Published in final edited form as:

Behav Brain Res. 2014 April 1; 262: 101–108. doi:10.1016/j.bbr.2013.12.014.

Ethanol Conditioned Place Preference and Alterations in FosB Following Adolescent Nicotine Administration Differ in Rats Exhibiting High or Low Behavioral Reactivity to a Novel Environment

Rex M. Philpot, Melanie E. Engberg, and Lynn Wecker

Department of Psychiatry and Behavioral Neurosciences University of South Florida Morsani College of Medicine, Tampa, FL

Abstract

This study determined the effects of adolescent nicotine administration on adult alcohol preference in rats exhibiting high or low behavioral reactivity to a novel environment, and ascertained whether nicotine altered FosB in the ventral striatum (vStr) and prefrontal cortex (PFC) immediately after drug administration or after rats matured to adulthood. Animals were characterized as exhibiting high (HLA) or low (LLA) locomotor activity in the novel open field on postnatal day (PND) 31 and received injections of saline (0.9%) or nicotine (0.56 mg free base/kg) from PND 35–42. Ethanol-induced conditioned place preference (CPP) was assessed on PND 68 following 8 days conditioning in a biased paradigm; FosB was measured on PND 43 or PND 68. Following adolescent nicotine exposure, HLA animals demonstrated a CPP when conditioned with ethanol; LLA animals were unaffected. Further, adolescent nicotine exposure for 8 days increased levels of FosB in limbic regions in both HLA and LLA rats, but this increase persisted into adulthood only in LLA animals. Results indicate that adolescent nicotine exposure facilitates the establishment of an ethanol CPP in HLA rats, and that sustained elevations in FosB are not necessary or sufficient for the establishment of an ethanol CPP in adulthood. These studies underscore the importance of assessing behavioral phenotype when determining the behavioral and cellular effects of adolescent nicotine exposure.

Keywords

Addiction; Adolescent; FosB; Ethanol; Nicotine; Reward

© 2013 Elsevier B.V. All rights reserved

Corresponding Author: Rex M. Philpot, Ph.D. Department of Psychiatry & Behavioral Neurosciences USF Morsani College of Medicine 3515 East Fletcher Avenue Tampa, FL 33613 (813) 390-2031 rphilpot@health.usf.edu.

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

1. Introduction

Numerous studies have indicated that high novelty-seeking and exploration are associated with increased sensitivity to drug reward [1–8]. Adolescents have been shown to exhibit greater novelty-seeking and exploration than adults [9–11], and several reports demonstrate that adolescents are more likely than adults to progress to addiction when initiating drug use [12–18]. Thus, adolescents may be more susceptible to the reinforcing and rewarding effects of abused drugs, and adolescents with a high sensation-seeking profile may represent the most vulnerable population.

The two drugs most commonly used by adolescents are nicotine and alcohol [19, 20], and evidence suggests that the use of nicotine affects alcohol consumption. Smoking and drinking behaviors often occur together, with the frequency of either behavior associated with the frequency of the other [21]. Grant [22] reported that nearly 29% of individuals who begin smoking before the age of 14 become alcohol dependent and 8% progress to alcohol abuse during their lifetime. Further, 19% of those who initiate smoking between 14 and 16 become alcohol dependent, with 7% of these individuals progressing to alcohol abuse. Interestingly, individuals who do not initiate smoking until 17 years of age are half as likely to become alcohol dependent or progress to addiction. Thus, early onset smoking is a strong predictor of lifetime drinking, and alcohol dependence and abuse [22].

Adolescent nicotine exposure has been shown to increase the rewarding effects of several drugs in adult laboratory animals, including nicotine, cocaine and diazepam [23–26]. Further, Riley et al. [27] demonstrated that the administration of nicotine to mice during adolescence, but not adulthood, increases sensitivity to ethanol withdrawal when measured in adulthood, and suggested that adolescence represents a critical period of sensitivity to nicotine that results in changes in the brain that persist into adulthood. This idea is supported by several studies demonstrating that adolescent exposure to nicotine leads to an anxiogenic state in adulthood [28–30]. It is possible that enduring alterations following adolescent nicotine exposure involve the transcription factor FosB, which has been shown to produce persistent sensitization of the mesolimbic pathway and to heighten sensitivity to the motivational properties of several drugs of abuse, including alcohol [31–34], and whose overexpression in the limbic system enhances drug preferences [31, 35]. Interestingly, adolescent animals exhibit greater increases than adults in FosB in the nucleus accumbens (NAcc) in response to the administration of cocaine or amphetamine [36]; the effect of nicotine administration during adolescence on FosB has not been examined. Because adolescent animals exhibit enhanced up regulation of FosB relative to adults in response to abused drugs, they may be more sensitive to rewarding stimuli following repeated exposure than similarly exposed adults. This idea is supported by studies indicating that adolescent rats that establish a nicotine-induced conditioned place preference (CPP) following 4 injections exhibit an increase in FosB immunoreactivity (the FosB splice variant was not specifically measured) in the ventral tegmental area (VTA), NAcc and prefrontal cortex (PFC) immediately following behavioral testing [37].

Despite evidence that adolescence is a period of increased sensation-seeking and first time drug use, that nicotine use is linked with increased ethanol use, and that an increased

sensitivity to drugs of abuse is associated with FosB accumulation [31], the impact of adolescent nicotine exposure on FosB levels and its long term consequences on ethanol reward are unclear. Therefore, this study: 1) determined the effects of adolescent nicotine administration on adult alcohol preference in rats characterized during adolescence by their behavioral reactivity to a novel environment, viz., exhibiting high or low locomotor activity; and 2) ascertained whether nicotine altered FosB in the ventral striatum (vStr) and PFC of these animals immediately after administration in adolescence or after rats matured to adulthood.

