nose-poke holes side-by-side in the door. A 10-ml syringe was mounted in an infusion pump, located outside the test chamber, and connected by polyethylene tubing to a feed-through swivel located above the test chamber. The other side of the feed-through swivel was connected to the infusion cannula on the animal's back with polyethylene tubing covered by a steel spring to prevent puncture from biting. The syringe was filled with enough solution to provide a maximum of 100,100-µl injections. The control of all experimental parameters and the collection of all data were controlled by a multi-channel computer system (MED Associates, Inc., St. Albans, VT).

Animals were tested in daily 2-h sessions with a fixed ratio 1 where each nose-poke at the reinforced hole delivered 100 µl of a mixture of nicotine (30 µg/kg) plus acetaldehyde (16 µg/kg). During each 5.6-s infusion, an 1800-Hz tone sounded and, immediately after the drug infusion, the signal light over the hole went out for 60 s during which time responses were counted but had no effect. To control for nonspecific activating effects of drugs, nose-pokes were counted at the non-reinforced hole where responses had no programmed consequences. The more natural nose-poke response was chosen in an attempt to compensate for the absence of food-rewarded response training.

2.6. Materials

Nicotine ((−)nicotine ditartrate) was purchased from Sigma-Aldrich; all doses are reported as free base. Acetaldehyde was purchased from Supelco in 1-ml sealed vials.

2.7. Data analysis

For open field locomotion and center time, the data were analyzed by handling × drug treatment × sex × time ANOVA with repeated measures on time. Significant main or interaction effects were further analyzed by subsequent ANOVAs and post-hoc tests where appropriate. For corticosterone levels, the data were analyzed by handling × drug treatment × sex ANOVA. For correlational analyses of behavior and corticosterone levels, both locomotion and center time values were taken during the initial 5 min period, during which significant drug effects were observed. Both locomotion and center times were correlated with corticosterone levels from the same animals using Pearson correlation analysis. For self-administration studies, the data were initially analyzed by five-way ANOVA on age × handling × sex × day × reinforced/non-reinforced responses with repeated measures on reinforced/non-reinforced responses and days. Significant main or interaction effects were further analyzed by subsequent ANOVAs with Dunnett's-corrected post-hoc comparisons. Data with more than two grouping variables were analyzed using SYSTAT 9.0 (SPSS, Inc.) and all other analyses were preformed with Prism 4.0 (GraphPad, Inc.) statistical software.

3. Results

3.1. Experiment 1. Open field locomotor and endocrine responses to nicotine/acetaldehyde (NicAc)

Overall analysis indicated that ambulatory activity (Fig. 1) was significantly affected by drug ($F_{1,45}=6.045$, $p<0.02$), sex ($F_{1,45}=8.402$, $p<0.01$) and time ($F_{5,225}=139.146$, $p<0.0001$), with significant interactions between time × sex ($F_{5,225}=3.351$, $p<0.01$) and time × drug ($F_{5,225}=10.298$, $p=0.001$), and a nearly significant interaction between

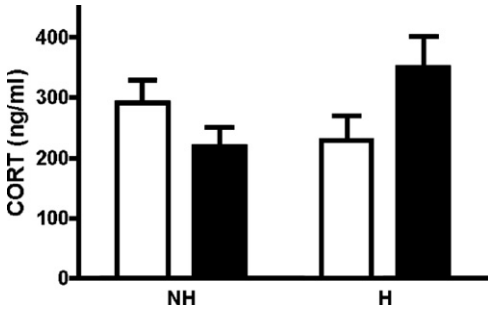

Fig. 3. Endocrine responses of non-handled (NH) and handled (H) male (M) and female (F) adolescents after administration of nicotine plus acetaldehyde (NicAc). Male and female adolescents received two consecutive i.v. injections of either saline (open bar) or NicAc (closed bar). A significant interaction between handling and drug was observed. $n=6–7$/group.

