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Dopamine synthesis in alcohol drinking-prone and -resistant mouse strains

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ABSTRACT

Alcoholism is a prevalent and debilitating neuropsychiatric disease, and much effort has been aimed at elucidating the neurobiological mechanisms underlying maladaptive alcohol drinking in an effort to design rational treatment strategies. In preclinical literature, the use of inbred mouse lines has allowed for the examination of ethanol effects across vulnerable and resistant phenotypes. C57BL/6J mice consistently show higher rates of ethanol drinking compared to most mouse strains. Conversely, DBA/2J mice display low rates of ethanol consumption. Given that the reinforcing and rewarding effects of ethanol are thought to be in part mediated by its actions on dopamine neurotransmission, we hypothesized that alcohol-preferring C57BL/6J and alcohol-avoiding DBA/2J mice would display basal differences in dopamine system function. By administering an L-aromatic acid decarboxylase inhibitor and measuring L-Dopa accumulation via high-performance liquid chromatography as a measure of tyrosine hydroxylase activity, we found no difference in dopamine synthesis between mouse strains in the midbrain, dorsal striatum, or ventral striatum. However, we did find that quinpirole-induced inhibition of dopamine synthesis was greater in the ventral striatum of C57BL/6J mice, suggesting increased presynaptic D2-type dopamine autoreceptor sensitivity. To determine whether dopamine synthesis or autoreceptor sensitivity was altered by a history of ethanol, we exposed C57BL/6J mice to one or two weekly cycles of chronic intermittent ethanol (CIE) exposure and withdrawal. We found that there was an attenuation of baseline dopamine synthesis in the ventral striatum after two cycles of CIE. Finally, we examined tissue content of dopamine and dopamine metabolites across recombinant inbred mice bred from a C57BL/6J × DBA/2J cross (BXD). We found that low dopaminergic activity, as indicated by high dopamine/metabolite ratios, was positively correlated with drinking. Together, these findings show differential autoreceptor effects on dopamine synthesis between C57BL/6J and DBA/2J mice, and suggest that decreased dopaminergic activity is associated with excessive drinking.

Introduction

Alcohol-use disorders are among the most prevalent and damaging neuropsychiatric disorders, resulting in over 100,000 deaths per year in the United States alone (McGinnis & Foege, 1999; SAMHSA, 2012), and intense efforts have been aimed at elucidating the neurobiological basis of alcohol-use disorders in an effort to identify effective treatment strategies. In this search, comparisons of inbred mouse strains with preference for or aversion to ethanol provide a means to study vulnerability to ethanol abuse and dependence. It is well documented that C57BL/6J (C57) and DBA/2J (DBA) mice display high- and low-ethanol drinking preference, respectively (Belknap, Crabbe, & Young, 1993; Meliska, Bartke, McGlacken, & Jensen, 1995; Mittleman, Van Brunt, & Matthews, 2003; Yoneyama, Crabbe, Ford, Murillo, & Finn, 2008). Despite higher intake and preference in ethanol drinking tasks in C57 mice, DBA mice show greater conditioned place preference for ethanol,

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greater ethanol-induced locomotion, locomotor sensitization to ethanol, and anxiety-like behaviors during ethanol withdrawal (Cunningham, Niehus, Malott, & Prather, 1992; McCool & Chappell, 2015; Phillips, Dickinson, & Burkhart-Kasch, 1994; Rose, Calipari, Mathews, & Jones, 2013). Thus, comparison of these two strains provides a powerful model for elucidating the pre-existing neurochemical underpinnings of differential responses to ethanol.

One area of particular interest is the dopamine system, as it is thought to play a role in ethanol reward and reinforcement. Years of research have highlighted the role of dopamine in regulating motivated behaviors, mood, and arousal as well as many other processes which are essential for organisms to perform advantageous behaviors (Schultz, 2007; Schultz, Dayan, & Montague, 1997). Further, much work has highlighted changes in this system as key factors in the acute effects of ethanol as well as long-term neuroadaptations which may contribute to alcoholism (Siciliano et al., 2015; Volkow et al., 1996, 2007). Although C57 and DBA mice have differential responses to ethanol, they do not differ in midbrain dopamine neuron firing rates (Brodie & Appel, 2000), or in basal dopamine levels in the nucleus accumbens (Kapasova & Szumlinski, 2008). However, given that the two strains exhibit different phenotypes for several dopamine-mediated behaviors, we sought to examine two aspects of dopamine system function that are known to play a role in ethanol actions: dopamine synthesis and D2-type autoreceptor regulation of the dopamine system.

