Published in final edited form as:

Psychopharmacology (Berl). 2016 February ; 233(3): 361–371. doi:10.1007/s00213-015-4106-8.

Adolescent binge-like alcohol alters sensitivity to acute alcohol effects on dopamine release in the nucleus accumbens of adult rats

Tatiana A. Shnitko¹, Linda P. Spear³, and Donita L. Robinson^{1,2,*}

¹Bowles Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC, USA

²Department of Psychiatry, University of North Carolina, Chapel Hill, NC, USA

³Center for Development and Behavioral Neuroscience, Department of Psychology, Binghamton University, Binghamton, NY 13902, USA

Abstract

Rationale—Early onset of alcohol drinking has been associated with alcohol abuse in adulthood. The neurobiology of this phenomenon is unclear, but mesolimbic dopamine pathways, which are dynamic during adolescence, may play a role.

Objectives—We investigated the impact of adolescent binge-like alcohol on phasic dopaminergic neurotransmission during adulthood.

Methods—Rats received intermittent intragastric ethanol, water or nothing during adolescence. In adulthood, electrically-evoked dopamine release and subsequent uptake were measured in the nucleus accumbens core at baseline and after acute challenge of ethanol or saline.

Results—Adolescent ethanol exposure did not alter basal measures of evoked dopamine release or uptake. Ethanol challenge dose-dependently decreased the amplitude of evoked dopamine release in rats by 30–50% in control groups, as previously reported, but did not alter evoked release in ethanol-exposed animals. To address the mechanism by which ethanol altered dopamine signaling, the evoked signals were modeled to estimate dopamine efflux per impulse and the velocity of the dopamine transporter. Dopamine uptake was slower in all exposure groups after ethanol challenge compared to saline, while dopamine efflux per pulse of electrical stimulation was reduced by ethanol only in ethanol-naïve rats.

Conclusions—The results demonstrate that exposure to binge levels of ethanol during adolescence blunts the effect of ethanol challenge to reduce the amplitude of phasic dopamine release in adulthood. Large dopamine transients may result in more extracellular dopamine after alcohol challenge in adolescent-exposed rats, and may be one mechanism by which alcohol is more reinforcing in people who initiated drinking at an early age.

Corresponding author: Donita L. Robinson, PhD, Bowles Center for Alcohol Studies, CB #7178, University of North Carolina, Chapel Hill, NC 27599-7178; DLR@unc.edu; Phone: 919-966-9178; Fax: 919-966-5679.

Authors have no conflict of interests to declare.

Keywords

adolescent binge alcohol; dopamine release and uptake; accumbens; fast-scan cyclic voltammetry

Introduction

One hallmark of adolescence is sexual and cognitive maturation, which has been associated with risk-taking behavior in teens and exploration of novel experiences, including alcohol drinking (Spear 2014). Compared to adults, adolescents are less responsive to many effects of alcohol intoxication (motor impairment, sedation, analgesia) and withdrawal (hangover, anxiety), but are more sensitive to positive effects of alcohol like euphoria and facilitation of social interaction (Wood et al. 1992, Varlinskaya et al. 2001, Spear and Varlinskaya 2005). This combination of sensitivities to alcohol enables alcohol drinking by adolescents at well above binge levels (Patrick et al. 2013), defined by National Institute on Alcohol Abuse and Alcoholism as intake of 4–5 drinks per session producing blood alcohol concentrations of at least 0.08 g/dL (NIAAA 2004).

The vulnerability of adolescents to excessive alcohol use is thought to be promoted by an immature balance between cortical and limbic brain systems, including cortical control over dopaminergic pathways (Crews et al. 2007, Ernst and Fudge 2009, Chartier et al. 2010). Many aspects of dopamine transmission are dynamic during adolescence; for example, the density of dopamine receptors typically increases (Andersen et al. 2000, McCutcheon and Marinelli 2009, Jucaite et al. 2010) and spontaneous firing rates of dopamine neurons are higher (McCutcheon et al. 2012). This up-regulated dopaminergic activity in the brain may contribute to alcohol abuse in adolescents, impacting neuronal development and leading to alcohol use disorder in some individuals. Indeed, epidemiological data show that the earlier one starts drinking, the more likely one is to develop alcohol use disorder, although this association is not necessarily causal (Grant and Dawson 1998).

Studies using microdialysis revealed that acute ethanol increases tonic concentrations of dopamine in the brain of the adult rat (Di Chiara and Imperato 1985, Schier et al. 2013). In addition, acute ethanol increases the number of dopamine transients (Robinson et al. 2009), which are brief, and high concentrations of dopamine resulting from burst firing of dopamine neurons (Sombers et al. 2009) and measured with fast-scan cyclic voltammetry. While spontaneous dopamine release is enhanced, ethanol decreases the concentration of dopamine release evoked by electrical stimulation of dopamine neurons (Budygin et al. 2001, Robinson et al. 2005, Jones et al. 2006, Shnitko et al. 2014). In other words, while dopamine transients are more frequent (Robinson et al. 2009), they would be smaller. This reduction in the amount of dopamine efflux per impulse is likely a negative feedback mechanism to compensate for the high extracellular dopamine levels, potentially via activation of D2-receptor autoinhibition. Moreover, some studies found that ethanol challenge concurrently slows dopamine clearance (Robinson et al. 2005, Shnitko et al. 2014), which would permit dopamine to accumulate in the extracellular space. Thus, studies of acute ethanol effects on dopaminergic neurotransmission revealed complexity of its action on dopamine release and uptake.

Effects of chronic ethanol exposure on dopamine transmission are also under intensive investigation. Human imaging studies demonstrated that expression of D2 receptors is down-regulated in the striatum of abstinent alcoholics (Volkow et al. 1996). *In vitro* studies found that chronic intermittent ethanol exposure in adult mice reduces dopamine release, increases uptake and enhances D2-autoreceptor activity in the NAc (Karkhanis et al. 2015). While less is known about the consequences of adolescent ethanol exposure on dopamine systems, Badanich and colleagues reported that tonic dopamine levels were higher in the nucleus accumbens (NAc) of rats exposed to ethanol during adolescence compared to controls (Badanich et al. 2007).

These data led us to hypothesize that chronic ethanol exposure during adolescence disrupts the mechanisms of dopamine release and uptake. This study evaluated how binge-like ethanol exposure in adolescent rats affects electrically-evoked dopamine release and uptake in the NAc in adulthood, both at baseline and after ethanol challenge. We predicted that acute ethanol challenge would decrease electrically-evoked dopamine release and inhibit the rate of dopamine uptake in the NAc of ethanol-naïve rats, as previously demonstrated (Budygin et al. 2001, Robinson et al. 2005, Jones et al. 2006, Shnitko et al. 2014). However, in rats exposed to binge-like ethanol during adolescence, we predicted that the same challenge would not affect dopamine release, and would either not alter or enhance uptake via the dopamine transporter.

