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SOCIAL ISOLATION REARING INCREASES NUCLEUS ACCUMBENS DOPAMINE AND NOREPINEPHRINE RESPONSES TO ACUTE ETHANOL IN ADULthood

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Abstract

BACKGROUND—Early-life stress is associated with increased vulnerability to alcohol addiction. However, the neural substrates linking chronic childhood/adolescent stress and increased risk of alcohol addiction are not well understood. In the nucleus accumbens (NAc), dopamine (DA) and norepinephrine (NE) signaling can be profoundly influenced by stress, anxiety, and drugs of abuse, including ethanol. Here, we employed a rodent model of early-life stress that results in enduring increases in behavioral risk factors of alcoholism to gain a better understanding of how chronic adolescent stress may impact the ethanol sensitivity of DA and NE release in the NAc.

METHODS—Male Long Evans rats were either group housed (GH; 4 rats/cage) or socially isolated (SI; 1 rat/cage) for six weeks beginning on postnatal day 28. SI and GH rats were tested in adulthood for anxiety-like behaviors (elevated plus-maze), and the effects of ethanol (1 and 2 g/kg; i.p.) on NAc DA and NE were assessed by microdialysis.

RESULTS—SI animals showed increased anxiety-like behavior compared to GH animals. Although SI had no effect on baseline levels of DA or NE, baseline DA levels were positively correlated with anxiety measures. In addition, while no significant differences were observed with 1 g/kg ethanol, the 2 g/kg dose induced significantly greater DA release in SI animals. Moreover, EtOH (2 g/kg) only elevated NAc NE levels in SI rats.

CONCLUSIONS—These results suggest that chronic early-life stress sensitizes accumbal DA and NE release in response to an acute ethanol challenge. A greater EtOH sensitivity of DA and NE release dynamics in the NAc may contribute to increases in behavioral risk factors of alcoholism, like greater ethanol self-administration, that are observed in SI rats.

INTRODUCTION

Exposure to adverse experiences during development, for example childhood neglect, often results in psychiatric disorders and increased probability of drug and alcohol abuse problems that persist throughout adulthood (Anda et al., 2002). Similar to humans, early adolescence is a critical time period for rodents. Rats that have been socially isolated (SI) during adolescence spend less time on the open arm of an elevated plus maze (Hall et al., 1998; McCool and Chappell, 2009), display reduced social interaction (Green et al., 2012), and increased immobility during the forced swim test compared to group housed (GH) rats (Kokare et al., 2010), exemplifying anxiety- and depression-like phenotypes. SI rats also show pre-pulse inhibition deficits (Han et al., 2012) and reduced habituation to an open field (Lapiz et al., 2000) demonstrating a reduced ability to adapt to environmental changes.

These behavioral abnormalities are associated with increased vulnerability to alcoholism. For example, increased anxiety-like behavior is positively correlated with increased intake of ethanol (EtOH; Chappell et al., 2013). Isolation rearing also increases the self-administration of EtOH (Wolffgramm and Heyne, 1991; McCool and Chappell, 2009) and conditioned place preference acquisition for EtOH (Whitaker et al., 2013). However, the effects of early-life stress on the neural correlates of alcoholism are not fully understood. For example, dopamine (DA; Fitzgerald et al., 2013) and norepinephrine (NE; Lapiz et al., 2000) play integral roles in regulating behaviors affected by stress and anxiety, suggesting that DA and NE signaling may contribute to some of the behavioral changes associated with social isolation. Specifically, *in vivo* microdialysis studies report elevated baseline DA levels in the nucleus accumbens (NAc) of SI compared to GH rats (Hall et al., 1998; Han et al., 2011), while NE levels are decreased in the ventral striatum of SI rats (Brenes et al., 2008), but depleting NE in SI, but not GH, rats increases exploration of an open field (Lapiz et al., 2000).

Acute EtOH elevates DA in the NAc when administered systemically (Yim et al., 2000), primarily via the activation of DA neurons in the ventral tegmental area (VTA) (Brodie et al., 1999; Ding et al., 2009). In contrast to a large body of literature investigating the effects of acute EtOH on DA, there is only one report on the effects of acute EtOH on NE in the NAc (Marinelli et al., 2003). Microdialysis in normal animals has shown that EtOH does not induce a NE response in the NAc (Marinelli et al., 2003). However, electrophysiology studies in anesthetized animals have shown that acute EtOH decreases locus coeruleus (LC) neuron activity and increases synthesis of NE in the hypothalamus and midbrain (Pohorecky and Jaffe, 1975). Thus, the acute effects of EtOH on NE release are unclear.