Previous work from our lab and others has highlighted changes in dopamine release as well as the ability of presynaptic dopamine D2-type autoreceptors to regulate dopamine release as neuroadaptations induced by chronic ethanol exposure, and important factors in the development of excessive drinking behaviors (Dutton, Chen, You, Brodie, & Lasek, 2016; Karkhanis, Rose, Huggins, Konstantopoulos, & Jones, 2015; Narita, Soma, Tamaki, Narita, & Suzuki, 2002; Rossetti, Melis, Carboni, Diana, & Gessa, 1992; Siciliano, Calipri, Yorgason, Mateo, et al., 2016). One possible explanation for altered dopamine release following ethanol exposure is that ethanol dysregulates the dopamine synthesis process. Further, dopamine synthesis is tightly regulated by dopamine autoreceptors. While ethanol-induced changes in autoreceptor effects on dopamine release have been well studied, it is unknown if differences in autoreceptor regulation of dopamine synthesis affect ethanol consumption, or if this regulation is altered by chronic ethanol exposure. While dopamine metabolites have been shown to differ between C57 and DBA mice (Cabib & Puglisi-Allegra, 1991), it is unclear if synthesis is disparate between the two strains. Here we examined rates of dopamine synthesis and autoreceptor regulation of dopamine synthesis between DBA and C57 mice, as well as the effects of ethanol exposure. In addition, we performed an analysis of a genetic cross between these two mouse strains (BXD lines) to determine the contribution of striatal dopamine signaling to drinking behaviors.

Methods

Animals

Male C57BL/6J and DBA/2J mice were maintained on a 12:12 h light/dark cycle (3:00 AM lights on; 3:00 PM lights off) with food and water *ad libitum*. All animals were maintained according to the National Institutes of Health guidelines in Association for Assessment and Accreditation of Laboratory Animal Care accredited facilities. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Wake Forest School of Medicine.

HPLC analysis of tissue L-Dopa content

To measure rates of dopamine synthesis, mice were injected with the L-aromatic acid decarboxylase inhibitor 3-hydroxybenzylhydrazine (NSD-1015) (100 mg/kg, intraperitoneally [i.p.]) and γ-butyrolactone (GBL) (750 mg/kg, i.p.) (Jones et al., 1999; Walters & Roth, 1976). Autoreceptor regulation of dopamine synthesis was probed with a challenge dose of quinpirole (0.25 mg/kg). Quinpirole, NSD-1015, and GBL were injected at 50, 45, and 40 min prior to sacrifice and tissue dissection. NSD-1015 blocks the activity of L-aromatic acid decarboxylase to prevent the transformation of L-Dopa into dopamine, and GBL blocks dopamine neuron firing, to reduce extracellular levels to near zero and remove any tonic autoreceptor activation by dopamine. The magnitude of L-Dopa accumulation under these conditions is a reliable measure of maximal tyrosine hydroxylase activity. Mice were sacrificed and brains were removed and dissected for midbrain, ventral striatum, and dorsal striatum.

Tissue was dissected, snap-frozen, and samples were homogenized in 250 μL of 0.1 M $HClO_4$ and analyzed for protein concentration by the BCA method (Thermo Scientific). Extracts were centrifuged and the supernatants removed and analyzed for L-Dopa using HPLC coupled to electrochemical detection at +220 mV (ESA, Inc.) and separated on a Luna 50 × 2.0 mm C18 3 μm reverse-phase column (Phenomenex). The mobile phase consisted of 49.9 mM sodium dihydrogen phosphate, 200 μM EDTA, 9.9 mM sodium chloride, 0.2 mM octyl sulfate sodium salt, 100 mL methanol, 900 mL ultrapure water (pH 2.6). Analytes were quantified using PowerChrom software (eDAQ) and a calibration curve.

Ethanol-vapor chamber

For ethanol exposure experiments in C57 mice, a loading dose of 1 g/kg ethanol and the alcohol dehydrogenase inhibitor pyrazole (85 mg/kg) in 0.9% saline was administered i.p. to the mice prior to entering the ethanol-vapor inhalation chamber. Ethanol was delivered to the chamber by volatilizing 190 proof ethanol for 16 h followed by an 8 h period where only air was delivered. Animals were subjected to either one or two weekly cycles of ethanol exposure. Each cycle consisted of 16 h of ethanol exposure followed by 8 h of withdrawal for 4 days, followed by 3 days of withdrawal. The control group was treated identically to the ethanol group, except that they received a pyrazole injection alone and were placed in a chamber that received only air.