Methods

Animals

Male Sprague-Dawley rats (N=39) were bred and reared at the University of North Carolina at Chapel Hill. Litters were culled to 10 pups with no more than 6 males per litter. They were weaned on postnatal day (P) 21 and pair-housed in a temperature- and humidity-controlled room on a 12-h light-dark schedule with food and water available *ad libitum*. All procedures complied with the Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of North Carolina.

Adolescent intermittent ethanol exposure

Adolescent intermittent ethanol (AIE) exposure occurred from P25–P45; this period was previously defined as early to middle adolescence in rats (Spear 2015). On P25, pairs of rats (sibling cage-mates) were assigned to an experimental group: non-manipulated control (NM), water-exposed control (WAT) and ethanol-exposed (AIE). Rats were assigned in a balanced order, such that only one pair of rats per litter was assigned to a particular group. AIE rats received 4g/kg ethanol (25% v/v solution in water) intragastrically (i.g.) every other day, as previously described (McClory and Spear 2014). WAT rats were given i.g. water at volumes equivalent to the ethanol doses. In total, 11 doses were administered from P25 to P45, 48 hours apart, to rats in WAT and AIE groups. NM rats were weighed on P25 and P45, but were otherwise unhandled by researchers. After the final administration on P45, rats were allowed to reach adulthood (P75). The periods of adolescent exposure and voltammetric measurement are indicated in the timeline of the experiment in Figure 1A. As

demonstrated in Figure 1B, body weights were similar across the three groups during the exposure period.

Fast scan cyclic voltammetry

Dopamine measurements were conducted in the NAc core of adult rats (P75 to 85) by using fast-scan cyclic voltammetry and carbon-fiber microelectrodes ($87 \pm 16 \mu\text{m}$ length, $6\text{--}7 \mu\text{m}$ diameter). On the day of the experiment, rats were anesthetized with urethane (50% w/w in saline, 1.5g/kg, i.p.) and placed in a stereotaxic frame on a heated pad for the surgery and experiment. The skull was exposed and holes were drilled above the NAc core (AP +1.6mm, ML –1.8mm) and the VTA (AP –5.2mm, ML –1.0mm). The stimulating electrode (bipolar, parallel, stainless-steel, 0.2mm diameter/tip; Plastics One, Roanoke, VA) was initially lowered to the VTA at DV –8.0mm from bregma while the carbon-fiber electrode was initially placed in the NAc at DV –6.0mm from the skull surface. An Ag/AgCl reference electrode was positioned in the cortex contralateral to the carbon-fiber electrode and secured with a stainless-steel screw and dental cement.

Next, a triangle-waveform potential ramping from –0.4V to +1.3V and back to –0.4V at 400V/s (vs Ag/AgCl reference electrode) was applied to the carbon-fiber electrode at 10Hz. The carbon-fiber and stimulating electrodes were lowered at 0.2mm increments into the NAc and the VTA to optimize the VTA-evoked dopamine release signal to >30-fold higher than the root mean square of the background. Dopamine release in the NAc was evoked by electrical stimulation (24 biphasic, square-wave pulses, 2ms/phase, 125–185 μA , 60Hz) delivered to the VTA every 5 min. Carbon-fiber electrodes were calibrated post-experimentally using 1 μM dopamine solution in buffer as previously described (Robinson et al. 2009).

Experimental design

In order to investigate the effect of AIE on changes in dopamine release and uptake evoked in the NAc by ethanol challenge, rats were given ethanol or saline during the experiment. One rat per cage was randomly assigned to the ethanol-challenge or saline-challenge experimental group, and its cage-mate sibling was assigned to the alternate group. In each rat, three baseline (BL) evoked dopamine signals were recorded, followed by saline (SAL) injection (intraperitoneal, i.p) and three subsequent evoked dopamine recordings. The saline injections were given to provide within-subject analysis and control for the effect of an injection. Next, rats in the saline-challenge groups received three saline injections while rats in the ethanol-challenge groups received three ethanol injections. The injections were given 15 min apart in cumulative dosing: 1g/kg, 1g/kg (cumulative 2g/kg), 2g/kg (cumulative 4g/kg) ethanol or equivalent volumes of saline, similar to previous studies (Robinson et al. 2009, Schier et al. 2013, Shnitko et al. 2014). The period of 15 min was chosen based on the evidence that ethanol at various doses reaches near-maximal effects on evoked DA release in 10 min after injection (Budygin et al. 2001, Robinson et al. 2005). Moreover, both microdialysis and proton magnetic resonance spectroscopic analysis yielded peak ethanol concentrations in the rat brain within 10–15 min after ~1g/kg dose (Crippens et al. 1999, Adalsteinsson et al. 2006). VTA-evoked dopamine signals continued every 5 min. Thus, the

design was a group (NM, WAT, AIE) $\times$ challenge (ethanol, saline) $\times$ dose (BL, SAL, 1.0, 2.0, 4.0) factorial.

Blood ethanol concentration (BEC) assessment

In order to evaluate ethanol dosing, tail blood samples were collected for subsequent analysis using AM1 Analox Alcohol Analyzer (Analox Instruments, MA). During the period of adolescent exposure, blood samples were collected on P35 and P45 in rats from AIE groups 30 min after i.g. injections. Blood samples were also collected in WAT rats to control for any effects of sampling. During voltammetric experiments, blood samples were collected immediately after the last dopamine signal (i.e., 15 min after the final dose). Plasma was separated using centrifugation and stored at -80°C until analyzed and compared to an ethanol standard of 100mg/dl.

Data Analysis

Electrochemical signals detected upon electrical stimulation were analyzed by using color plots (TarHeel CV 6.0, Department of Chemistry, UNC Chapel Hill). In the color plots, changes in current are plotted as a function of applied potential over time. An increase in current at approximately 0.65V vs Ag/AgCl reference electrode was considered to be due to oxidation of dopamine after confirmation by analysis of cyclic voltammograms averaged over 500 ms around the peak of oxidation current. The oxidation current was converted to dopamine concentration, or [DA], by using calibration factors obtained post-experimentally and plotted as a function of time. All analyses of release and uptake were conducted on these concentration-versus-time traces. In these traces, the maximal [DA] was used as a parameter of dopamine release ([DA]_{max}). The traces were also fit to a model describing evoked dopamine signals as a balance of efflux and uptake with the Michaelis-Menten kinetic equation as described previously (Wu et al. 2001). We used the equation

$$d[\text{DA}]/dt = [\text{DA}]_p * f - V_{\text{max}} / (K_m / [\text{DA}] + 1),$$

where [DA]_p is dopamine concentration released per pulse of electrical stimulation, *f* is frequency of electrical stimulation and *V*_{max} and *K*_m are the Michaelis-Menten uptake rate constants describing velocity and affinity of dopamine transporter, respectively. The time course of DA release and decay was fit to the model with the *K*_m held at 200 nM and [DA]_p and *V*_{max} adjusted to achieve a correlation coefficient of *r* > 0.8 between the model and the experimental data.