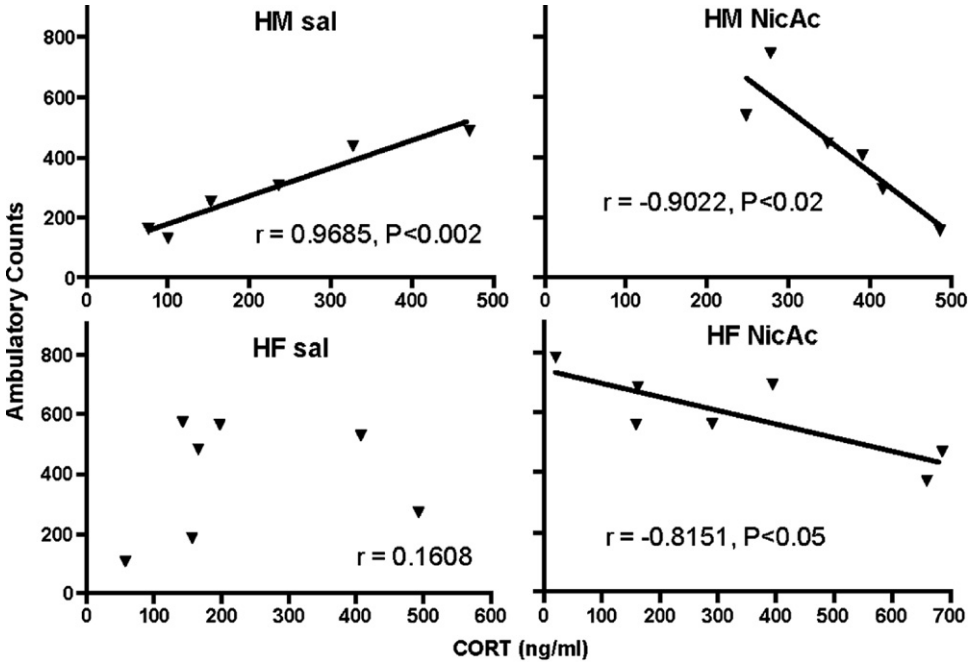

Fig. 4. Correlations between locomotion and corticosterone levels in H rats. Ambulatory counts during initial 5 min in an open field are correlated with corticosterone levels in the same animals. Pearson coefficient (r) is shown for each group with a linear regression plot when the linear correlation is statistically significant.

handling × drug ($F_{1,45}=3.219$, $p=0.0795$). Baseline locomotion was higher in females than males ($p<0.01$), with no differences between handling groups. There was a significant increase in ambulatory activity in response to drug during the initial 15 min period in NH males, but not in H males. NicAc also induced increases in initial 5 min locomotor activity in both H and NH females (Fig. 1).

In evaluating time spent in the center of the open field, which is inversely correlated with anxiety/fear, there was a significant effect of sex ($F_{1,45}=5.944$, $p<0.02$) and significant time × drug ($F_{5,225}=2.496$, $p<0.05$) and time × drug × sex ($F_{5,225}=2.367$, $p<0.05$) interactions. Post-hoc analysis showed that NicAc administration had a significant initial anxiolytic effect, as indicated by a significant increase in center time during the first 5 min, in NH but not H males ($p<0.001$; Fig. 2). Saline-treated NH males also spent significantly less time in the center during the initial 5 min period as compared to H males ($p<0.05$; Fig 2). There were no baseline differences or drug effects in the two female groups (Fig. 2).

In the overall analysis of plasma corticosterone levels, a significant drug × handling interaction ($F_{1,53}=5.3117$, $p<0.05$; Fig. 3) was observed. Whereas post-hoc analyses did not reach significance, there was a trend towards drug-induced increases in plasma corticosterone levels in H animals, but the opposite in NH.

Correlation of endocrine and behavioral response in the same animals revealed a significant relationship between plasma corticosterone levels and initial locomotor response to the novel environment in H, but not NH, animals (Fig. 4; Table 1). In saline-treated H males, there was a highly significant ($p<0.01$) positive correlation between these parameters. Although NicAc did not induce a significant change in locomotor activity within the H male group (Fig. 2), it resulted in a negative correlation between plasma corticosterone and initial locomotor response ($p<0.05$). Whereas saline-treated H females did not show any correlation between plasma corticosterone and initial locomotor activity, NicAc treatment of this group also induced a negative correlation between these factors ($p<0.05$). No such correlations were observed in NH animals (Table 1). Furthermore, with the exception of a nearly significant positive correlation between initial center time and corticosterone levels in saline-treated H males ($p=0.056$), there was no observed interrelationship