Previous data suggest that changes in DA and NE signaling may contribute to SI-associated increases in anxiety-like behavior and EtOH drinking. Although several studies have documented changes in DA and NE signaling following social isolation, the EtOH-induced modulation of these catecholamines in awake, behaving animals is not fully understood. An examination of the ethanol sensitivity of accumbal DA and NE systems in SI rats may provide insights into the neurobiological underpinnings of increased risk of alcohol use disorders. Therefore, the primary goal of the current study was to use *in vivo* microdialysis to characterize the effects of adolescent social isolation on EtOH-stimulated DA and NE

release in the NAc. The present data show, for the first time, that social isolation results in a sensitized response of both catecholaminergic systems to acute EtOH administration.

MATERIALS AND METHODS

Group and isolation rearing

Male Long-Evans rats were purchased from a commercial supplier (Harlan Laboratories, IN) at postnatal day (PD) 21. Following a week of acclimation in standard housing conditions (four animals per cage, food and water *ad libitum*; 12 h light/dark cycle), at PD 28, rats were randomly assigned to one of two experimental groups: group housed (GH; $n = 15$) and socially isolated (SI; $n = 17$). Rats in the GH condition were housed in groups of four in guinea pig cages (33 cm X 50.7 cm; Nalgene, Rochester, NY); SI rats were housed individually in rat cages (20.3 cm X 26.7 cm; Allentown, Allentown, NJ). Rats were maintained in their respective housing conditions for at least six weeks before behavioral testing and microdialysis experiments (Fig. 1).

Anxiety assay

Following six weeks of group and isolation rearing, anxiety-like behavior was assessed using the standard elevated plus maze (EPM) apparatus (Med Associates, St. Albans, VT) on PD 74 and 75. The EPM assay was performed as previously described (McCool and Chappell, 2009). Briefly, the anxiety assay was conducted during the first two hours of the light phase of the light/dark cycle. Lighting was provided by two standard 60 W incandescent lights placed at the ends of each open arm. Rats were placed at the junction of the EPM facing the open arm. Each animal was assessed on the EPM for five mins. Behavioral measures were scored using an automated infrared beam detection system (Med Associates, St. Albans, VT). Animals were tested concurrently on two mazes placed approximately 60 cm apart from each other. GH rats were alone on the EPM for no more than five mins. The amount of time spent on the open arms and the number of entries onto the open arms were used as measures of anxiety-like behavior. The number of closed arm entries was used as a measure of locomotor activity (Holmes and Rodgers, 1999). The EPM was used to assess and confirm the differences in anxiety-like behavior observed between GH and SI animals by previous studies (Hall et al., 1998; McCool and Chappell, 2009; Chappell et al., 2013).

Blood EtOH elimination

For a separate group of rats, following six weeks of the housing paradigm, EtOH (1.5 g/kg; i.p.) was administered to SI and GH rats ($n = 7$ in each group). Tail blood samples (10 μ l per sample) were collected over a period of six hours (every 20 mins for the first hour, every 30 mins for the second hour, and every hour for the following four hours) and analyzed for blood ethanol concentration (BEC) using a commercially available ethanol assay kit (229-29; Sekisui Diagnostics LLC, Lexington, MA).

Microdialysis

Surgery—All rats were anesthetized with ketamine (100 mg/kg; i.p.) and xylazine (10 mg/kg; i.p.). A guide cannula with a stylet (MD-2250; BASi Instruments, West Lafayette,