Initially, evoked [DA]_{max}, [DA]_p and *V*_{max} at baseline were analyzed among groups with a one-way ANOVA. The acute effects of ethanol challenge on evoked [DA]_{max}, [DA]_p and *V*_{max} were analyzed with three-way repeated-measures (RM) ANOVA with group (NM, WAT and AIE) and challenge (saline and ethanol) as the between-subject factors and dose (SAL, 1, 2 and 4g/kg ethanol) as the within-subject factor. As data were non-normally distributed (all *p* < 0.05, Shapiro-Wilk test), we transformed them using a log₁₀ transformation so they fit the assumptions of the parametric test. The Tukey test was used for post-hoc, pairwise comparisons. BECs collected during adolescent exposure and after voltammetric measurements were analyzed with a paired t-test and one-way ANOVA,

respectively. All statistical analyses were performed using STATISTICA 12.0 (StatSoft, Inc., USA). Results with $p < 0.05$ were considered significant.

Results

The concentration of ethanol in the blood of rats 30 min after administration during AIE exposure is demonstrated in Figure 1C. BEC in AIE rats were 118 ± 12 mg/dl and 140 ± 17 mg/dl on P35 and P45, respectively, indicating that binge-like ethanol levels (> 80 mg/dl) were reached in this study. No significant difference was found in the BEC between P35 and P45 (paired t-test, $t = -1.00$, $p > 0.05$). BEC was also measured after the voltammetric recordings, shown in Figure 1D. No BEC differences among the NM, WAT and AIE groups were found after ethanol challenge during FSCV measurements (one-way ANOVA, $F_{2,10} = 0.7$, $p > 0.05$).

As previous studies reported persistent alterations in dopamine neurotransmission after chronic ethanol exposure (Badanich et al. 2007, Budygin et al. 2007, Trantham-Davidson et al. 2014), we evaluated whether AIE exposure affected basal evoked dopamine release and clearance in the NAc core of rats. A representative VTA-evoked dopamine signal measured with FSCV is shown as a color plot in Figure 2A, where the increase in current due to oxidation of dopamine occurs at ~ 0.65 V vs the Ag/AgCl reference electrode immediately upon electrical stimulation. Figure 2B displays the concentration-versus-time trace for the same electrically-evoked signal, and the amplitude of the signal is indicated by $[DA]_{\max}$. The corresponding background-subtracted cyclic voltammogram (inset) confirms the catecholaminergic nature of the evoked signal. Composite data are shown in Figure 2C, and there were no significant differences in baseline $[DA]_{\max}$ among the groups (one-way ANOVA, $F_{2,36} = 0.87$, $p > 0.05$). Next, these data were modeled using Michaelis-Menten uptake kinetics, and neither $[DA]_p$ nor $V_{\max}$ differed across exposure groups (Table 1; $[DA]_p$: $F_{2,36} = 0.96$, $p > 0.05$; $V_{\max}$: $F_{2,36} = 1.96$, $p > 0.05$).

Ethanol at a variety of doses increases ambient concentrations of dopamine in the striatum by increasing the firing rate of dopamine neurons (e.g. Di Chiara and Imperato 1985, Gessa et al. 1985). When dopamine release is then electrically evoked, ethanol reliably reduces the amount of dopamine released per electrical impulse (e.g. Budygin et al. 2001), presumably as readily releasable pools are diminished and negative feedback via autoreceptors is engaged. To test our hypothesis that AIE reduces the sensitivity of dopamine neurotransmission to acute ethanol challenge, rats from each exposure group received sequential ethanol or saline challenges. Cumulative ethanol challenge reduced the VTA-evoked dopamine signal in control groups, but not in the AIE group, as shown in Figure 3. A three-way RM ANOVA yielded a significant interaction among challenge, dose and group ($F_{6,99} = 2.6$, $p < 0.05$), indicating that changes in $[DA]_{\max}$ varied by time and challenge differently across the groups. To identify what dose of ethanol affected $[DA]_{\max}$ in each group, we performed post-hoc comparisons by using the Tukey test. In the NM group (Figure 3A), 4 g/kg ethanol significantly decreased $[DA]_{\max}$ by 35% versus SAL ($p < 0.01$). In the WAT group (Figure 3B), $[DA]_{\max}$ was significantly and dose-dependently decreased at both 2 and 4 g/kg ethanol by 38 and 55% (2 g/kg vs SAL and 4 g/kg, $p < 0.05$; 4 g/kg vs all other doses of WAT ethanol challenge as well as the WAT saline challenge, all p 's < 0.05). In

contrast, in the AIE group (Figure 3C) no significant differences were found between ethanol and saline challenges or across ethanol doses. Thus, these results demonstrate that while acute ethanol challenge inhibited evoked dopamine release in ethanol-naïve rats, consistent with previous studies, it had no such effect on evoked dopamine release in AIE animals.

Many factors could contribute to the observed effect of chronic and acute ethanol on VTA-evoked dopamine signals in this study. Dopamine release is dependent on the density of dopaminergic terminals in the recording site activated by the electrical stimulation, the regulatory function of D2 autoreceptors and the rate of impulse flow. Moreover, any changes in the rate of dopamine uptake directly influences evoked dopamine signals. Therefore, we further investigated the mechanism by which acute ethanol challenge altered evoked dopamine signals by fitting them to the Michaelis-Menten model described above. Figure 4 displays evoked dopamine signals (green lines) recorded in the NAc of individual rats from WAT and AIE groups, modeled to estimate $[DA]_p$ and V_{max} of the dopamine transporter (K_m was held constant at 200nM). The model traces are overlaid on the raw data (blue dashed lines), indicating a good fit between the model and the data. The traces recorded in the WAT rat (left) illustrate the dose-dependent reduction of the amplitude of evoked dopamine signals. However, ethanol did not alter the amplitude of the evoked dopamine signals recorded in the AIE-treated animal (right). Kinetic analysis revealed that ethanol challenge decreased $[DA]_p$ in the NAc of ethanol-naïve but not AIE rats (Table 2). Three-way RM ANOVA revealed a significant group $\times$ challenge $\times$ dose interaction ($F_{6,99}=2.2$, $p<0.05$), indicating that changes in $[DA]_p$ varied by time and challenge differently across the groups. To identify what dose of ethanol affected $[DA]_p$ in each group, we performed Tukey post-hoc comparisons. In the NM group, ethanol at 4g/kg decreased $[DA]_p$ by 37% ($p<0.05$ vs SAL). In the WAT group, both 2 and 4g/kg ethanol attenuated $[DA]_p$ by 37 and 52% (2g/kg vs SAL, $p<0.05$; 4g/kg vs SAL and 1g/kg as well as the WAT saline challenge at all doses, all p 's <0.05). In contrast, there were no significant effects of ethanol challenge found on $[DA]_p$ in the AIE group of rats.