2. Methods

2.1 Materials

Ethanol was obtained from AAPER Alcohol and Chemical Company (Shelbyville, KY). All other reagents were purchased from Sigma-Aldrich Life Sciences (St. Louis, MO) unless otherwise noted.

2.2 Subjects

The male and female offspring (n=89) of timed pregnant rats (n=10) were used as subjects; the day of birth was defined as postnatal day 0 (PND 0). To assure similar development across litters, all litters were culled to 10–12 pups (5–6 males/5–6 females) on PND 1, and remained housed with their respective dams until PND 21, at which time animals were weaned and housed in same sex groups of 3 in standard polypropylene cages with corncob bedding. All animals were housed at the University of South Florida in a temperature and humidity-controlled vivarium on a 12:12-hr light–dark cycle (7 a.m./7 p.m.). Experiments were conducted during the light phase, and the care and use of animals was in accordance with guidelines set by the Institutional Animal Care and Use Committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. In accordance with these guidelines, experiments utilized the fewest number of animals per group necessary to obtain meaningful data.

2.3 Characterization of Behavioral Reactivity to a Novel Environment

Locomotor activity was used to characterize the behavioral reactivity of rats to a novel environment. To accomplish this, on PND 31, animals were removed from their home cage and placed in a circular arena (100 cm diameter) under moderate illumination (20 lux) for 5 min. The total distance moved (TDM) was recorded automatically with a video camera and analyzed using EthoVision software (Noldus Information Technology, Leesburg, VA) as described [38]. Animals were classified as exhibiting either high (HLA) or low (LLA) locomotor activity in the novel open field using a median split strategy, with the former exhibiting activity in the upper 50%, and the latter in the lower 50% relative to their littermates [4].

2.4 Nicotine Injections

Animals received injections (s.c.) of either phosphate-buffered saline (PBS, 0.9%), or nicotine hydrogen bitartrate in PBS (0.56 mg free base nicotine/kg) once daily for 4 or 8 days beginning on PND 35. This dose of nicotine has been demonstrated to increase

responding for conditioned stimuli [39, 40] and increase breakpoints for reinforced responding [41] indicating that it is rewarding and reinforcing, and was used in a prior study of adolescents [38]. For each injection, animals were transported in their home cage to a dimly lit procedure room, placed in a new cage lined with fresh bedding, injected, and returned to their home cage.

2.5 Conditioned Place Preference (CPP)

For measures of CPP, rats received injections of nicotine from PND 35–42 and 18 days following the last injection of nicotine, on PND 60, animals ($n=40$; 4–5 per group) were allowed free access to two interconnected Plexiglas chambers (each chamber: 21 cm wide $\times$ 18 cm long $\times$ 21 cm high) containing distinct visual (vertical or horizontal black and white stripes) and tactile cues (rubberized or sandpaper flooring) for three 5 min intervals. The mean time spent on each side of the apparatus was used to determine baseline chamber preference for each animal. Although each animal exhibited a side preference at baseline, there was no tendency within the population for a particular chamber to be preferred. Over the next 8 days, from PND 61 to 68, a biased conditioning paradigm was used wherein animals were trained to associate the non-preferred chamber with the subjective effects of ethanol. For conditioning, each animal received an injection of ethanol (17%; 1.0 g/kg, i.p.) and was subsequently confined to the initially non-preferred chamber for 15 min. This dose and concentration of ethanol has been shown to establish a CPP during late adolescence [42] and to significantly elevate dopamine in the NAcc of adolescent and young adult animals [43, 44]. Control animals were confined for 15 min to the initially non-preferred chamber following an injection of saline (0.9%, i.p.). Both ethanol-conditioned and control animals received saline injections prior to being confined to the initially preferred chamber for 15 min each day. Thus, each animal received 2 training sessions per day, one for the initially non-preferred and one for the preferred chamber. The order of these sessions was alternated on each day and occurred in the morning and afternoon, separated by at least 5 hours. On PND 69, approximately 16–18 hours after the last training session, animals were allowed free access to both chambers for 5 min and the time spent in each chamber was measured to assess the CPP. A preference score was calculated by subtracting the time spent in the initially preferred chamber from the time spent in the initially non-preferred chamber.

2.6 Western Blot Analyses

For immunoblot analyses, rats were decapitated rapidly and the vStr and PFC isolated 24 hrs after either the 4th or 8th nicotine injection on PND 39 or 43, respectively, ($n=32$; 4 per group) or 26 days following the 8th injection on PND 69 ($n=16$; 4 per group), corresponding to the day that CPP was assessed in a separate group of animals. Tissue was quick frozen on dry ice and stored at -80°C until homogenized as described [38]. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (10% polyacrylamide) and transferred electrophoretically to polyvinylidene fluoride membranes. The membranes were blocked for 1 hour in Tris-buffered saline containing 0.1% Tween 20 and 5% nonfat dry milk. Subsequently, primary antibody [FosB (5G4) #2251, 1:4000; Cell Signaling, Danvers, MA], which produces robust labeling of FosB [45], was added in blocking solution and the membranes were incubated overnight at 4°C . Sixteen hours later, the membranes were washed and incubated with secondary antibody [goat anti-rabbit IgG-HRP, 1:2000, Santa

Cruz Biotechnology, Inc., Santa Cruz, CA] in blocking solution for 1 hour at room temperature, and signals visualized using enhanced chemiluminescence. After immunodetection, blots were stripped, blocked and incubated with a primary antibody directed against β -tubulin [H-235, Santa Cruz Biotechnology, Inc., 1:16,000] as a loading control. The 35/37 kDa band representing FosB and the 50 kDa band corresponding to β -tubulin were quantified on each blot using a densitometer and Un-Scan-It gel digitizing software (Silk Scientific Inc., Orem, Utah). The optical density of the former was normalized to the latter for each sample, and results are expressed as percent of corresponding saline controls on each blot to eliminate variability across blots.