IN) was implanted over the NAc (anterior, +2.2; lateral, +0.7; ventral, −5.4 with respect to bregma; Fig. 2). The NAc coordinates were determined using a rat atlas (Paxinos and Watson, 2007). The guide cannula was anchored to the skull and the exposed skull was covered using fast-drying dental acrylic (Lang Dental Manufacturing Co., Inc., Wheeling, IL). Ketoprofen (5 mg/ml; 1 ml/kg, s.c.) was administered post-surgery. A microdialysis probe with a membrane length of 2 mm (MD-2200, BASi Instruments, West Lafayette, IN), was inserted through the guide cannula into the NAc immediately after the surgery. Prior to inserting the microdialysis probe into the cannula, it was connected to a syringe pump (A-99; Razel Scientific Instruments, Inc., Stamford, CT) and perfused with artificial cerebrospinal fluid (aCSF; 148 mM NaCl; 2.7 mM KCl; 1.2 mM CaCl₂; 0.85 mM MgCl₂; pH 7.4) at a flow rate of 1 µl/min for a minimum of one hour. Following microdialysis experiments, all rats were perfused transcardially with saline followed by paraformaldehyde (10%; SF100-4; Fischer Scientific, Fair Lawn, NJ) and the brains were removed for probe placement confirmation.

Experimental design—Microdialysis experiments were conducted following the EPM behavioral assay (between PD 80 and 110). GH rats were placed in separate chambers and run at the same time. The post-surgery recovery time was between 18 and 24 hrs for all rats. EtOH (1 and/or 2 g/kg) was administered intraperitoneally (i.p.). A subset of animals received 1 g/kg, 2 g/kg, or both doses of EtOH. Overnight, the aCSF flow through the probe was maintained at a rate of 0.4 µl/min. Following the recovery period, the flow rate was increased to 1 µl/min for two hours prior to baseline sample collections. The samples were collected with an interval of 20 mins for two hours prior to EtOH administration and are referred to as baseline. Dialysate samples were collected for 100 mins following EtOH administration (1 or 2 g/kg; 20% w/v). A different group of rats were treated identically but received saline injections instead of EtOH to examine the effects of i.p. injection stress on DA and NE levels. The volume of the saline injection matched that of the 2 g/kg EtOH dose. Perchloric acid (10 µl) was added to all sample tubes before collection. All samples were frozen at −80° C until analysis.

High Performance Liquid Chromatography—The dialysate samples were analyzed using high performance liquid chromatography (HPLC; ESA/Thermo Scientific, Chelmsford, MA) with electrochemical detection. Dialysate samples (10 µl) were injected onto a reverse-phase microbore column (Luna 3µ, C18(2), 100 Å; 100 × 4.60 mm, 3 µm; 00D-4251-E0; Phenomenex, Torrance, CA) for separation. Two separate systems with identical mechanical configurations were used for DA and NE detection. The following mobile phase was used for DA detection: 75 mM monohydrate sodium dihydrogen phosphate, 1.9 mM 1-octanesulfonic acid sodium salt, 100 µl/L triethylamine, 25 µM EDTA, 10% acetonitrile, adjusted to a pH of 3 using NaOH. The mobile phase used in the current study was a modified version of the MD-TM mobile phase (70-1332; Thermo Fisher Scientific; Sunnyvale, CA).

The mobile phase used for NE detection was modified from a previously published study (Lapiz et al., 2000): 1.65 mM octyl sodium sulfate, 9.2 mM citric acid, 1 ml EDTA, 14%

acetonitrile, adjusted to a pH of 5.0 using NaOH. NE detection was conducted in a subset of animals ($n = 8$ in each group).

Statistical analysis

All statistical analyses were conducted using GraphPad Prism 5 (GraphPad Software, La Jolla, CA) and R (R Core Team, Vienna, Austria). All comparisons to assess anxiety-like behavior were analyzed using one-tailed Students' *t*-tests. The microdialysis data were analyzed using a two-way analysis of variance (ANOVA) with Bonferroni's *post hoc* analysis. The two independent variables were time and EtOH dose. The dependent variable was either extracellular DA or NE. In a second comparison, the two independent variables were housing and EtOH dose. For this comparison, DA and NE responses were quantified by an area under the curve analysis (AUC). AUC was calculated individually for each animal and averaged for grouped data. AUC included the entire area on the Y-plane (DA or NE levels) ranging from the baseline (0% mark) to the peak of catecholamine for that particular animal at any given unit in time. This was calculated across the entire X-plane (time) which was held constant for all animals (100 mins). Peaks of DA and NE that did not exceed a 5% increase over baseline were considered noise and were not included in the AUC. A one-way ANOVA followed by Dunnett's Multiple Comparison *post hoc* test was used for the within-group comparison of DA and NE levels before and after saline or EtOH administration. All data are reported as mean $\pm$ standard error of the mean. The significance level for all statistical measures was set at $p < 0.05$.