We also evaluated the effect of acute ethanol challenge on the velocity of the dopamine transporter, V_{max} , in the same model (Table 3). Three-way RM ANOVA analysis yielded a significant dose $\times$ challenge interaction ($F_{3,99}=3.22$, $p<0.05$) and a significant main effect of dose ($F_{3,99}=19.67$, $p<0.001$); all other interactions and main effects did not reach significance. Thus, we collapsed the data across adolescent exposure groups before calculating post-hoc comparisons. In rats receiving saline challenge, V_{max} was slower after the final dose of saline ($p<0.05$ vs SAL), indicating an effect of time. However, ethanol challenge produced a dose-dependent effect beyond that observed in the saline-challenge rats, as both the 2 and 4g/kg doses significantly reduced V_{max} (2g/kg vs SAL, $p<0.05$; 4g/kg vs SAL and 1g/kg, $p<0.05$).

Discussion

Binge-like drinking is characterized by intermittent, high ethanol concentrations in the blood over a short period of time and is a prevalent pattern of drinking among adolescents (White et al. 2006, Patrick et al. 2013). Early onset of ethanol drinking is a risk factor for alcohol

use disorder, and while many people who drink as adolescents “age out” of binge drinking, some adolescent users will experience problematic drinking in adulthood. Dopamine neural systems are still developing during adolescence, and ethanol exposure to these systems might have consequences that ultimately increase vulnerability to alcohol use disorder in adulthood. Thus, this study aimed to investigate the effects of binge-like ethanol exposure during adolescence on the dynamics of dopamine release and uptake in the NAc of rats in adulthood. Dopamine release was elicited by electrical stimulation consisting of trains of 24 pulses delivered to the VTA to model burst-firing of dopamine neurons (Somers et al. 2009), and the resulting dopamine release events were analyzed to evaluate ethanol effects on phasic dopamine.

Initially, we evaluated baseline dopamine release elicited in the NAc by electrical stimulation of the VTA, and we found no significant difference between control rats and those exposed to intermittent ethanol during adolescence. A previous microdialysis study reported that adolescent ethanol exposure enhances basal dopamine concentrations in the NAc of adult rats, and this change was not due to alterations in dopamine reuptake (Badanich et al. 2007). As extracellular dopamine results from a balance between release and uptake, these findings suggest that adolescent ethanol exposure enhances an aspect of release, such as the number of active dopamine neurons, their firing frequency, or the amount of dopamine release per impulse. Our finding that electrically-evoked dopamine signals were not altered at baseline demonstrates that the kinetics of phasic dopamine transmission were maintained. Interestingly, other studies found that immediately after multiple days of vapor ethanol exposure in adulthood, dopamine uptake was faster in the NAc of rodents (Budygin et al. 2007, Karkhanis et al. 2015). However, the present and previous data (Badanich et al. 2007) indicated that if adolescent ethanol exposure has a similar effect on uptake, it apparently does not persist into adulthood. Future studies could more thoroughly compare adolescent versus adult ethanol exposures on baseline tonic and phasic neurotransmission of dopamine in dorsal and ventral striatum in order to reveal any age-specific elements of ethanol consequences on basal dopamine function.

Next, we evaluated the effect of acute ethanol challenge on electrically evoked dopamine release in ethanol-naïve and AIE rats. In ethanol-naïve control rats, cumulative ethanol dosing dose-dependently decreased the amplitude of the evoked dopamine signals. This result corresponds to the previous evidence obtained *in vivo* in the striatum of both urethane-anesthetized and awake rats (Budygin et al., 2001, Robinson et al., 2005, Jones et al., 2006, Shnitko et al., 2014). In contrast, and supporting our hypothesis, cumulative ethanol dosing had no effect on electrically evoked dopamine efflux in the NAc of AIE rats. As previous studies have shown, ethanol challenge increases spontaneous firing rate of dopamine neurons and increases *tonic* dopamine concentration in the brain. In the present study, the firing of dopamine neurons was elicited by electrical stimulation that was kept consistent during the experiments; thus, the reduction of evoked dopamine signals might occur due to ethanol effects on $[DA]_p$ or activity of dopamine transporter rather than the firing pattern producing each dopamine release event.

To evaluate the mechanism by which ethanol altered VTA-evoked dopamine release, we modeled the data to extract estimates of efflux and uptake parameters. The analysis revealed

that ethanol decreased $[DA]_p$ in the NAc of control animals but had no effect in rats exposed to ethanol during adolescence. We suggest that in ethanol-naïve rats the reduction in $[DA]_p$ partially compensates for the well known increase in tonic dopamine concentration induced by alcohol challenge, by simultaneously decreasing the amount of dopamine release per impulse. Our study demonstrates that adolescent binge-like ethanol disrupts this compensatory mechanism, as acute ethanol challenge had no significant effect on $[DA]_p$. Thus, it would effectively increase the size of individual dopamine transients in the NAc core of rats in response to ethanol challenge later in adulthood, potentially allowing higher tonic dopamine concentrations. As the mesolimbic dopamine system is thought to mediate the positive reinforcing actions of ethanol especially during the early stages of alcohol use disorder (Alaux-Cantin et al. 2013), larger phasic dopamine transients might impact subsequent drinking and response to alcohol-associated cues. Consistent with this hypothesis, a recent study demonstrated that rats exposed to ethanol during adolescence are more sensitive to the reinforcing effects of ethanol, as even small doses of ethanol delivered directly to the VTA support self-administration in these rats (Toalston et al. 2014). Moreover, adolescent alcohol exposure resulted in enhanced dopamine transients to reward-predictive cues (Spoelder et al. 2015) and greater c-Fos activation in the NAc and the VTA after ethanol challenge later in adulthood (Liu and Crews 2015). More broadly, behavioral studies demonstrate that adolescent rats are more sensitive to the positive-reinforcing properties of ethanol compared to adults (for review, see (Spear and Varlinskaya 2010)). It is possible that AIE results in the persistence of an adolescent-like phenotype in adulthood regarding dopamine sensitivity to ethanol, as has been shown with other adolescent-like phenotypes (Spear and Swartzwelder 2014). However, critical measurements of ethanol effects on $[DA]_p$ and V_{max} in adolescence that would allow this conclusion have yet to be made.