2.7 Statistical Analyses

A 4 factor analysis of variance (ANOVA) was used to determine effects on CPP [(male or female) $\times$ (HLA or LLA) $\times$ (saline or nicotine exposure) $\times$ (saline or ethanol conditioning)] and Tukey's test was used post hoc to ascertain significant differences between groups. A 3 factor ANOVA was used to determine differences in FosB between male and female HLA and LLA animals [(male or female) $\times$ (HLA or LLA) $\times$ (saline or nicotine)] with the Student's t-test performed post hoc to ascertain significant differences between groups. A level of $p < 0.05$ was accepted as evidence of a significant effect. Because the sample size in these studies was small, leading to reduced statistical power, effect size (η_p^2) or Cohen's D) was determined for all analyses and non significant effects with an effect size greater than 0.06 (η_p^2) or 0.4 (D) are reported.

3. Results

3.1 Behavioral Reactivity to a Novel Environment

The locomotor activity exhibited by adolescent rats in a novel open field for 5 min is shown in Figure 1. The TDM was normally distributed (Kolmogorov-Smirnov $D = 0.083$, $p > 0.05$), with animals exhibiting a range of movement between 4339 and 7739 cm/5 min. The median TDM was 5936 cm/5 min with one animal at the median (shown in the grey circle), which was removed from further study. The TDM for HLA and LLA groups was significantly different [$t(86) = 12.15$, $p < 0.05$; Cohen's $D = 2.56$] with a TDM of 6621 ± 71 cm/5 min for HLA animals and 5499 ± 59 cm/5 min for LLA animals. Animals were systematically assigned to experimental groups according to behavioral reactivity to the novel environment to ensure that all groups exhibited equivalence in novel open field activity, and contained equal numbers of HLA and LLA animals (Table 1). Further, no more than 1 male and 1 female from a given litter were assigned to each group.

3.2 Ethanol CPP in Adulthood Following Nicotine Exposure During Adolescence

The first set of experiments determined whether nicotine exposure during adolescence increased vulnerability to the rewarding effects of alcohol in adulthood, and ascertained whether responses were dependent on the behavioral reactivity of the rats to a novel environment. Following classification of rats as HLA or LLA, animals received injections of saline or nicotine from PND 35–42, and CPP to ethanol was determined when rats were young adults on PND 69. Results are shown in Figure 2. ANOVA indicated a significant 3-

way interaction among novel open field activity (HLA or LLA), nicotine exposure, and ethanol conditioning [$F(1,19) = 5.165$, $p < 0.05$], with an observed power of 0.578 and an estimated effect size (η_p^2) of 0.214. No significant differences were observed between males and females as a main effect or interaction and the effect size (η_p^2) was less than 0.06 in all cases, indicating that this variable had little impact on the observed results. HLA animals exposed to nicotine during adolescence and conditioned with ethanol in adulthood exhibited a preference for the ethanol-paired compartment when compared to HLA animals that were either nicotine exposed and saline-conditioned or saline exposed and ethanol conditioned [$p < 0.05$]. Nicotine exposed LLA animals appeared to exhibit an aversion to the ethanol-paired chamber when compared to corresponding saline exposed animals with an effect size (Cohen's D) of 0.80, but this effect did not reach significance [$t(7) = 1.346$, $p > 0.05$] at an observed power of 0.425. Thus, data indicated that HLA adolescents possess a vulnerability to ethanol reward that can be primed, or initiated, by adolescent exposure to nicotine, while LLA and saline exposed HLA animals exhibit responses to ethanol typical of adult rats [42, 46].

3.3 FosB in Adolescence During Repeated Nicotine Exposure

Because increases in FosB in limbic structures enhance drug preference [15,16], experiments determined whether adolescent nicotine exposure had a differential effect on levels of this transcription factor in vStr and PFC from HLA and LLA rats. Following behavioral classification, male and female rats received injections of either saline or nicotine for 4 or 8 days beginning on PND 35. Brain samples were isolated 24 hours after the final injection on PND 39 or 43, respectively, and subjected to Western immunoblot analyses. Results of FosB measurements in the vStr (Figure 3) indicated a significant main effect of both the number of days of injections [$F(1, 16) = 4.542$, $p < 0.05$; $\eta_p^2 = 0.221$] and drug exposure [$F(1, 16) = 18.132$, $p < 0.05$; $\eta_p^2 = 0.531$] and an interaction between drug exposure and phenotype that approached significance [$F(1, 16) = 3.594$, $p = 0.076$; $\eta_p^2 = 0.183$]. There were no significant differences observed between males and females as a main effect or interaction, and effect size (η_p^2) was less than 0.025 in all cases, indicating that sex had little effect on the observed results. Four days of nicotine exposure increased FosB levels significantly ($p < 0.05$) only in the vStr of HLA rats, and this increase persisted following 8 days of nicotine exposure, a time when nicotine also significantly ($p < 0.05$) increased FosB levels in vStr from LLA rats. Analysis of FosB in the PFC revealed a significant interaction between the number of days of injections and drug exposure [$F(1, 16) = 7.912$, $p = 0.05$; $\eta_p^2 = 0.331$]. There were no significant differences observed between males and females as a main effect or interaction; however, the interaction of sex with days of injection and drug exposure did approach significance ($p = 0.055$; $\eta_p^2 = 0.211$) with males tending to exhibit higher FosB values following 4 days of nicotine than females. Overall the levels of FosB in PFC were unaltered following 4 days of nicotine exposure in either HLA or LLA animals, but 8 days of nicotine exposure led to similar significant ($p < 0.5$) increases in FosB in tissue from both HLA and LLA rats. Thus, nicotine had a differential time effect on levels of FosB in vStr from HLA and LLA rats, but not on levels in PFC.