BXD experiment

Male and female mice representing 21 BXD recombinant inbred strains along with progenitor strains (C57 and DBA), F2 generation, were included in the design of the study. All these mice were obtained from R. Williams' lab at the University of Tennessee and were 12–16 weeks old upon arrival. Adult (10 weeks old upon arrival) C57 mice obtained from Jackson Labs (Bar Harbor, ME) served as the positive control condition (n = 8/group). The general study design typically involved one or two mice per experimental cell defined by genotype, sex, and group. Mice were individually housed with free access to food (Harland Teklad, Madison, WI) and water throughout all phases of the experiments. Body weights were recorded weekly during ethanol drinking weeks or daily during CIE or air exposure (detailed below). Mice were housed in a temperature- and humidity-controlled animal facility under a reversed 12 h light/dark cycle (lights on at 2:00 AM). Mice were not food- or water-deprived at any time during the study. All procedures were approved by the Medical University of South Carolina Institutional Animal Care

and Use Committee and followed the NIH Guide for the Care and Use of Laboratory Animals (8th edition, National Research Council, 2011).

Mice were individually housed and were allowed to drink ethanol (15% v/v vs. water) for 2 h each day starting 30 min before lights off (Becker & Lopez, 2004; Griffin, Lopez, & Becker, 2009; Griffin, Lopez, Yanke, Middaugh, & Becker, 2009; Lopez & Becker, 2005). The study involved first recording baseline levels of intake for 6 weeks. Mice representative of each strain were then separated into two groups to be exposed to either weekly cycles of CIE exposure (CIE group) or air-control exposure (air group) as described below. Seventy-two hours after CIE exposure (or air-control exposure), mice resumed ethanol drinking for 5 consecutive days. This pattern of CIE or air-control exposure followed by 5 days of ethanol self-administration was repeated for four cycles. A fifth cycle of CIE exposure (or air exposure) followed the last ethanol intake evaluation, and mice were sacrificed for tissue collection at the 72 h withdrawal time point. Dorsal striatum samples were then analyzed via HPLC for dopamine and dopamine metabolite concentrations. Correlations were performed between baseline drinking rates (average intake over the 6 weeks of baseline consumption) and dopamine tissue content values. Complete drinking data from these experiments were reported by Lopez, Miles, Williams, and Becker (2016).

For tissue content analysis in BXD mice, the caudate was microdissected by hand and flash-frozen in liquid nitrogen. Tissue was stored at −80 °C until the time of analysis. Samples were homogenized in 250 μL of 0.1 M $HClO_4$ and analyzed for protein concentration by the BCA method (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific, Inc., Rockford, IL). Extracts were centrifuged and the supernatants were removed and analyzed for dopamine and its metabolites using HPLC. The mobile phase consisted of 50 mM citric acid, 90 mM sodium dihydrogen phosphate, 1.7–2.0 mM 1-octanesulfonic acid, 50 μM ethylenediaminetetraacetic acid, 10–12% acetonitrile, and 0.3% triethylamine in a volume of 1 L (pH 3.0). All other aspects of detection and analysis were identical to L-Dopa methods.

Statistics

All comparisons of C57 to DBA groups as well as CIE to air groups comparisons were made using two-tailed Student's *t* tests. Within-strain comparisons across regions were made using a one-way analysis of variance (ANOVA), and differences between groups were tested using a Bonferroni *post hoc* test. Spearman's correlation coefficients were used to test the strength of tissue content to drinking relationships across BXD strains. All p values < 0.05 were considered to be statistically significant.