Table 1
Correlation coefficient (r) between either ambulatory (Amb.) counts or center time and corticosterone levels (CORT) in the same animals

Group	NHM		NHF		HM		HF	
Treatment	Sal	NicAc	Sal	NicAc	Sal	NicAc	Sal	NicAc
r: Amb. Counts vs. CORT	−0.151	0.688	0.019	−0.135	0.969**	−0.902*	0.161	−0.815*
r: Center Time vs. CORT	−0.026	0.463	0.003	0.329	0.799	−0.716	0.030	−0.298

Non-handled (NH) and handled (H) male (M) and female (F) rats received two consecutive i.v. injections of either saline (Sal) or nicotine/acetaldehyde (NicAc) and were placed in an open field for 30 min before collecting blood samples for CORT analysis. Correlation coefficients for ambulatory counts and center time during the initial 5 min vs. CORT levels are shown. *$p<0.05$, **$p<0.01$.

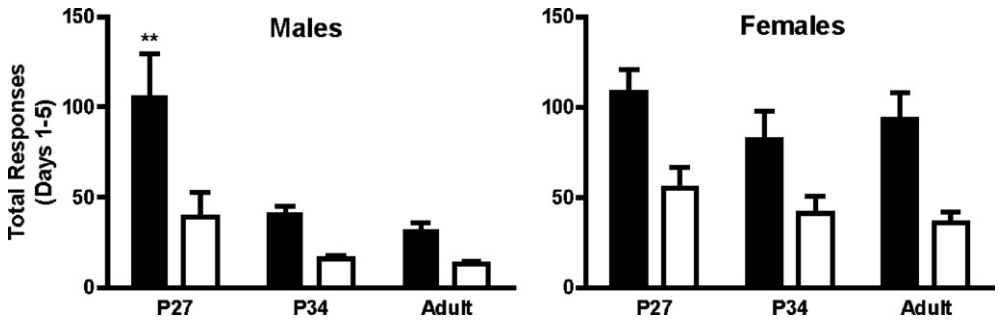

Fig. 5. Age and sex differences in NicAc self-administration. Both male and female NH and H rats were catheterized and tested in daily 2-hr self-administration sessions on a FR1 schedule for 5 days at the age of P27, P34 and adult (P90 or older). Mean 5-day total reinforced (closed bar) and non-reinforced (open bar) responses for males and females for all ages, respectively. Since no significant handling effect was observed, data are collapsed across H and NH groups. $^{**}p<0.01$ vs. P34 and P90. Data shown are mean+SEM, $n=11–19$/group.

between fear/anxiety levels and HPA activity in any animal group (Table 1).

3.2. Experiment 2. Self-administration of nicotine/acetaldehyde (NicAc)

Three age groups, prepubescent (P27–31), pubescent (P34–P38) and adult (P90 or over), were tested for acquisition of NicAc self-administration. Overall analysis indicated that both males and females (Fig. 5) of all age groups responded significantly more at the reinforced than at the non-reinforced hole ($F_{1,85}=84.334$, $p<0.0001$). There were also significant effects of age ($F_{2,85}=6.653$, $p=0.002$) and sex ($F_{1,85}=14.475$, $p<0.0005$), but not of handling ($F_{1,85}=0.0111$, $p=0.916$). Therefore, data were collapsed across handling groups and total responses at reinforced and non-reinforced holes during the 5-day acquisition period were compared between males and females at different ages.

Males aged P27–31 self-administered NicAc substantially more than those aged P34–38 ($p<0.01$) or adult ($p<0.01$). This age-related decline in males was also evident in non-reinforced responding ($p<0.01$). Such age-related differences in 5-day total responding were not observed in females. Total responding at the reinforced hole was similar across female age groups and was not significantly different from that of prepubescent males.