3.4 FosB in Adulthood Following Nicotine Exposure During Adolescence

To determine whether the nicotine-induced elevations in FosB observed in adolescence persisted through young adulthood, following the behavioral classification of rats, animals received injections of saline or nicotine for 8 days from PND 35–42, and 27 days later, on PND 69, the vStr and PFC were isolated and FosB quantified. Results of FosB measurements in the vStr (Figure 4) indicated a significant main effect of both phenotype [$F(1, 16) = 14.349, p < 0.05; \eta_p^2 = 0.642$] and drug exposure [$F(1, 16) = 7.368, p < 0.05; \eta_p^2 = 0.479$]. Similarly, results of FosB measurements in the PFC indicated a significant main effect of phenotype [$F(1, 16) = 9.17, p < 0.05; \eta_p^2 = 0.534$] and drug exposure [$F(1, 16) = 10.129, p < 0.05; \eta_p^2 = 0.559$]. There were no significant differences observed between males and females as a main effect or interaction for FosB measurements in the vStr or PFC.

However, the effect size (η_p^2) for the main effect of sex was 0.143 and 0.191 for the vStr and PFC respectively, with males tending to exhibit higher FosB values than females. The levels of FosB were unaltered in both the vStr and PFC of HLA animals who received nicotine during adolescence relative to their saline-exposed counterparts. In contrast, the levels of FosB in both vStr and PFC from LLA rats who received nicotine during adolescence were significantly ($p < 0.05$) greater than those from either saline-injected LLA animals [vStr $t(3) = 2.47, p < 0.05$; PFC $t(3) = 2.013, p < 0.05$] or nicotine-injected HLA animals [vStr $t(6) = 3.925, p < 0.05$; PFC $t(6) = 2.864, p < 0.05$]. Thus, although 8 days of adolescent nicotine exposure led to immediate increases in FosB levels in vStr and PFC from both HLA and LLA animals, this effect persisted into adulthood only in LLA animals.

4. Discussion

The present study demonstrates that exposure to nicotine during adolescence has differential effects on ethanol CPP and alterations in FosB in limbic regions from rats with different behavioral reactivities to a novel environment. Adolescent nicotine exposure facilitated the establishment of an ethanol CPP in adulthood only in animals who exhibited high locomotor activity in the novel environment in adolescence. Further, although adolescent nicotine exposure increased levels of FosB in the vStr and PFC following 8 days of administration, this increase persisted into adulthood only in animals who exhibited low locomotor activity in a novel environment. Thus, results indicate that the effects of adolescent nicotine exposure on ethanol CPP in adulthood depend on the behavioral phenotype of the animals, and suggest that sustained elevations in FosB in limbic regions are not necessary or sufficient to facilitate an ethanol CPP in adulthood.

The finding that adolescent nicotine exposure facilitates a CPP to ethanol in adulthood in HLA animals agrees with findings that individuals with increased behavioral reactivity to novel stimuli exhibit a greater sensitivity to the rewarding effects of abused compounds than individuals with lower reactivity [1–8]. However, it should be noted that a CPP can be produced by the reinforcement of specific behaviors during conditioning or result from conditioned drug effects [47], and thus, caution should be used when interpreting CPP results as indicative of heightened drug reward. Indeed, Smith et al. [48] did not observe increased ethanol intake in adult Sprague-Dawley rats following adolescent nicotine

exposure, suggesting that the rewarding properties of ethanol were not changed by prior experience with nicotine. However, these authors used a continuous exposure paradigm over 21 days and did not distinguish animals based on locomotor activity in a novel environment. The results of the present study suggest that the consequences of daily injections of nicotine may differ from those produced by continuous nicotine exposure and demonstrate the importance of distinguishing between HLA and LLA rats, a distinction that may be particularly important when studying adolescents. Although many investigators have reported that the adolescent population may be more sensitive to the rewarding and reinforcing effects of drugs [49–51], this observation likely reflects the developmental tendency for adolescents to possess characteristics of HLA animals [10]. Indeed, studies in the human population have demonstrated that sensation-seeking peaks during adolescence and declines thereafter, with those maintaining adolescent-like sensation-seeking most likely to escalate alcohol use [52].

Results indicating a differential effect of adolescent nicotine exposure on FosB in brain from HLA and LLA rats underscore inherent differences between these groups of animals. Results show a clear increase in FosB levels in the vStr and PFC from both groups of rats following 8 days of adolescent nicotine exposure, but this effect persisted into adulthood only in brain from LLA rats. Soderstrom et al. [53] demonstrated that 10 days of nicotine exposure (0.4 mg/kg, i.p.) from PND 34–43 increased FosB immunoreactivity in the NAcc at 37 days following the last nicotine injection, but these authors did not specifically measure FosB or characterize the behavioral phenotype of the animals. Results indicating that prolonged elevations in FosB following adolescent nicotine exposure occur only in LLA adolescents suggest that LLA adolescents are more “adult-like” than their HLA counterparts. Indeed, a prolonged elevation of FosB following drug administration has been demonstrated repeatedly in adult animals [31, 33, 34].