The concentration of released dopamine per impulse is regulated by D2-autoreceptors located on the terminals and cell bodies of dopamine neurons (Kennedy et al. 1992). Thus, the inability of ethanol challenge to diminish the size of dopamine transients in AIE rats might be due to alterations in D2 receptors and ineffective mechanisms of autoinhibition. As the dopaminergic projection neurons undergo maturation processes during adolescence, including pruning of dopamine receptors in the NAc and the mPFC (Andersen et al. 2002, Benoit-Marand and O'Donnell 2008, Naneix et al. 2012), chronic ethanol may disrupt some aspect of that process, resulting in the long-lasting reductions in D2 receptors observed in subjects with alcohol use disorder (Volkow et al., 1996). Moreover, adolescent binge-like ethanol exposure in mice has been demonstrated to reduce gene expression of dopamine receptors in adulthood (Coleman et al. 2011). Thus, a future direction for this research is to quantify persistent AIE effects on dopamine terminals and receptors that may correspond to the changes in dopamine release reported here.

An alternative mechanism by which ethanol might diminish evoked dopamine signals is to enhance uptake via the dopamine transporter (Wightman and Zimmerman 1990). In contrast, analysis of V_{max} of the transporter in this study demonstrated an ethanol-induced *decrease* in the rate of dopamine uptake, and this effect was not group-dependent. Ethanol challenge has previously been shown to slow the velocity of the dopamine transporter in the ventral striatum of ethanol-naïve rats (Robinson et al. 2005). In contrast, other *in vitro* studies report

no effect of ethanol challenge on the rate of dopamine uptake in striatal slices (.Budygin et al. 2001, Budygin et al. 2005, Mathews et al. 2006) or increased rates of uptake when human dopamine transporter is expressed in cell expression systems (Eshleman et al. 1994, Mayfield et al. 2001, Maiya et al. 2002). Which differences among the various preparations contribute to the different ethanol effects are unclear, although one potential factor in the present preparation is the anesthesia. Specifically, ethanol at higher doses could contribute to the sedation produced by the anesthetic and thereby reduce uptake. In support of this interpretation, the single study of ethanol effects on electrically-evoked dopamine release in awake rats did not find changes in dopamine uptake (Budygin et al. 2001), although that study was primarily looking for increases rather than decreases in uptake that would explain the reduced electrically-evoked dopamine signal. Arguing against that interpretation, similar decreases in dopamine transporter velocity were observed when measured in striatal tissue suspensions as were found in anesthetized rats (Robinson et al. 2005). It is likely that this issue must be resolved with measurements in awake rats, although it is difficult to evoke sufficient quantities of endogenous dopamine release in awake rats to allow for good modeling without altering rat behavior. Improvements in the sensitivity of FSCV or selective, optogenetic activation of dopamine release may allow such measurements in the future.

Recently, we found an attenuating effect of cumulative dosing of ethanol on rate of dopamine clearance in the mPFC of ethanol-naïve rats (Shnitko et al. 2014). In that study, a single dose (4g/kg) of ethanol decreased evoked dopamine release and attenuated clearance in the mPFC of both ethanol-naïve rats and animals treated with ethanol during adolescence. Interestingly, while the present study demonstrated a lack of acute ethanol effect on evoked dopamine release in the NAc of AIE rats, the previous study found that acute ethanol decreased evoked dopamine release in the mPFC of AIE-exposed rats. A caveat to direct comparison of the studies is that in the mPFC study a lower dose of ethanol was used for the AIE regimen, and we may have missed a critical amount of ethanol exposure required to persistently alter mesocortical dopamine release after alcohol challenge. However, an alternative explanation is that the timing of the alcohol exposure was optimized to affect mesolimbic rather than mesocortical dopamine development, as developmental changes in the mesocortical dopaminergic system occur in late adolescence and early adulthood (Andersen et al. 2000). In any case, these findings provide a strong rationale for a direct comparison of AIE effects on mesocortical and mesolimbic dopamine transients, as the resulting data would inform predicted consequences of binge drinking in humans.

There are two caveats to the present study. First, ethanol significantly reduced dopamine release at 2 and 4g/kg in the WAT control group while only 4g/kg ethanol affected NM rats, suggesting that the handling or stress associated with the gavage procedure accentuated sensitivity to a later alcohol challenge. Gavage treatment is not necessarily more stressful than other routes of administration, as Turner and coauthors (2012) found no significant effects of 28 days of repeated gavage of water on a range of physiological measures and behaviors compared to a non-manipulated control group (Turner et al. 2012). However, that study began gavage treatment on P37–42, while we began treatment at P25, and early adolescence may be a time of particular sensitivity to stressful events, including gavage and/or handling. For example, behavioral and neuronal changes in adult rats were

demonstrated after exposure to juvenile stress on P27–29 (Jacobson-Pick and Richter-Levin 2012). However, a recent study from Varlinskaya and colleagues found no difference in social behavior of adult male rats after repeated intragastric exposure to water from P25–45 when compared to males who were not manipulated at that time (Varlinskaya et al. 2014). In any case, these results illustrate why it is important to include a non-manipulated control group in adolescent treatment studies. A second caveat is that the BACs reported during AIE administration are lower than might be expected after 4g/kg ethanol. Other studies have shown that this dose of ethanol has little sedative and intoxicating effects on adolescent rats, especially by P35 when rats have experienced multiple doses (Broadwater and Spear 2013). However, it is possible that we missed the peak BAC as we took blood samples at only one time point.

In summary, acute ethanol has many documented effects on dopamine neurotransmission, including a reduction in dopamine release per impulse as described here and previously. We hypothesize that the diminished dopamine release per impulse is a compensatory mechanism in response to increases in firing rate and extracellular dopamine, potentially regulated by the D2-type autoreceptor. The critical contribution of the present study is the finding that adolescent binge-like ethanol exposure blunts this reduction in dopamine release per impulse in the NAc after alcohol challenge in adulthood, without changing ethanol's effects to slow dopamine uptake, and the net effect would be higher and more persistent dopamine concentrations in AIE rats compared to controls after an alcohol challenge. Future studies can directly test this refined hypothesis by recording spontaneous dopamine release events in awake AIE and control-exposed rats after alcohol challenge and self-administration.