4. Discussion

Substantial individual differences in the onset of smoking behavior have been documented in the clinical literature (Schepis and Rao, 2005; Turner et al., 2004). Whereas social influences have a major role in shaping these differences, biological factors may also be critical. Although animal studies are valuable in clarifying underlying biological mechanisms, few such studies have evaluated individual differences in behavioral response to nicotine, particularly during adolescence, a developmental period with high rates of smoking initiation in humans (Eissenberg and Balster, 2000) and increased sensitivity to the rewarding effect of nicotine and tobacco constituents in animals (Adriani et al., 2002; Belluzzi et al., 2004, 2005; Vastola et al., 2002).

The aim of the present study was to systematically evaluate the influence of sex and stress reactivity on behavioral and endocrine responses to tobacco constituents in adolescent and adult rats. We have used an early environmental manipulation model (Liu et al., 1997), which has been shown to produce substantial changes in stress responsiveness of adolescent male, but not female, rats (Park et al., 2003). Whereas the majority of animal studies have focused on nicotine alone, we have recently shown that other constituents of tobacco smoke can significantly enhance nicotine's rewarding effects (Belluzzi et al., 2005; Villegier et al., 2006). We have therefore used a mixture of nicotine and acetaldehyde (NicAc) in the current study, which more closely mimics smoking-induced biological effects.

4.1. Locomotor and endocrine effects of NicAc

In our first experiment, we evaluated whether differences in sex and/or stress reactivity influence adolescent locomotor and endocrine response to NicAc. Our findings support and extend earlier observations in adult Long Evans rats that early handling induces persistent changes in behavioral responses to abused drugs (Brake et al., 2004; Kalinichev et al., 2002). Although there were no differences in basal locomotor activity of H and NH males when placed in an open field, NicAc induced increases in initial locomotor response only in NH males. Such observations are consistent with earlier findings that adult NH males are more sensitive to the locomotor activating effects of cocaine (Brake et al., 2004). The mechanism underlying these handling-induced differences in locomotor response to psychostimulants in male rats is unclear, but may reflect changes in dopaminergic signaling within the striatum, as has been shown previously in NH males (Brake et al., 2004). Consistent with our earlier observations that adolescent females do not respond to neonatal handling in the same way as males (Park et al., 2003), we did not observe early handling effects on the locomotor response of female rats. Basal locomotor activity was elevated in both H and NH females as compared to males, and NicAc induced a significant increase in initial locomotor response in both female groups.

It has been shown previously that NH adult males are more fearful when exposed to a novel environment, and that this

behavior is related to decreased GABAergic tone within mPFC and brainstem noradrenergic nuclei (Caldji et al., 2000). We now show similar initial fearful behavior in NH adolescent males, as measured by decreased time spent in the center of an open field. This fearfulness is reversed by administration of NicAc. In contrast to the males, the female groups did not show early handling-induced differences in initial center time in either the absence or presence of drug. Although clinical data have shown that trait anxiety is highly predictive of tobacco dependence in adolescents (DiFranza et al., 2004), animal studies have yielded conflicting data as to whether or not nicotine is anxiolytic in adolescent male or female rats (Cheeta et al., 2001; Elliott et al., 2004; Genn et al., 2003). These inconsistencies are believed to reflect a number of factors such as rat strain, nicotine dose, behavioral test and housing and dietary conditions. Our data are consistent with those of Elliott et al. (2004), who have shown that nicotine is anxiolytic in male but not female adolescent Sprague Dawley rats. However, we have shown that this is only the case in the subset of NH males with increased fearful behavior in response to a novel environment. In both our study and that of Elliott et al. (2004), female adolescents had lower baseline anxiety levels than did the males who exhibited an anxiolytic response to drug treatment. Thus, an anxiolytic effect of nicotine or NicAc in adolescents may be seen only in animals in which there is an increased basal anxiety level.

Handling, but not sex, altered the endocrine response to NicAc administration in adolescent rats. Although nicotine induces substantial activation of the HPA axis in adult rats (Matta et al., 1998; Rhodes et al., 2001), we observed a minimal drug response in adolescents, with a trend towards increased plasma corticosterone levels in H animals and decreased levels in NH. The HPA axis is still maturing during adolescence (Romeo et al., 2006) and the corticosterone response to nicotine is different from that of adults (Cruz et al., 2005). Our present findings are consistent with other studies in our laboratory which have shown that nicotine and NicAc do not induce HPA activation in adolescents under the same experimental conditions in which there is robust activation in the adult (Cao et al., 2004). Although there are substantial sex differences in nicotine-induced corticosterone release in adult rats (Rhodes et al., 2001), sex differences in the HPA axis do not emerge until puberty (Spear, 2000). Since the present studies were carried out in pre-pubescent animals, no sex differences were observed.