It was expected that HLA animals exposed to nicotine during adolescence would demonstrate both an ethanol-induced CPP in adulthood and a sustained elevation of FosB that presumably sensitized the reward pathways. However, results indicate that persistent elevations in FosB following adolescent nicotine exposure are neither necessary nor sufficient for the establishment of an ethanol CPP in adulthood. Because the biased CPP paradigm used in this study is sensitive to the anxiolytic effects of ethanol [54, 55], the ethanol-induced CPP observed following adolescent nicotine exposure may be mediated by changes in sensitivity to ethanol's anxiolytic effects, rather than the result of a sensitized reward pathway. Adult animals exposed to nicotine during adolescence exhibit increased sensitivity to stress and anxiety in adulthood, as evidenced by elevated corticosterone [28], decreased exploration of the novel open field and decreased time in the open arms of the elevated plus maze [29, 30]. Thus, it seems likely that adult animals exposed to nicotine as adolescents may exhibit an ethanol CPP in a biased paradigm as a consequence of the anxiolytic properties of ethanol. Interestingly, animals exhibiting elevated FosB expression may be less sensitive to stress and anxiety as indicated by increased time spent in the open arms of the elevated plus maze [56], increase swim time in the Porsolt forced swim test [56], increased resilience following social defeat stress [57] and a diminished corticosterone response to restraint stress [58]. Thus, nicotine exposed LLA animals, who exhibit sustained FosB expression as adults, may not find the anxiolytic effects of ethanol rewarding, and as

a consequence, fail to exhibit a CPP in the biased paradigm. Indeed, ethanol-injected LLA animals exhibited a large reduction ($D = 0.80$) in time spent on the ethanol-paired side when compared to saline-injected LLA animals, suggestive of an ethanol-induced conditioned place aversion. Further studies are necessary to confirm differences between HLA and LLA animals in anxious behavior and stress sensitivity following adolescent nicotine exposure.

Although no statistically significant differences were observed between male and female animals, some moderate to large sex-related effects were present. FosB measurements in the PFC were about 25% lower in male adolescents than their female counterparts after 4 saline injections, and about 19% higher in male than female adolescents following 4 nicotine injections, suggesting that adolescent males may exhibit an increase in FosB following fewer exposures to nicotine than adolescent females. Additionally, FosB measurements were 15–17% higher in the vStr and PFC of adult males than observed in adult females regardless of whether these animals were exposed to saline or nicotine as adolescents. The latter finding is consistent with a report demonstrating that adult males exhibit slightly higher levels of FosB in the nucleus accumbens core and shell regions than their female counterparts and that this difference is present in animals injected with either saline or cocaine (15 mg/kg) for 2 weeks indicating that this difference is independent of drug exposure [45]. To our knowledge, no studies of adolescent or adult animals have examined sex differences in FosB expression following nicotine exposure; these findings warrant further investigation.

In sum, adolescent animals demonstrating differences in behavioral reactivity to a novel environment also exhibit differences in: 1) the long term consequences of nicotine exposure on sensitivity to ethanol's effects in adulthood; 2) the induction of FosB during repeated exposure to nicotine; and 3) the persistence of FosB following repeated nicotine exposure. These findings provide a foundation for investigating differences in the inherent vulnerabilities of adolescent animals, characteristics that can be screened using relatively simple behavioral measures.

Acknowledgments

Research was supported by the State of Florida and NIAAA of the National Institutes of Health under award number F32AA016449. The content is solely the responsibility of the authors and does not necessarily represent the official views of the State of Florida or National Institutes of Health.

References

- [1]. Dellu F, Piazza PV, Mayo W, Le Moal M, Simon H. Novelty-seeking in rats--biobehavioral characteristics and possible relationship with the sensation-seeking trait in man. *Neuropsychobiology*. 1996; 34:136–45. [PubMed: 8916071]
- [2]. Deminiere JM, Piazza PV, Le Moal M, Simon H. Experimental approach to individual vulnerability to psychostimulant addiction. *Neurosci Biobehav Rev*. 1989; 13:141–7. [PubMed: 2682400]
- [3]. Klebaur JE, Bardo MT. Individual differences in novelty seeking on the playground maze predict amphetamine conditioned place preference. *Pharmacol Biochem Behav*. 1999; 63:131–6. [PubMed: 10340533]