RESULTS

Anxiety-like behavior

Following six weeks of either group or isolation rearing, rats were evaluated on the EPM to assess anxiety-like behavior. The SI rats spent significantly less time on the open arms of the EPM compared to their GH counterparts (Fig. 3A; $t = 3.30$, $p < 0.01$, *t*-test). Furthermore, the SI rats entered the open arms a fewer number of times compared to the GH rats (Fig. 3B; $t = 2.94$, $p < 0.01$, *t*-test). No significant difference was found in the number of entries onto the closed arm of the EPM (Fig. 3C; $t = 1.43$, $p > 0.05$, *t*-test), confirming that housing condition did not alter overall locomotor activity in this assay.

Blood EtOH elimination

Following EtOH administration, tail blood samples were collected and analyzed to quantify BEC over a period of six hours. No significant differences were found in the BECs of GH and SI rats (Fig. 4; $F = 1.81$; $p > 0.05$). The EtOH elimination curves were conducted in a distinct cohort of SI/GH rats from the ones used for neurochemical analysis. In order to reduce the number of animals needed for this experiment, an EtOH dose (1.5 g/kg, i.p.) that was intermediate between the two doses tested for microdialysis studies was used.

Effects of acute EtOH on extracellular levels of DA

Extracellular DA at baseline—Baseline samples were collected for two hours prior to EtOH administration to ensure that the DA level in the NAc was stable. A mean of three samples was used to compare baseline extracellular DA levels in GH and SI rats, and no

significant difference was found (Fig. 5A; $t = 0.53$, $p > 0.05$, t -test). However, a significant correlation was observed between increased open arm time and the baseline DA level in the NAc (Fig. 5B; black solid line; $r^2 = 0.1544$, $p < 0.05$). A positive significant correlation was observed within the GH animals (Fig. 5B; red dotted line; $r^2 = 0.2922$, $p < 0.05$). No significant correlation was observed within the SI animals (Fig. 5B; blue dotted line; $r^2 = 0.0468$, $p > 0.05$) potentially due to a floor effect of number of open arm entries.

Social isolation results in an augmentation of DA response to the 2 g/kg dose of EtOH in SI rats

—In GH rats, a comparison across 1 and 2 g/kg of EtOH showed that the two doses of EtOH did not differentially alter the peak extracellular level of DA (Fig. 5C) immediately following EtOH challenge with both doses producing an approximately 50% increase ($F = 1.07$; $p > 0.05$). In SI animals at the 20min sampling time, the 2 g/kg ethanol dose increased the extracellular levels of DA almost two-fold above the 1g/kg dose (Fig. 5D; $F = 8.42$; $p < 0.001$). A two-way ANOVA indicated that the augmentation of the DA response was dependent on the housing condition and the EtOH dose (Fig. 5E; $F = 5.86$; $p < 0.01$). The *post hoc* analysis confirmed that EtOH (2 g/kg) resulted in a larger DA response in the NAc of SI compared to GH rats. These data show that SI rats have an augmented DA response to EtOH (2 g/kg). In order to verify that the effects of EtOH observed in SI and GH animals are due to the pharmacological effects of EtOH, saline was administered in a separate group of SI and GH animals. Saline administration did not result in a significant increase in extracellular DA levels in GH and SI animals (Fig. 5F; $F = 0.37$; $p > 0.05$).

Acute EtOH effects on NE in the NAc

Extracellular NE at baseline—Baseline samples from NAc were analyzed to confirm that the extracellular NE level was stable prior to EtOH administration. Three samples preceding EtOH challenge were averaged to obtain the baseline NE level in the NAc of SI and GH rats. A comparison of the two groups showed that isolation rearing does not affect NE levels at baseline (Fig. 6A; $t = 0.35$, $p > 0.05$, t -test). Furthermore, the baseline NE level did not correlate with anxiety-like behavior (data not shown; $p > 0.05$).