Acknowledgments

Authors thank Dr. Elena Varlinskaya for comments on the results in this study. This research was funded by the National Institute of Alcoholism and Alcohol Abuse NADIA project (U01 AA019972 to LPS) and the UNC Bowles Center for Alcohol Studies. Salary support for DLR was also provided by grant U24 AA020024 of the NADIA project and Project 3 of P60 AA011605.

References

- Adalsteinsson E, Sullivan EV, Mayer D, Pfefferbaum A. In vivo quantification of ethanol kinetics in rat brain. *Neuropsychopharmacology*. 2006; 31(12):2683–2691. [PubMed: 16407891]
- Alaux-Cantin S, Warnault V, Legastelois R, Botia B, Pierrefiche O, Vilpoux C, Naassila M. Alcohol intoxications during adolescence increase motivation for alcohol in adult rats and induce neuroadaptations in the nucleus accumbens. *europarmacology*. 2013; 67:521–531.
- Andersen SL, Thompson AP, Krenzel E, Teicher MH. Pubertal changes in gonadal hormones do not underlie adolescent dopamine receptor overproduction. *Psychoneuroendocrinology*. 2002; 27(6): 683–691. [PubMed: 12084661]
- Andersen SL, Thompson AT, Rutstein M, Hostetter JC, Teicher MH. Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. *Synapse*. 2000; 37(2):167–169. [PubMed: 10881038]
- Badanich KA, Maldonado AM, Kirstein CL. Chronic ethanol exposure during adolescence increases basal dopamine in the nucleus accumbens septi during adulthood. *Alcohol Clin Exp Res*. 2007; 31(5):895–900. [PubMed: 17391340]
- Benoit-Marand M, O'Donnell P. D2 dopamine modulation of corticoaccumbens synaptic responses changes during adolescence. *Eur J Neurosci*. 2008; 27(6):1364–1372. [PubMed: 18331340]

- Broadwater M, Spear LP. Consequences of ethanol exposure on cued and contextual fear conditioning and extinction differ depending on timing of exposure during adolescence or adulthood. *Behav Brain Res.* 2013; 256:10–19. [PubMed: 23938333]
- Budygin EA, Mathews TA, Lapa GB, Jones SR. Local effects of acute ethanol on dopamine neurotransmission in the ventral striatum in C57BL/6 mice. *Eur J Pharmacol.* 2005; 523(1–3):40–45. [PubMed: 16226738]
- Budygin EA, Oleson EB, Mathews TA, Lack AK, Diaz MR, McCool BA, Jones SR. Effects of chronic alcohol exposure on dopamine uptake in rat nucleus accumbens and caudate putamen. *Psychopharmacology (Berl).* 2007; 193(4):495–501. [PubMed: 17492432]
- Budygin EA, Phillips PE, Robinson DL, Kennedy AP, Gainetdinov RR, Wightman RM. Effect of acute ethanol on striatal dopamine neurotransmission in ambulatory rats. *J Pharmacol Exp Ther.* 2001; 297(1):27–34. [PubMed: 11259524]
- Budygin EA, Phillips PE, Wightman RM, Jones SR. Terminal effects of ethanol on dopamine dynamics in rat nucleus accumbens: an in vitro voltammetric study. *Synapse.* 2001; 42(2):77–79. [PubMed: 11574942]
- Chartier KG, Hesselbrock MN, Hesselbrock VM. Development and vulnerability factors in adolescent alcohol use. *Child Adolesc Psychiatr Clin N Am.* 2010; 19(3):493–504. [PubMed: 20682217]
- Coleman LG Jr, He J, Lee J, Styner M, Crews FT. Adolescent binge drinking alters adult brain neurotransmitter gene expression, behavior, brain regional volumes, and neurochemistry in mice. *Alcohol Clin Exp Res.* 2011; 35(4):671–688. [PubMed: 21223304]
- Crews F, He J, Hodge C. Adolescent cortical development: a critical period of vulnerability for addiction. *Pharmacol Biochem Behav.* 2007; 86(2):189–199. [PubMed: 17222895]
- Crippens D, White ML, George MA, Jaworski JN, Brunner LJ, Lancaster FE, Gonzales RA. Gender differences in blood levels, but not brain levels, of ethanol in rats. *Alcohol Clin Exp Res.* 1999; 23(3):414–420. [PubMed: 10195812]
- Di Chiara G, Imperato A. Ethanol preferentially stimulates dopamine release in the nucleus accumbens of freely moving rats. *Eur J Pharmacol.* 1985; 115(1):131–132. [PubMed: 4043232]
- Ernst M, Fudge JL. A developmental neurobiological model of motivated behavior: anatomy, connectivity and ontogeny of the triadic nodes. *Neurosci Biobehav Rev.* 2009; 33(3):367–382. [PubMed: 19028521]
- Eshleman AJ, Henningsen RA, Neve KA, Janowsky A. Release of dopamine via the human transporter. *Mol Pharmacol.* 1994; 45(2):312–316. [PubMed: 7906856]
- Gessa GL, Muntoni F, Collu M, Vargiu L, Mereu G. Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. *Brain Res.* 1985; 348(1):201–203. [PubMed: 2998561]
- Grant BF, Dawson DA. Age of onset of drug use and its association with DSM-IV drug abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *J Subst Abuse.* 1998; 10(2):163–173. [PubMed: 9854701]
- Jacobson-Pick S, Richter-Levin G. Short- and long-term effects of juvenile stressor exposure on the expression of GABAA receptor subunits in rats. *Stress.* 2012; 15(4):416–424. [PubMed: 22044189]
- Jones SR, Mathews TA, Budygin EA. Effect of moderate ethanol dose on dopamine uptake in rat nucleus accumbens in vivo. *Synapse.* 2006; 60(3):251–255. [PubMed: 16752364]
- Jucaite A, Forssberg H, Karlsson P, Halldin C, Farde L. Age-related reduction in dopamine D1 receptors in the human brain: from late childhood to adulthood, a positron emission tomography study. *Neuroscience.* 2010; 167(1):104–110. [PubMed: 20109534]
- Karkhanis AN, Rose JH, Huggins KN, Konstantopoulos JK, Jones SR. Chronic intermittent ethanol exposure reduces presynaptic dopamine neurotransmission in the mouse nucleus accumbens. *Drug Alcohol Depend.* 2015; 150:24–30. [PubMed: 25765483]
- Kennedy RT, Jones SR, Wightman RM. Dynamic observation of dopamine autoreceptor effects in rat striatal slices. *J Neurochem.* 1992; 59(2):449–455. [PubMed: 1352798]
- Liu W, Crews FT. Adolescent intermittent ethanol exposure enhances ethanol activation of the nucleus accumbens while blunting the prefrontal cortex responses in adult rat. *Neuroscience.* 2015; 293:92–108. [PubMed: 25727639]