- [4]. Klebaur JE, Bevins RA, Segar TM, Bardo MT. Individual differences in behavioral responses to novelty and amphetamine self-administration in male and female rats. *Behav Pharmacol.* 2001; 12:267–75. [PubMed: 11548112]
- [5]. Nadal R, Armario A, Janak PH. Positive relationship between activity in a novel environment and operant ethanol self-administration in rats. *Psychopharmacology (Berl).* 2002; 162:333–8. [PubMed: 12122492]
- [6]. Piazza PV, Deminiere JM, Le Moal M, Simon H. Factors that predict individual vulnerability to amphetamine self-administration. *Science.* 1989; 245:1511–3. [PubMed: 2781295]
- [7]. Zheng X, Ke X, Tan B, Luo X, Xu W, Yang X, et al. Susceptibility to morphine place conditioning: relationship with stress-induced locomotion and novelty-seeking behavior in juvenile and adult rats. *Pharmacol Biochem Behav.* 2003; 75:929–35. [PubMed: 12957238]
- [8]. Zheng XG, Tan BP, Luo XJ, Xu W, Yang XY, Sui N. Novelty-seeking behavior and stress-induced locomotion in rats of juvenile period differentially related to morphine place conditioning in their adulthood. *Behav Processes.* 2004; 65:15–23. [PubMed: 14744543]
- [9]. Crawford AM, Pentz MA, Chou CP, Li C, Dwyer JH. Parallel developmental trajectories of sensation seeking and regular substance use in adolescents. *Psychol Addict Behav.* 2003; 17:179–92. [PubMed: 14498812]
- [10]. Philpot RM, Wecker L. Dependence of adolescent novelty-seeking behavior on response phenotype and effects of apparatus scaling. *Behav Neurosci.* 2008; 122:861–75. [PubMed: 18729640]
- [11]. Spear LP. The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev.* 2000; 24:417–63. [PubMed: 10817843]
- [12]. Anthony JC, Petronis KR. Early-onset drug use and risk of later drug problems. *Drug Alcohol Depend.* 1995; 40:9–15. [PubMed: 8746919]
- [13]. Bonomo YA, Bowes G, Coffey C, Carlin JB, Patton GC. Teenage drinking and the onset of alcohol dependence: a cohort study over seven years. *Addiction.* 2004; 99:1520–8. [PubMed: 15585043]
- [14]. Grant BF, Stinson FS, Harford TC. Age at onset of alcohol use and DSM-IV alcohol abuse and dependence: a 12-year follow-up. *J Subst Abuse.* 2001; 13:493–504. [PubMed: 11775078]
- [15]. Kandel DB, Yamaguchi K, Chen K. Stages of progression in drug involvement from adolescence to adulthood: further evidence for the gateway theory. *J Stud Alcohol.* 1992; 53:447–57. [PubMed: 1405637]
- [16]. Lynskey MT, Heath AC, Bucholz KK, Slutske WS, Madden PA, Nelson EC, et al. Escalation of drug use in early-onset cannabis users vs co-twin controls. *Jama.* 2003; 289:427–33. [PubMed: 12533121]
- [17]. Patton GC, McMorris BJ, Toumbourou JW, Hemphill SA, Donath S, Catalano RF. Puberty and the onset of substance use and abuse. *Pediatrics.* 2004; 114:e300–6. [PubMed: 15342890]
- [18]. 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. [PubMed: 1881424]
- [19]. Johnston, LD.; O'Malley, PM.; Bachman, JG.; Schulenberg, JE. National results on adolescent drug use: Overview of key findings, 2008. NIH Publication; Bethesda, MD: 2009.
- [20]. Johnston, LD.; O'Malley, PM.; Bachman, JG.; Schulenberg, JE. Monitoring the Future national results on adolescent drug use: Overview of key findings, 2011. Institute for Social Research, The University of Michigan; Ann Arbor: 2012.
- [21]. Johnson KA, Jennison KM. The drinking-smoking syndrome and social context. *Int J Addict.* 1992; 27:749–92. [PubMed: 1618582]
- [22]. Grant BF. Age at smoking onset and its association with alcohol consumption and DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *J Subst Abuse.* 1998; 10:59–73. [PubMed: 9720007]
- [23]. Adriani W, Spijker S, Deroche-Gamonet V, Laviola G, Le Moal M, Smit AB, et al. Evidence for enhanced neurobehavioral vulnerability to nicotine during periadolescence in rats. *J Neurosci.* 2003; 23:4712–6. [PubMed: 12805310]