Social isolation results in a NE response following the 2 g/kg dose of EtOH

—In GH rats, there was no elevation of NE following either of the two doses of EtOH (Fig. 6B; $F = 0.13$; $p > 0.05$). A comparison of doses showed that NE levels were elevated following both 1 and 2 g/kg doses of EtOH in SI rats, however this elevation was found to be significantly greater following the higher dose of EtOH (Fig. 6C; $F = 14.33$; $p < 0.05$). A two-way ANOVA indicated that the robust increase in NE following acute EtOH was dependent on the EtOH dose and the housing condition (Fig. 6D; $F = 12.04$; $p < 0.01$). The *post hoc* analysis confirmed that EtOH (2 g/kg) resulted in an augmentation of NE response in the NAc of SI compared to GH rats. Saline was administered in a separate group of GH and SI animals in order to verify that the augmentation of NE response observed following EtOH was due to the pharmacological effects of EtOH and not a response to injection stress. Saline administration did not elevate NE levels in SI or GH animals (Fig. 6E; $F = 0.32$; $p > 0.05$).

DISCUSSION

The current data show that early-life stress in the form of social isolation rearing resulted in increased anxiety-like behavior during adulthood, a finding that has been observed in several laboratories (Pisu et al., 2011; McCool and Chappell, 2009). This anxiety-like behavior was positively correlated with baseline DA levels in the NAc. Furthermore, social isolation was also associated with an increased response of DA and NE in the NAc to an acute EtOH challenge, which was not due to alterations in EtOH metabolism. First, baseline extracellular DA was not significantly different in SI and GH rats. Second, DA elevations were augmented following 2 g/kg (but not 1 g/kg) acute EtOH challenge in SI compared to GH rats. Third, social isolation did not significantly affect baseline extracellular NE. Fourth, similar to DA, EtOH-induced NE elevations were augmented following 2 g/kg, but not 1 g/kg doses in SI compared to GH rats. Lastly, the mild stress of saline administration did not increase DA or NE levels in the NAc of SI or GH animals. Overall, isolation rearing stress increased sensitivity to EtOH-mediated stimulation of NAc DA and NE release.

DA responsivity to EtOH is increased by social isolation

SI and GH rats had equivalent baseline DA levels prior to EtOH injection. These data are consistent with other studies which have shown no baseline differences in DA in SI and GH rats (Fabricius et al., 2011; Wang et al., 2012). However, others have reported that SI rats have elevated extracellular DA compared to GH rats (Hall et al., 1998; Han et al., 2011). Recently, it has been shown that SI rats have increased DA release and uptake in the NAc (Yorgason et al., 2013). Thus, it is likely that DA uptake offsets the increase in DA release, leading to a lack of difference in baseline DA levels in SI and GH animals. Despite the lack of baseline differences in DA levels in SI and GH animals in the current study, a significant positive correlation between this measure and anxiety-like behavior was observed. The correlation between anxiety and baseline DA levels within SI animals was not significant; however, one potential problem with the SI correlations is a lack of a sufficient dynamic range in the anxiety measure. Within the GH rats, which had a greater range of anxiety-related scores, rats with comparatively higher DA levels spent less time on the open arm of the EPM. This suggests that relatively higher baseline DA levels in the NAc may be associated with increases in anxiety-like behavior, independent of adolescent housing condition.

Previous studies involving psychostimulants show an augmentation of DA elevations in SI compared to GH rats following amphetamine administration (Hall et al., 1998) and an increased locomotor response to cocaine administration (Phillips et al., 1994). Moreover, there is evidence that social isolation results in increased conditioned place preference acquisition for amphetamine and ethanol in rats (Whitaker et al., 2013). These observations were attributed to an increased sensitivity to substances of abuse because repeated stress cross-sensitizes to stimulants. Consistent with these results, EtOH (2 g/kg) increased DA more in SI relative to GH animals in the current study. The current 2 g/kg data in GH animals are consistent with previous work showing an equal increase in DA following 1 and 2 g/kg EtOH in rats housed under standard conditions (Yim et al., 2000). The augmented DA elevations in the SI animals at a 2 g/kg dose of EtOH may be involved in the increased

consumption and seeking of EtOH observed in SI rats (McCool and Chappell, 2009; Chappell et al., 2013). Based on reports of sensitized responses to psychostimulants such as cocaine and amphetamine, it is likely that chronic stress also cross-sensitizes to EtOH, resulting in an augmentation of EtOH-induced DA elevations in the NAc.