Results

Autoreceptor-induced inhibition of dopamine synthesis is greater in the ventral striatum of C57 mice

To examine differences in dopamine synthesis, animals were injected with the L-aromatic acid decarboxylase inhibitor NSD-1015, which blocks the production of dopamine from L-Dopa and instead allows accumulation of L-Dopa. Because tyrosine hydroxylase is the rate-limiting enzyme responsible for the production of dopamine, and is feedback-inhibited by dopamine, the degree of this accumulation is a measure of maximal tyrosine hydroxylase activity and thereby maximal dopamine synthesis rate. Animals were also injected with GBL to remove any differences in dopamine tone that could influence synthesis rates. GBL prevents dopamine cell firing, and thus dopamine release, allowing for the pharmacologic isolation of autoreceptor activity separately from any possible differences in endogenous dopamine activation of autoreceptors (Jones et al., 1999; Walters & Roth, 1976). To measure autoreceptor actions on dopamine synthesis, a separate group of animals was injected with quinpirole in addition to NSD-1015 and GBL. It is

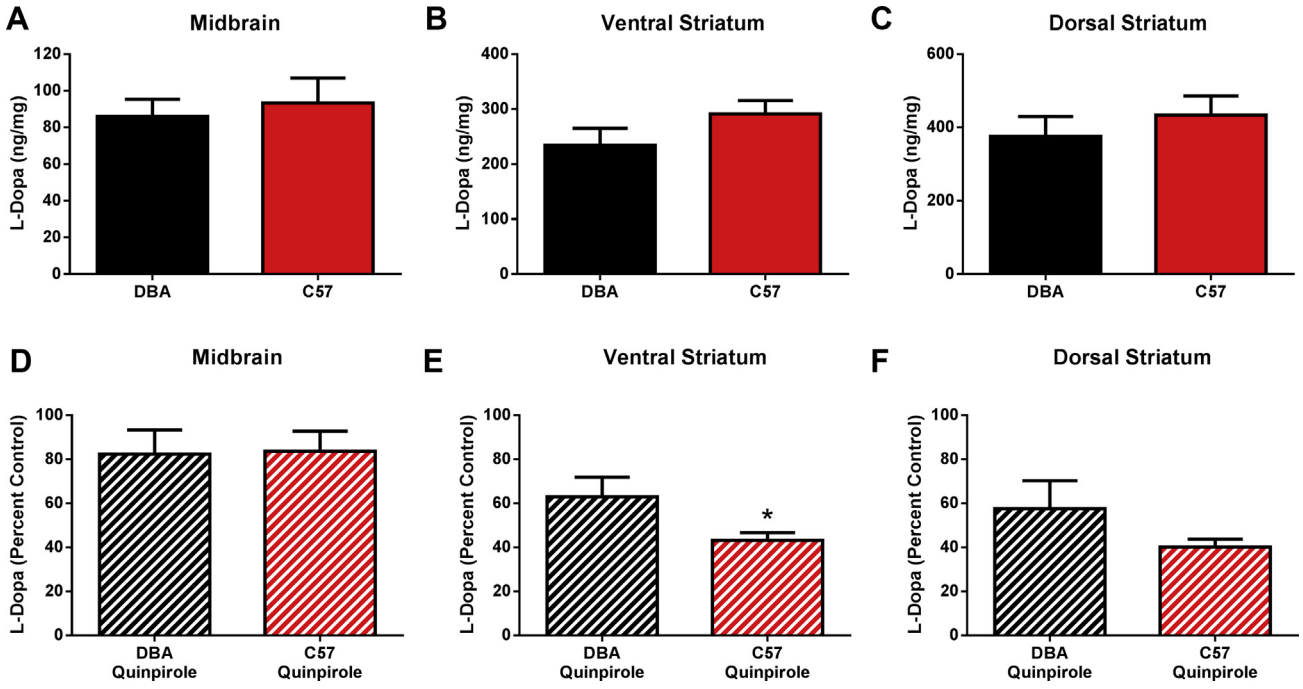

Fig. 1. Autoreceptor inhibition of dopamine synthesis is greater in C57 than DBA mice. (A) Midbrain L-Dopa accumulation (dopamine synthesis) did not differ between strains. (B) In the ventral striatum we found no difference in dopamine synthesis between strains. (C) No difference in dopamine synthesis in the dorsal striatum between strains. (D) Autoreceptor inhibition of dopamine synthesis in the midbrain is similar between C57 and DBA mice. (E) Autoreceptor control of dopamine synthesis is greater in the ventral striatum of C57 mice as compared to DBA mice. (F) No effect of strain on autoreceptor regulation of dopamine synthesis in the dorsal striatum. $^*p < 0.05$.

important to measure autoreceptor actions separately from changes in dopamine levels, as basal dopamine is decreased by ethanol exposure (Rossetti, Isola, De Vry, & Fadda, 1999; Rossetti et al., 1992).