Within-animal correlational analysis of behavioral data and plasma corticosterone levels yielded complex findings. Overall, however, no significant correlations were found in the NH groups. Furthermore, initial locomotion in H animals was more highly correlated with plasma corticosterone levels than was center time. This suggests that HPA activity is more closely associated with motor response to novelty stress than with fear/anxiety in adolescent rats. Although surprising, this finding is consistent with that of a recent study showing a significant correlation between corticosterone levels and initial locomotion in a novel environment in adult male rats, but not with time spent in the open arms of an elevated plus maze (Marquez et al., 2006). Furthermore, neonatal handling and NicAc treatment appear to be important in modulating the interrelationship between HPA activity and initial locomotor response to novelty stress. In saline-injected H males, but not females, there was a highly significant positive correlation between initial locomotor activity and plasma corticosterone. This finding suggests that neonatal handling of male rats may alter development of the limbic system such that there is a stronger integration of the HPA axis and the dopamine circuitry that mediates locomotor response to novelty. Consistent with earlier studies that neonatal handling does not produce the same changes in limbic system development as occurs in males (Park et al., 2003), we did not see this striking interrelationship in H females. However in both H males and females, NicAc produced a strong negative correlation between HPA activity and locomotor response to novelty. The mechanism underlying this drug effect is unknown, but may reflect the unique actions of nicotine on adolescent prefrontal cortex (Schochet et al., 2005).

4.2. Self-administration

The groundbreaking work of Piazza and Le Moal (1996) has shown a strong correlation between locomotor response to a novel environment, HPA activation and acquisition of psychostimulant self-administration. Furthermore, a positive correlation between high responding to novelty and acquisition of nicotine self-administration has previously been shown in adult male rats (Suto et al., 2001). It was therefore surprising that, despite the enduring effect of neonatal handling on open field behavior and HPA axis reactivity, no handling effect on NicAc self-administration was observed. However, the correlation between HPA activity and novelty response that we observed was only within H groups, whereas our comparison of self-administration scores was between H and NH animals. It has previously been suggested that NH animals are aberrant in that they do not receive a routine amount of laboratory care (Pryce and Feldon, 2003). Since prior studies relating individual differences in locomotor response to novelty and acquisition of drug self-administration were done on laboratory-maintained animals, these animals had received handling that was more akin to that of H rats. Thus, early rearing conditions may be a critical variable in the outcome of such studies.

Our present data do indicate that both sex and age regulate total intake of NicAc in H and NH animals. We have replicated our earlier finding (Belluzzi et al., 2005) that prepubescent males self-administer significantly more NicAc as compared to older ages. However, females showed no such age-dependent change in behavioral response. In fact, females maintained high responding for both reinforced and non-reinforced holes, as well as time-out responding (data not shown), at all ages tested. This increased activity in adult females has also been observed by others in nicotine self-administration studies (Donny et al., 2000). The age-dependent decline seen in males is not due to an inhibitory effect of testosterone since gonadectomy did not affect total NicAc intake in pubescent males (data not shown). Interestingly, estradiol levels in both males and females during pubertal development show a pattern similar to the total NA intake seen in the current study (Hodes and Shors, 2005). Estradiol is known to regulate nicotinic receptors (Svensson and

Nordberg, 1999) and nicotine-induced DA release (Dluzen and Anderson, 1997). Therefore, decreased estradiol levels in older males may inhibit nicotine intake. Further studies will be needed to evaluate the mechanisms underlying these sex differences in reward-related behavior.

In summary, our data indicate that there is a complex interaction between sex, age and early environment in mediating biological responses to tobacco constituents. These findings are consistent with clinical observations of differential contributions to smoking behavior of genetic heritability, environment, age and sex (Shenassa et al., 2003). Our present findings suggest that neonatal manipulations in rodents may provide a useful experimental model for studying individual differences in biological response to tobacco constituents.

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