Social isolation-induced NE response to acute EtOH

Although acute stressors typically increase NE levels, chronic stress has been shown to deplete NE tissue content in the hippocampus, hypothalamus (Fulford and Marsden, 1997) and NAc (Brenes et al., 2008) but have no effects on NE content in the cortex, hippocampus, and hypothalamus (Lapiz et al., 2000) following social isolation. The present data indicated that baseline extracellular NE levels were not different between SI and GH rats. As mentioned above, social isolation increases the uptake and release of DA (Yorgason et al., 2013). It is possible that NE is regulated in the NAc such that both uptake and release are increased following social isolation, similar to DA, although further experiments are required to confirm such changes.

Although baseline NE levels were not altered by social isolation, this model of early-life stress did significantly increase the acute ethanol sensitivity of the accumbal NE system, as EtOH (2 g/kg) resulted in a robust increase in NAc NE only in SI animals. Many studies have demonstrated that the NE system regulates EtOH drinking. For example, NE depletion as a result of blockade of synthetic pathways attenuates EtOH self-administration in mice (Weinshenker et al., 2000). Dopamine- β -hydroxylase knockout mice, which lack the ability to synthesize NE, also consume less EtOH (Weinshenker et al., 2000). In rats, administration of propranolol, a beta-adrenergic receptor antagonist, reduced operant responding for alcohol (Gilpin and Koob, 2010). Moreover, increasing NE transmission via inactivation of presynaptic alpha-2 adrenergic autoreceptors potentiates reinstatement of EtOH self-administration, while activating this receptor attenuates EtOH drinking (Le et al., 2005). Furthermore, prazosin, an alpha-1 adrenergic receptor antagonist, reduces EtOH drinking in alcohol-dependent (Walker et al., 2008) and -preferring rats (Verplaetse et al., 2012), and reduces stress-induced reinstatement of alcohol seeking in rats (Le et al., 2011). In addition, chronic infusion of prazosin significantly decreased anxiety measures and ethanol self-administration in rats with relatively high levels of basal anxiety-like behavior (Skelly et al., 2014). Importantly, administration of prazosin in alcohol dependent individuals results in decreased alcohol consumption, suggesting that this may be a promising pharmacotherapy to treat alcoholism (Simpson et al., 2009). Thus it appears that reductions in NE signaling may decrease EtOH intake. Furthermore, it is possible that in the current SI model, the documented augmentation of EtOH intake is mediated, in part, by increased sensitivity of the noradrenergic system. A previous report showed an amphetamine-induced augmentation of NE response in the NAc of rats exposed to prenatal restraint stress (Silvagni et al., 2008) suggesting that chronic stress increases the sensitivity of the NE system. Consistent with the psychostimulant data, the current data show an increased NE response following EtOH (2 g/kg) in SI, but not GH rats. The current findings showing that EtOH (1 and 2 g/kg) does not significantly increase NE in GH rats are consistent with others showing no significant changes in NE levels in the NAc following acute EtOH administration in rats housed under standard conditions (Marinelli et al., 2003).

Dopamine and norepinephrine interactions

DA and NE increase in tandem following acute EtOH (2 g/kg) administration only in SI rats. Such DA and NE responses may be independent of each other or may result from an interaction between the two catecholaminergic systems. DA and NE terminals are both present in the NAc shell region (McKittrick and Abercrombie, 2007; Park et al., 2010). The NAc shell receives major NE innervation from the nucleus tractus solitarius (Delfs et al., 1998) and minor innervation from the LC (Berridge et al., 1997). Moreover, the norepinephrine transporter may be involved in the reuptake of DA in regions where NE and DA terminals are both present, such as the NAc shell (Carboni et al., 2006). More recently it has been shown that pre-synaptic α 1ARs in the NAc shell influence cocaine- and morphine-induced behavior and DA transmission (Mitrano et al., 2012). Additionally, blockade of alpha-1 adrenergic receptors inhibits amphetamine-induced locomotion (Dickinson et al., 1988). Therefore it is likely that the augmentation of EtOH-induced NE increases observed in the current data may influence DA release.