We found that in samples taken from the midbrain of C57 and DBA mice, there was no difference in baseline dopamine synthesis (Fig. 1A). Similarly, we found no differences in dopamine synthesis in the ventral (Fig. 1B) or dorsal striatum (Fig. 1C) between C57 and DBA mice. In the midbrain, quinpirole-induced inhibition of dopamine synthesis did not differ between strains (Fig. 1D). However, C57 mice exhibited increased quinpirole-induced inhibition of dopamine synthesis in ventral striatum as compared to DBA mice (Fig. 1E; *t* test: $t_{21} = 2.126$, $p < 0.05$), demonstrating that autoreceptor regulation of terminal dopamine synthesis in this area is greater in C57 mice. In dorsal striatum, quinpirole-induced inhibition of dopamine synthesis did not differ between strains (Fig. 1F).

We then compared between regions to determine if there were differences in dopamine synthesis rates within each strain. We found that in C57 mice, dopamine synthesis increased in a stepwise fashion with the lowest rates in the midbrain and the highest in the dorsal striatum (Fig. 2A; one-way ANOVA: $F[2,33] = 25.13$, $p < 0.0001$). Quinpirole-induced inhibition of dopamine synthesis did not differ between ventral and dorsal striatum, although both were greater than in the midbrain (Fig. 2B; one-way ANOVA: $F[2,33] = 16.20$, $p < 0.0001$). DBA mice exhibited the same topography of baseline dopamine synthesis rates as C57 mice, with the lowest in the midbrain and highest in the dorsal striatum (Fig. 2C; one-way ANOVA: $F[2,32] = 14.60$, $p < 0.0001$). Unlike C57s, DBAs showed no differences in quinpirole-induced inhibition of dopamine synthesis across regions (Fig. 2D).

Two cycles of chronic intermittent ethanol (CIE) exposure decreased dopamine synthesis in the ventral striatum of C57 mice

Given that C57 mice show greater autoreceptor sensitivity in the ventral striatum, a trait that is associated with ethanol use (Siciliano, Calipari, Yorgason, Lovinger, et al., 2016), we next sought to determine the effects of a history of ethanol exposure on dopamine synthesis and autoreceptor sensitivity in C57 mice. Following exposure to one cycle of ethanol (4 days of 16 h ethanol vapor/8 h of withdrawal followed by 3 days of withdrawal), we found that there was no change in dopamine synthesis in the ventral striatum (Fig. 3A). However, there was a trend toward a decrease in synthesis in the dorsal striatum (Fig. 3B; *t* test: $t_7 = 1.88$, $p < 0.10$). Quinpirole-induced attenuation of dopamine synthesis was not altered by CIE in the ventral (Fig. 3C) or dorsal (Fig. 3D) striatum.

We then exposed a second group of C57 mice to two cycles of CIE. Following two cycles of exposure we found that dopamine synthesis was unchanged in the midbrain of ethanol-exposed animals as compared to air-exposed controls (Fig. 4A). However, we found that CIE decreased dopamine synthesis in the ventral striatum as compared to air-exposed controls (Fig. 4B; *t* test: $t_{18} = 1.947$; $p < 0.05$). The synthesis rate was unchanged in the dorsal striatum between ethanol and control animals (Fig. 4C). Although CIE decreased quinpirole-induced inhibition of dopamine synthesis in the midbrain (Fig. 4D; *t* test: $t_{13} = 2.488$, $p < 0.05$), we found no change in sensitivity to