- [24]. James-Walke NL, Williams HL, Taylor DA, McMillen BA. Periadolescent nicotine exposure produces sensitization to reinforcement by diazepam in the rat. *Neurotoxicol Teratol.* 2007; 29:31–6. [PubMed: 17097852]
- [25]. McMillen BA, Davis BJ, Williams HL, Soderstrom K. Periadolescent nicotine exposure causes heterologous sensitization to cocaine reinforcement. *Eur J Pharmacol.* 2005; 509:161–4. [PubMed: 15733551]
- [26]. McQuown SC, Belluzzi JD, Leslie FM. Low dose nicotine treatment during early adolescence increases subsequent cocaine reward. *Neurotoxicol Teratol.* 2007; 29:66–73. [PubMed: 17174067]
- [27]. Riley HH, Zalud AW, Diaz-Granados JL. The influence of a chronic adolescent nicotine exposure on ethanol withdrawal severity during adulthood in C3H mice. *Alcohol.* 2010; 44:81–7. [PubMed: 20113876]
- [28]. Klein LC. Effects of adolescent nicotine exposure on opioid consumption and neuroendocrine responses in adult male and female rats. *Exp Clin Psychopharmacol.* 2001; 9:251–61. [PubMed: 11534535]
- [29]. Slawecki CJ, Gilder A, Roth J, Ehlers CL. Increased anxiety-like behavior in adult rats exposed to nicotine as adolescents. *Pharmacol Biochem Behav.* 2003; 75:355–61. [PubMed: 12873627]
- [30]. Slawecki CJ, Thorsell AK, El Khoury A, Mathe AA, Ehlers CL. Increased CRF-like and NPY-like immunoreactivity in adult rats exposed to nicotine during adolescence: relation to anxiety-like and depressive-like behavior. *Neuropeptides.* 2005; 39:369–77. [PubMed: 16038974]
- [31]. Nestler EJ, Kelz MB, Chen J. DeltaFosB: a molecular mediator of long-term neural and behavioral plasticity. *Brain Res.* 1999; 835:10–7. [PubMed: 10448191]
- [32]. Nestler EJ. Molecular neurobiology of addiction. *Am J Addict.* 2001; 10:201–17. [PubMed: 11579619]
- [33]. Nestler EJ. Molecular basis of long-term plasticity underlying addiction. *Nat Rev Neurosci.* 2001; 2:119–28. [PubMed: 11252991]
- [34]. Hope BT, Nye HE, Kelz MB, Self DW, Iadarola MJ, Nakabeppu Y, et al. Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron.* 1994; 13:1235–44. [PubMed: 7946359]
- [35]. Colby CR, Whisler K, Steffen C, Nestler EJ, Self DW. Striatal cell type-specific overexpression of DeltaFosB enhances incentive for cocaine. *J Neurosci.* 2003; 23:2488–93. [PubMed: 12657709]
- [36]. Ehrlich ME, Sommer J, Canas E, Unterwald EM. Periadolescent mice show enhanced DeltaFosB upregulation in response to cocaine and amphetamine. *J Neurosci.* 2002; 22:9155–9. [PubMed: 12417638]
- [37]. Pascual MM, Pastor V, Bernabeu RO. Nicotine-conditioned place preference induced CREB phosphorylation and Fos expression in the adult rat brain. *Psychopharmacology (Berl).* 2009; 207:57–71. [PubMed: 19711055]
- [38]. Philpot RM, Engberg ME, Wecker L. Effects of nicotine exposure on locomotor activity and pCREB levels in the ventral striatum of adolescent rats. *Behav Brain Res.* 2012; 230:62–8. [PubMed: 22301350]
- [39]. Raiff BR, Dallery J. Effects of acute and chronic nicotine on responses maintained by primary and conditioned reinforcers in rats. *Exp Clin Psychopharmacol.* 2006; 14:296–305. [PubMed: 16893272]
- [40]. Raiff BR, Dallery J. The generality of nicotine as a reinforcer enhancer in rats: effects on responding maintained by primary and conditioned reinforcers and resistance to extinction. *Psychopharmacology (Berl).* 2008; 201:305–14. [PubMed: 18695928]
- [41]. Popke EJ, Mayorga AJ, Fogle CM, Paule MG. Effects of acute nicotine on several operant behaviors in rats. *Pharmacol Biochem Behav.* 2000; 65:247–54. [PubMed: 10672976]
- [42]. Philpot RM, Badanich KA, Kirstein CL. Place conditioning: age-related changes in the rewarding and aversive effects of alcohol. *Alcohol Clin Exp Res.* 2003; 27:593–9. [PubMed: 12711921]
- [43]. Philpot R, Kirstein C. Developmental differences in the accumbal dopaminergic response to repeated ethanol exposure. *Ann N Y Acad Sci.* 2004; 1021:422–6. [PubMed: 15251921]

- [44]. Philpot RM, Wecker L, Kirstein CL. Repeated ethanol exposure during adolescence alters the developmental trajectory of dopaminergic output from the nucleus accumbens septi. *Int J Dev Neurosci*. 2009; 27:805–15. [PubMed: 19712739]
- [45]. Sato SM, Wissman AM, McCollum AF, Woolley CS. Quantitative mapping of cocaine-induced DeltaFosB expression in the striatum of male and female rats. *PLoS One*. 2011; 6:e21783. [PubMed: 21747956]
- [46]. Asin KE, Wirtshafter D, Tabakoff B. Failure to establish a conditioned place preference with ethanol in rats. *Pharmacol Biochem Behav*. 1985; 22:169–73. [PubMed: 3983210]
- [47]. Huston JP, Silva MA, Topic B, Muller CP. What's conditioned in conditioned place preference? *Trends Pharmacol Sci*. 2013; 34:162–6. [PubMed: 23384389]
- [48]. Smith AM, Kelly RB, Chen WJ. Chronic continuous nicotine exposure during periadolescence does not increase ethanol intake during adulthood in rats. *Alcohol Clin Exp Res*. 2002; 26:976–9. [PubMed: 12170106]
- [49]. Adriani W, Laviola G. Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. *Behav Pharmacol*. 2004; 15:341–52. [PubMed: 15343057]
- [50]. Chambers RA, Taylor JR, Potenza MN. Developmental neurocircuitry of motivation in adolescence: a critical period of addiction vulnerability. *Am J Psychiatry*. 2003; 160:1041–52. [PubMed: 12777258]
- [51]. Crews F, He J, Hodge C. Adolescent cortical development: a critical period of vulnerability for addiction. *Pharmacol Biochem Behav*. 2007; 86:189–99. [PubMed: 17222895]
- [52]. Quinn PD, Harden KP. Differential changes in impulsivity and sensation seeking and the escalation of substance use from adolescence to early adulthood. *Dev Psychopathol*. 2012:1–17. [PubMed: 22292989]
- [53]. Soderstrom K, Qin W, Williams H, Taylor DA, McMillen BA. Nicotine increases FosB expression within a subset of reward- and memory-related brain regions during both peri- and post-adolescence. *Psychopharmacology (Berl)*. 2007; 191:891–7. [PubMed: 17333132]
- [54]. Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog Neurobiol*. 1998; 56:613–72. [PubMed: 9871940]
- [55]. Tzschentke TM. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addict Biol*. 2007; 12:227–462. [PubMed: 17678505]
- [56]. Ohnishi YN, Ohnishi YH, Hokama M, Nomaru H, Yamazaki K, Tominaga Y, et al. FosB is essential for the enhancement of stress tolerance and antagonizes locomotor sensitization by DeltaFosB. *Biol Psychiatry*. 2011; 70:487–95. [PubMed: 21679928]
- [57]. Vialou V, Robison AJ, Laplant QC, Covington HE 3rd, Dietz DM, Ohnishi YN, et al. DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nat Neurosci*. 2010; 13:745–52. [PubMed: 20473292]
- [58]. Christiansen AM, Dekloet AD, Ulrich-Lai YM, Herman JP. “Snacking” causes long term attenuation of HPA axis stress responses and enhancement of brain FosB/deltaFosB expression in rats. *Physiol Behav*. 2011; 103:111–6. [PubMed: 21262247]

Highlights

- Adolescent nicotine exposure results in an alcohol CPP in high sensation seeking adults
- Adolescent nicotine exposure increases FosB expression
- FosB expression following adolescent nicotine persists into adulthood in low sensation seekers

Fig. 1.