The VTA may serve as another source of DA and NE interactions. NE directly activates DA projection neurons from the VTA to the NAc via activation of α 1ARs. Blockade of α 1ARs attenuates the intra-VTA amphetamine-induced DA increases in the NAc (Pan et al., 1996). Local infusion of amphetamine in the VTA directly activates post-synaptic α 1ARs on DA neurons resulting in excitation (Paladini et al., 2001). EtOH activates DA projection neurons in the VTA (Brodie et al., 1999; Ding et al., 2009), and it is possible that this activation occurs, in part, as a result of increased NE transmission via α 1AR in the VTA. Therefore, elevated NE levels may drive the increase in DA following acute EtOH administration. Thus previous literature shows that the two catecholaminergic systems interact with each other and can modulate responsivity to stress and drugs of abuse. Alterations in the noradrenergic system as a result of chronic stress may increase EtOH-induced NE release in a dose dependent manner, further elevating DA transmission.

Chronic stress-induced sensitization to acute stressors and cross-sensitization to drugs of abuse

Chronic stress also causes cross-sensitization to substances of abuse (Kalivas and Stewart, 1991). Social isolation for an extended period of time during adolescence, a form of chronic early-life stress, results in an increased locomotor response (Lapiz et al., 2003) and increased DA release in the NAc (Fabricius et al., 2011) following amphetamine administration. Cocaine also causes a sensitized locomotor response in SI rats (Phillips et al., 1994). Therefore, similar to stimulants, it is likely that isolation-induced chronic stress results in increased sensitivity to EtOH. Present data showing increased DA responses to EtOH only in SI rats support this hypothesis. Studying the specific mechanisms involved in the augmentation of DA and NE response to acute EtOH (2 g/kg) in future studies could be important for establishing pharmacotherapies for alcoholism.

Alternative hypothesis: Neuroactive steroids

It is also possible that the sensitized effects of EtOH observed in SI animals may involve neuroactive steroids. For example, social isolation decreases the accumbal content of allopregnanolone, a neurosteroid synthesized from progesterone (Bortolato et al., 2011).

Depletion of allopregnanolone augments stress-induced DA release (Dazzi et al., 2002a). Moreover, animals with higher levels of allopregnanolone show an attenuation of DA response to EtOH compared to naïve animals (Dazzi et al., 2002b). Thus it is possible that the EtOH-induced augmented DA response observed in SI animals may in part be associated with depleted levels of allopregnanolone in SI animals.

Resiliency in GH animals

Although we have focused on the effects of social isolation stress, a recent study investigating differences in anxiety-like behavior and EtOH consumption in SI, GH, and “normal” adult animals procured from a standard commercial supplier showed that the normal adult animals have an anxiety-like and EtOH drinking phenotype comparable to age-matched SI rats (Chappell et al., 2013). It is possible that the catecholaminergic systems in GH rats develop resilience towards the effects of higher doses of EtOH due to the availability of increased social interaction. For example, most EtOH drinking studies are conducted in single housed rodents and these rats begin drinking during adolescence (Simms et al., 2008), which may influence the intake of EtOH such that there is an overall greater EtOH consumption. It is important to note that social isolation during adulthood does not alter NA DA signaling (Yorgason et al., 2013; Whitaker et al., 2013) or anxiety-like behaviors in Long Evans rats. Moreover, environmental enrichment decreases intake of ethanol in rodents (Rockman et al., 1983), and group housing is a form of environmental enrichment. This suggests that the EtOH (2 g/kg)-induced augmentation of NE and DA responses in SI rats and lack thereof in GH rats may reflect adaptive changes in both SI and GH rats.

Conclusions

In conclusion, the present findings demonstrated that both DA and NE in the NAc of SI rats have a sensitized response to EtOH (2 g/kg), presumably as a result of cross-sensitization to EtOH caused by chronic early-life stress. Importantly, these changes were likely due to pharmacodynamic alterations as no effects of social isolation on ethanol metabolism were observed. The current studies provide possible neural substrates that may contribute to the high co-occurrence of stress/anxiety and alcoholism. The sensitized EtOH-induced NAc DA and NE responses likely contribute to the increased EtOH intake observed in rodent models of early-life stress, as the NAc is involved in many aspects of addictive behaviors. These findings suggest that medications directed towards reducing DA and NE signaling may prove to be effective treatments of alcoholism, particularly in individuals exposed to early-life stress and/or suffering from a co-morbid anxiety disorder.