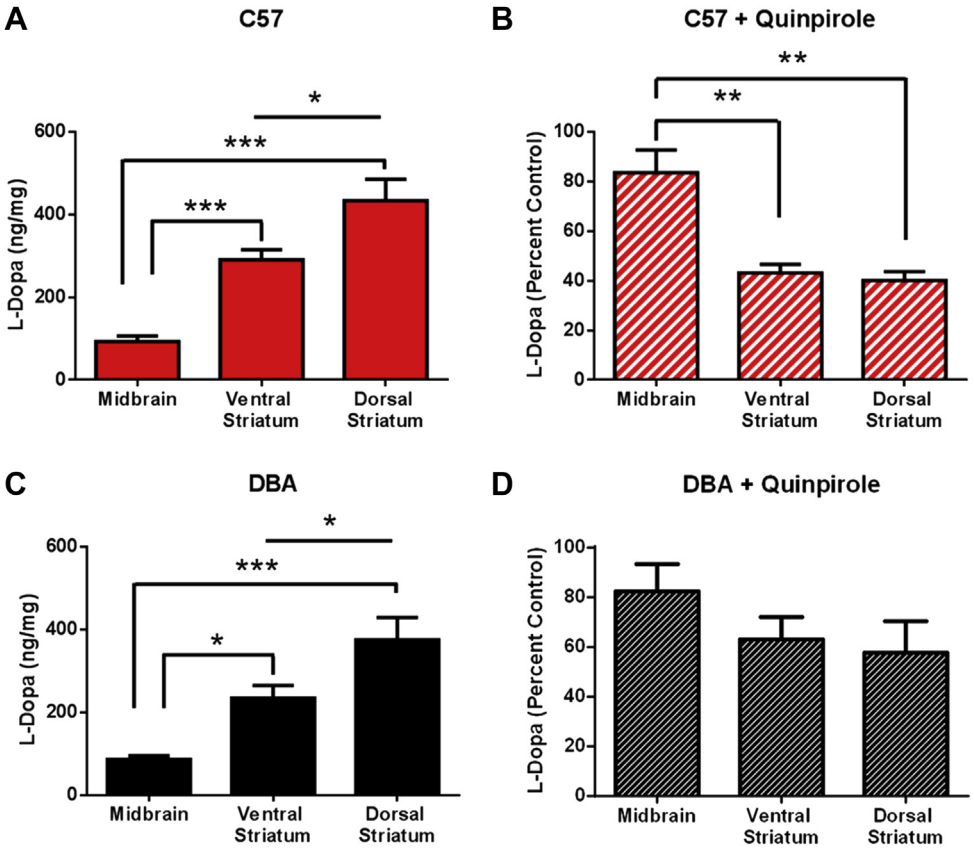

Fig. 2. Autoreceptor inhibition varies across region in C57 but not DBA mice. (A) Rate of dopamine synthesis varies across region in C57 mice. (B) In C57 mice, quinpirole-induced inhibition of dopamine synthesis is greater in the ventral and dorsal striatum as compared to midbrain. (C) Similar to C57 mice, DBA mice show differential dopamine synthesis rates across regions. (D) Unlike C57 mice, quinpirole does not have differential effects on dopamine synthesis rates across regions in DBA mice. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