Classification of behavioral reactivity of adolescent rats to a novel environment. The locomotor activity of adolescent animals ($N = 89$) was determined by measuring the total distance moved (TDM) in a novel open field for 5 min. Animals were classified as exhibiting high (HLA) or low (LLA) locomotor activity by applying a median split. The single animal exhibiting activity at the median value (5936 cm/5 min) was removed from further study.

Fig. 2.

Effects of adolescent nicotine exposure on ethanol-induced conditioned place preference (CPP) in adults. Rats were classified as exhibiting HLA or LLA on PND 31 as described, and received injections of either saline (0.9%) or nicotine (0.56 mg free base/kg) once daily for 8 days from PND 35–42. From PND 61–68, rats were trained using 1.0 g/kg ethanol (17%, i.p.) or saline in a biased CPP paradigm, and CPP determined on PND 69. Preference scores were calculated by subtracting the time spent in the initially preferred chamber (paired with saline injection) from the time spent in the initially non-preferred chamber (paired with either saline or ethanol). Bars represent the mean + sem of determinations from 5 rats/group, and significant ($p < 0.05$) differences between groups are noted with an asterisk.

Fig. 3.

Effects of adolescent nicotine exposure on levels of FosB in the ventral striatum and prefrontal cortex. Rats were classified as exhibiting HLA or LLA on PND 31 as described, received injections of either saline (0.9%) or nicotine (0.56 mg free base/kg) once daily for 4 or 8 days beginning on PND 35, and were decapitated 24 hrs after their final injection on PND 39 or PND 43, respectively. Levels of FosB were determined in ventral striatum and prefrontal cortex, were normalized to β -tubulin, and are expressed relative to saline control values from the same blot. Data were quantified using a densitometer and Un-Scan-It gel digitizing software (Silk Scientific Inc., Orem, Utah). The optical densities of FosB for saline controls did not differ significantly in brain regions from HLA and LLA rats either 4 or 8 days after saline administration and were: 1.34 ± 0.52 and 0.98 ± 0.37 (4 days) and 1.45 ± 0.70 and 1.09 ± 0.43 (8 days) for ventral striatum from HLA and LLA rats, respectively; and 1.87 ± 0.49 and 1.28 ± 0.36 (4 days) and 1.94 ± 0.43 and 1.46 ± 0.27 (8 days) for prefrontal cortex from HLA and LLA rats, respectively. Bars represent the mean + sem of determinations from 4 rats/group, and corresponding representative immunoblots are shown below. *Significantly different from corresponding saline controls, **Significantly different between corresponding HLA and LLA groups, $p < 0.05$

Fig. 4.

Effects of adolescent nicotine exposure on levels of FosB in the ventral striatum and prefrontal cortex of adults. Rats were classified as exhibiting HLA or LLA on PND 31, received 8 injections of either saline (0.9%) or nicotine (0.56 mg free base/kg) once daily beginning on PND 35, and were decapitated on PND 68, corresponding to the day CPP was assessed in a separate group of animals. Levels of FosB were determined and analyzed as per Fig. 3. Optical densities for saline controls did not differ significantly in brain regions from HLA and LLA rats and were: 1.20 ± 0.46 and 1.62 ± 0.55 for ventral striatum and 1.83 ± 0.12 and 2.20 ± 0.24 for prefrontal cortex, respectively. Bars represent the mean + sem of determinations from 4 rats/group, and corresponding representative immunoblots are shown below. *Significantly different from corresponding saline controls, $p < 0.05$.

Table 1

Novel open field activities exhibited by adolescent rats

Experimental Measure	Adolescent Locomotor Activity (cm/5 min)			
	LLA		HLA	
	Saline injected	Nicotine injected	Saline injected	Nicotine injected
CPP				
Saline conditioned	5760 ± 154 (n=5)	5711 ± 189 (n=5)	6766 ± 292 (n=5)	6760 ± 275 (n=5)
Ethanol conditioned	5788 ± 233 (n=5)	5860 ± 167 (n=5)	6883 ± 139 (n=5)	6826 ± 251 (n=5)
Adolescent Fos B				
4 days	5428 ± 106 (n=4)	5465 ± 82 (n=4)	6130 ± 179 (n=4)	6403 ± 341 (n=4)
8 days	5423 ± 176 (n=4)	5341 ± 230 (n=4)	6346 ± 251 (n=4)	6273 ± 250 (n=4)
Adult Fos B	5243 ± 354 (n=4)	5297 ± 356 (n=4)	6456 ± 156 (n=4)	6687 ± 230 (n=4)

Novel open field activity of animals used to assess the effect of adolescent nicotine exposure on ethanol conditioned place preference or FosB expression in adolescence or adulthood. Following behavioral characterization on PND 31, animals were systematically assigned to experimental groups to ensure that baseline activity was equivalent for groups receiving injections of saline or nicotine. Values represent group means ± sem.