- Maiya R, Buck KJ, Harris RA, Mayfield RD. Ethanol-sensitive sites on the human dopamine transporter. *J Biol Chem*. 2002; 277(34):30724–30729. [PubMed: 12070173]
- Mathews TA, John CE, Lapa GB, Budygin EA, Jones SR. No role of the dopamine transporter in acute ethanol effects on striatal dopamine dynamics. *Synapse*. 2006; 60(4):288–294. [PubMed: 16786536]
- Mayfield RD, Maiya R, Keller D, Zahniser NR. Ethanol potentiates the function of the human dopamine transporter expressed in *Xenopus* oocytes. *J Neurochem*. 2001; 79(5):1070–1079. [PubMed: 11739621]
- McClory AJ, Spear LP. Effects of ethanol exposure during adolescence or in adulthood on Pavlovian conditioned approach in Sprague-Dawley rats. *Alcohol*. 2014; 48(8):755–763. [PubMed: 25449366]
- McCutcheon JE, Conrad KL, Carr SB, Ford KA, McGehee DS, Marinelli M. Dopamine neurons in the ventral tegmental area fire faster in adolescent rats than in adults. *J Neurophysiol*. 2012; 108(6):1620–1630. [PubMed: 22723669]
- McCutcheon JE, Marinelli M. Age matters. *Eur J Neurosci*. 2009; 29(5):997–1014. [PubMed: 19291226]
- Naneix F, Marchand AR, Di Scala G, Pape JR, Coutureau E. Parallel maturation of goal-directed behavior and dopaminergic systems during adolescence. *J Neurosci*. 2012; 32(46):16223–6232. [PubMed: 23152606]
- NIAAA. Binge drinking defined. NIAAA Newsletter. 2004 Winter;(3):3.
- Patrick ME, Schulenberg JE, Martz ME, Maggs JL, O'Malley PM, Johnston LD. Extreme binge drinking among 12th-grade students in the United States: prevalence and predictors. *JAMA Pediatr*. 2013; 167(11):1019–1025. [PubMed: 24042318]
- Robinson DL, Howard EC, McConnell S, Gonzales RA, Wightman RM. Disparity between tonic and phasic ethanol-induced dopamine increases in the nucleus accumbens of rats. *Alcohol Clin Exp Res*. 2009; 33(7):1187–1196. [PubMed: 19389195]
- Robinson DL, Volz TJ, Schenk JO, Wightman RM. Acute ethanol decreases dopamine transporter velocity in rat striatum: in vivo and in vitro electrochemical measurements. *Alcohol Clin Exp Res*. 2005; 29(5):746–755. [PubMed: 15897718]
- Schier CJ, Dilly GA, Gonzales RA. Intravenous ethanol increases extracellular dopamine in the medial prefrontal cortex of the Long-Evans rat. *Alcohol Clin Exp Res*. 2013; 37(5):740–747. [PubMed: 23421849]
- Shnitko TA, Kennerly LC, Spear LP, Robinson DL. Ethanol reduces evoked dopamine release and slows clearance in the rat medial prefrontal cortex. *Alcohol Clin Exp Res*. 2014; 38(12):2969–2977. [PubMed: 25581652]
- Somers LA, Beyene M, Carelli RM, Wightman RM. Synaptic overflow of dopamine in the nucleus accumbens arises from neuronal activity in the ventral tegmental area. *J Neurosci*. 2009; 29(6):1735–1742. [PubMed: 19211880]
- Spear LP. Adolescents and alcohol: acute sensitivities, enhanced intake, and later consequences. *Neurotoxicol Teratol*. 2014; 41:51–59. [PubMed: 24291291]
- Spear LP. Adolescent alcohol exposure: Are there separable vulnerable periods within adolescence? *Physiol Behav*. 2015
- Spear LP, Swartzwelder HS. Adolescent alcohol exposure and persistence of adolescent-typical phenotypes into adulthood: a mini-review. *Neurosci Biobehav Rev*. 2014; 45:1–8. [PubMed: 24813805]
- Spear LP, Varlinskaya EI. Adolescence. Alcohol sensitivity, tolerance, and intake. *Recent Dev Alcohol*. 2005; 17:143–159. [PubMed: 15789864]
- Spear LP, Varlinskaya EI. Sensitivity to ethanol and other hedonic stimuli in an animal model of adolescence: implications for prevention science? *Dev Psychobiol*. 2010; 52(3):236–243. [PubMed: 20222058]
- Spoelder M, Tsutsui KT, Lesscher HM, Vanderschuren LJ, Clark JJ. Adolescent Alcohol Exposure Amplifies the Incentive Value of Reward-Predictive Cues Through Potentiation of Phasic Dopamine Signaling. *Neuropsychopharmacology*. 2015

- Toalston JE, Deehan GA Jr, Hauser SR, Engleman EA, Bell RL, Murphy JM, Truitt WA, McBride WJ, Rodd ZA. Reinforcing properties and neurochemical response of ethanol within the posterior ventral tegmental area are enhanced in adulthood by periadolescent ethanol consumption. *J Pharmacol Exp Ther.* 2014; 351(2):317–326. [PubMed: 25150280]
- Trantham-Davidson H, Burnett EJ, Gass JT, Lopez MF, Mulholland PJ, Centanni SW, Floresco SB, Chandler LJ. Chronic alcohol disrupts dopamine receptor activity and the cognitive function of the medial prefrontal cortex. *J Neurosci.* 2014; 34(10):3706–3718. [PubMed: 24599469]
- Turner PV, Vaughn E, Sunohara-Neilson J, Ovari J, Leri F. Oral gavage in rats: animal welfare evaluation. *J Am Assoc Lab Anim Sci.* 2012; 51(1):25–30. [PubMed: 22330864]
- Varlinskaya EI, Spear LP, Spear NE. Acute effects of ethanol on behavior of adolescent rats: role of social context. *Alcohol Clin Exp Res.* 2001; 25(3):377–385. [PubMed: 11290848]
- Varlinskaya EI, Truxell E, Spear LP. Chronic intermittent ethanol exposure during adolescence: effects on social behavior and ethanol sensitivity in adulthood. *Alcohol.* 2014; 48(5):433–444. [PubMed: 24928792]
- Volkow ND, Wang GJ, Fowler JS, Logan J, Hitzemann R, Ding YS, Pappas N, Shea C, Piscani K. Decreases in dopamine receptors but not in dopamine transporters in alcoholics. *Alcohol Clin Exp Res.* 1996; 20(9):1594–1598. [PubMed: 8986209]
- White AM, Kraus CL, Swartzwelder H. Many college freshmen drink at levels far beyond the binge threshold. *Alcohol Clin Exp Res.* 2006; 30(6):1006–1010. [PubMed: 16737459]
- Wightman RM, Zimmerman JB. Control of dopamine extracellular concentration in rat striatum by impulse flow and uptake. *Brain Res Brain Res Rev.* 1990; 15(2):135–144. [PubMed: 2282449]
- Wood MD, Nagoshi CT, Dennis DA. Alcohol norms and expectations as predictors of alcohol use and problems in a college student sample. *Am J Drug Alcohol Abuse.* 1992; 18(4):461–476. [PubMed: 1449125]
- Wu Q, Reith ME, Wightman RM, Kawagoe KT, Garriss PA. Determination of release and uptake parameters from electrically evoked dopamine dynamics measured by real-time voltammetry. *J Neurosci Methods.* 2001; 112(2):119–133. [PubMed: 11716947]