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Figure 1.

A schematic of the experimental paradigm. Male, Long Evans rats arrived at the facility on postnatal day (PD 21) and were maintained in group housing to acclimate for one week. On PD 28, half the rats were housed individually while the other half remained in group housing. The behavioral testing was conducted on PD 74 and microdialysis experiments were conducted between PD 80 and 110. EPM, elevated plus maze.

Figure 2.

Coronal sections showing microdialysis probe locations. Microdialysis probes were inserted in the NAc using the Paxinos and Watson (2007) rat atlas.

Figure 3.

An elevated plus maze (EPM) was used to phenotype for anxiety-like behavior in SI (n=17) and GH (n=15) rats. (A) Time spent on the open arm of the EPM. SI rats spent significantly less amount of time on the open arm compared to GH rats indicating increased anxiety-like behavior. (B) SI rats demonstrated fewer numbers of entries onto the open arm compared to GH rats exemplifying increased anxiety-like behavior. (C) No significant difference in number of closed arm entries was observed between SI and GH rats implying that locomotion was not affected due to the social isolation paradigm. GH, group housed; SI, socially isolated; **, $p < 0.01$.

Figure 4. Blood EtOH elimination curves. Blood samples collected from tails of SI (n=7) and GH (n=7) animals showed that there was no significant difference in the clearance of EtOH. GH, group housed; SI, socially isolated

Figure 5.

EtOH-induced DA response in the NAc of SI and GH rats. (A) Baseline levels of DA in SI (n=17) and GH (n=15) were not significantly different. DA concentrations were corrected for concentrations in the dialysate. (B) A significant positive correlation was observed between DA levels at baseline and anxiety-like behavior. Increased anxiety was associated with higher levels of DA. A significant positive correlation was observed within the GH animals, but no significant correlation was observed within the SI animals, potentially due to a floor effect for the number of open arm entries. (C) An EtOH dose comparison within the GH animals showed that the two doses of EtOH had comparable effects on DA release in the NAc. (D) An EtOH dose comparison within the SI animals demonstrated that at a higher dose (2 g/kg), acute EtOH resulted in an augmentation of the DA response in the NAc. (E) A two-way ANOVA between housing and dose comparison showed that social isolation results in an augmentation of EtOH-induced DA release only at a higher dose (2 g/kg) of EtOH and only in SI animals, confirming that both housing and dose affected DA responses to EtOH. (F) Saline comparison between GH and SI animals showed that saline did not significantly elevate DA levels in either group. SI, socially isolated; GH, group housed; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (1 g/kg vs. 2 g/kg); #, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$ (post-EtOH DA response vs. baseline). GH, group housed; SI, socially isolated. GH: 1 g/kg EtOH, n=9; 2 g/kg EtOH, n=8; saline, n=7. SI: 1 g/kg EtOH, n=9; 2 g/kg EtOH, n=9; saline, n=8.

Figure 6.

EtOH-induced NE response in the NAc of SI and GH animals. (A) Extracellular levels of NE at baseline in the NAc of SI (n=8) and GH (n=8) animals. Social isolation did not significantly alter the extracellular levels of NE in the NAc. NE concentrations were corrected for concentrations in the dialysate. (B) A dose comparison within the GH rats did not show a significant difference in 1 and 2 g/kg EtOH dose. (C) Acute EtOH (2 g/kg but not 1 g/kg) resulted in an augmentation of NE release in the SI animals. (D) A two-way ANOVA of dose and housing effects on the EtOH-induced NE response showed that a higher dose of EtOH (2 g/kg) induced increased NE response in the SI animals. (E) Saline comparison between GH and SI animals showed that saline administration did not significantly elevate NE levels in either group. SI, socially isolated; GH, group housed; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (1 g/kg vs. 2 g/kg); #, $p < 0.05$ (post-EtOH DA response vs. baseline). GH, group housed; SI, socially isolated. EtOH 1 and 2 g/kg: SI and GH, n=6 in all groups; saline: GH, n=6; SI, n=4.