Fig. 1.

Experimental design. (A) Timeline of the experiment with indicated periods of adolescent exposure and voltammetric measurements. (B) Changes in rat weight across adolescence. (C) BEC measured on P35 and P45 in AIE rats, 30 min after ethanol administration. (D) BEC measured 15 min after the final dose in rats given ethanol challenge during voltammetric dopamine measurements

Fig. 2.

Dopamine release in the NAc core of rats evoked by electrical stimulation of the VTA. (A) Representation of voltammetric signals of dopamine obtained in the NAc of an individual rat. In the color plot, changes in the current are depicted in color and plotted as function of applied potential on the y-axis and time on the x-axis. Changes in current due to oxidation and reduction of dopamine are plotted vs applied potential in the background-subtracted cyclic voltammogram on the top. Changes in current resulting from maximal oxidation of dopamine (at ~0.65V vs Ag/AgCl reference electrode) are converted to [DA] and plotted vs time in the concentration vs time trace demonstrated in B. In both the color plot and the [DA] vs time trace, green bars represent a delivery of electrical stimulation to the VTA (at 0

sec). (C) Baseline $[DA]_{\max}$ in the NAc core of rats from NM, WAT and AIE groups evoked by electrical stimulation in the VTA. Data are mean $\pm$ SEM, n=12–14 per group

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 3.

Effect of the ethanol challenge on evoked dopamine release in the NAc core of rats from three groups: NM, WAT and AIE. In x-axis of each graph, SAL indicates injection of the vehicle given to each rat prior to any experimental challenge (saline or ethanol). Experimental dosing was given cumulatively. Data are presented in percentage of baseline and as mean $\pm$ SEM ($n=12-14$ per group and $6-7$ per challenge). Results of Tukey tests are indicated by * ($p<0.05$ vs SAL), $\times$ ($p<0.05$ vs saline challenge) and + ($p<0.05$ vs 1.0 and 2.0 g/kg)

Fig. 4.

Effect of ethanol challenge on electrically evoked dopaminergic signals in the NAc core of individual rats exposed to water or ethanol during adolescence. Evoked dopamine signals are presented as [DA] vs time traces with corresponding cyclic voltammograms obtained from the peaks of the signals. Data were collected 10 min after injection of saline and indicated dose. Vertical dashed lines indicate onset of electrical stimulation (biphasic, 60Hz, 24pulses, 124 μ A). Scales demonstrated for the bottom voltammograms are valid for the rest of the voltammograms in the corresponding column. Traces of signals modeled with Michaelis-

Menten method are represented in blue in the corresponding dopaminergic signals. Traces were fitted to the dopaminergic signals via changing of $[DA]_p$ and V_{max} , while K_m was held at 200nM. Goodness of fit was estimated using Pearson correlation with coefficient $r > 0.8$

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Effect of adolescent intermittent ethanol exposure on VTA-evoked dopamine release and uptake in the NAc of adult rats.

Group	[DA] _p , nM	V _{max} , nM/s
NM	10.0 ± 0.7	299 ± 30
WAT	10.2 ± 1.3	356 ± 37
AIE	13.0 ± 1.9	406 ± 45

Data are mean ± SEM, n = 12–14/group.

Table 2

Effect of acute ethanol challenge on dopamine per pulse ([DA]_p, nM) in the NAc of adult rats exposed to alcohol during adolescence.

Dose (g/kg)	NM		WAT		AIE	
	Saline	Ethanol	Saline	Ethanol	Saline	Ethanol
SAL	8.5 ± 0.4	9.4 ± 1.0	11.6 ± 2.0	7.1 ± 0.8	12.1 ± 2.4	12.6 ± 2.3
1.0	8.0 ± 0.6	8.0 ± 1.0	12.4 ± 3.2	5.6 ± 0.6	11.4 ± 2.1	11.5 ± 2.2
2.0	7.8 ± 0.6	6.6 ± 0.6	11.4 ± 3.0	4.4 ± 0.8 ^a	10.2 ± 2.0	10.4 ± 1.9
4.0	7.3 ± 0.6	6.4 ± 0.8 ^a	11.0 ± 2.8	3.7 ± 1.2 ^{a,b}	9.4 ± 2.0	9.5 ± 1.8

Data are mean ± SEM, n = 6–7/cell.

^a significantly different from SAL, p<0.05.

^b significantly different from saline challenge, p<0.05.

Table 3

Effect of acute ethanol challenge on dopamine transporter velocity ($V_{\max}$, nM/s) in the NAc of adult rats exposed to alcohol during adolescence.

Dose (g/kg)	NM		WAT		AIE	
	Saline	Ethanol	Saline	Ethanol	Saline	Ethanol
SAL	285 ± 38	250 ± 38	358 ± 49	299 ± 33	373 ± 70	388 ± 49
1.0	287 ± 47	219 ± 40	352 ± 55	233 ± 30	357 ± 59	358 ± 48
2.0	275 ± 46	188 ± 33 ^a	318 ± 51	188 ± 30 ^a	339 ± 57	307 ± 40 ^a
4.0	242 ± 36 ^a	187 ± 24 ^a	294 ± 48 ^a	171 ± 33 ^a	312 ± 55 ^a	263 ± 34 ^a

Data are mean ± SEM, n = 6–7/cell.

^a collapsed across adolescent exposure group, significantly different from SAL, p<0.05.