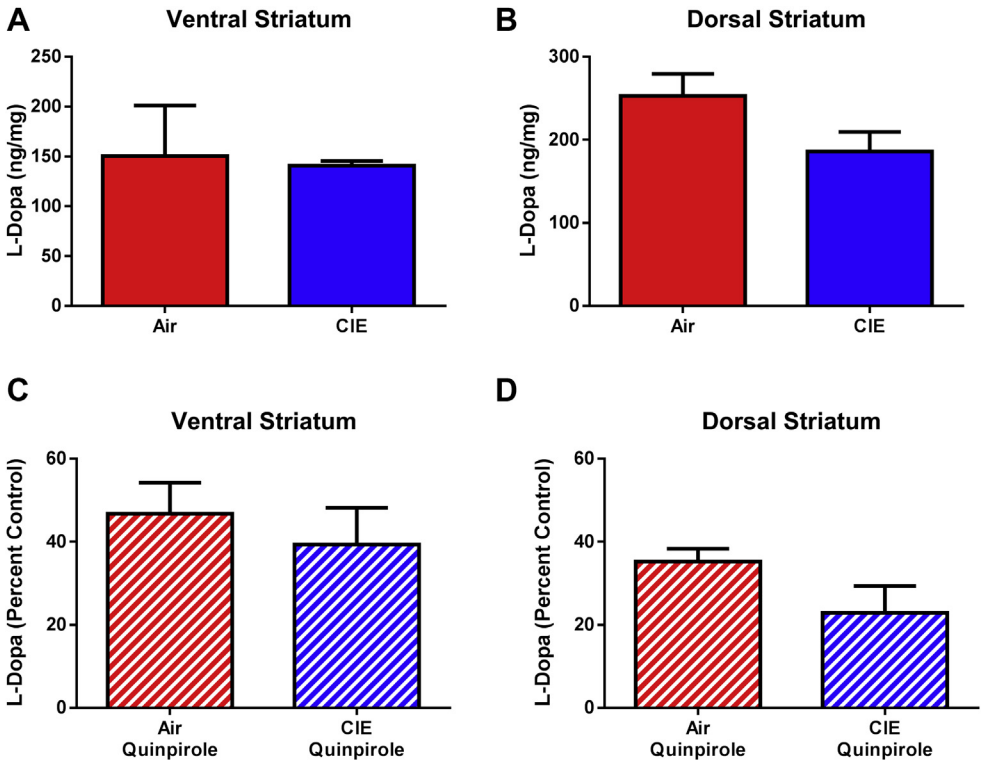

Fig. 3. One cycle of ethanol exposure and withdrawal does not alter dopamine synthesis or quinpirole-induced attenuation of dopamine synthesis. (A) No change in L-Dopa accumulation in the ventral striatum between air- and ethanol-exposed groups of C57 mice. (B) There was a non-significant trend ($p < 0.10$) toward decreased dopamine synthesis in the dorsal striatum of CIE mice as compared to air controls. (C) Quinpirole-induced reductions in L-Dopa accumulation in the ventral striatum were unchanged by ethanol exposure. (D) Quinpirole effects were unchanged in the dorsal striatum.

quinpirole in either the ventral (Fig. 4E) or dorsal (Fig. 4F) striatum of C57 mice exposed to two cycles of ethanol vapor and withdrawal.

Dopamine and metabolite tissue content levels are related to pre-CIE drinking across BXD mouse lines

Having elucidated dopamine synthesis rates between C57 and DBA mice, as well as ethanol-induced changes in dopamine synthesis rate in C57 mice, we next sought to determine how changes in dopamine tissue content and that of dopamine metabolites may relate to ethanol abuse vulnerability across genetically diverse mouse lines. To address this question, we took advantage of the many available BXD mouse lines. BXD mice are a recombinant inbred set of mice produced from a C57 × DBA cross. Here we allowed pairs of mice from across BXD mouse strains and allowed them to drink on a 2-bottle choice procedure for 6 weeks. Following baseline drinking, animals were exposed to 5 weeks of CIE and 2-bottle choice drinking while a second group was exposed only to air. Following vapor exposure and drinking, animals were killed and dorsal striatum tissue was harvested and analyzed for dopamine and dopamine metabolite tissue content via HPLC. Importantly, we found a positive correlation between dopamine content and pre-CIE drinking (baseline, averaged across the 6 weeks) across all of the strains (Fig. 5A; $r = 0.56$, $p < 0.01$). Further, we found that the dopamine/metabolite ratio was positively correlated with baseline drinking for the dopamine/3,4-Dihydroxyphenylacetic acid (DOPAC) ratio (Fig. 5B; $r = 0.53$, $p < 0.01$), and there was a strong trend toward a correlation between drinking and dopamine/homovanillic acid (HVA) ratios (Fig. 5C; $r = 0.39$, $p < 0.06$).

Discussion

Here we show that while C57 and DBA mice, two genetically distinct mouse lines with differential ethanol preference, do not differ in dopamine synthesis rates. However, C57 mice have greater autoreceptor regulation of dopamine synthesis in the ventral striatum. Further, we found that while both strains showed graded dopamine synthesis rates across regions (rank order from highest to lowest: dorsal striatum, ventral striatum, midbrain), C57 mice had greater autoreceptor sensitivity in the dorsal and ventral striatum as compared to the midbrain, while DBA mice did not display differential sensitivity across regions. We then explored the effects of CIE exposure on dopamine synthesis and D2-type autoreceptor regulation of dopamine synthesis in C57 mice, and found that neither measure was altered in any region tested following one cycle/week of 16 h of ethanol-vapor exposure and 8 h of withdrawal. In contrast, following two cycles of CIE in C57 mice, we found that dopamine synthesis was decreased in the ventral striatum, and autoreceptor regulation of dopamine synthesis was decreased in the midbrain. Together, these findings suggest that increased inhibitory autoregulation of striatal dopamine synthesis may play a role in the greater ethanol preference of C57 mice as compared to DBA mice. Further, these data suggest a role for changes in striatal dopamine synthesis and midbrain autoregulation of synthesis in the neurochemical adaptions induced by ethanol exposure.

The first finding of this study was that C57 and DBA mice, which vary greatly in their preference for ethanol, do not have differential basal rates of dopamine synthesis in any region tested. This is consistent with previous results demonstrating no differences in electrically evoked dopamine release in the nucleus accumbens of C57 or DBA mice (Rose et al., 2013). However, we found that in the

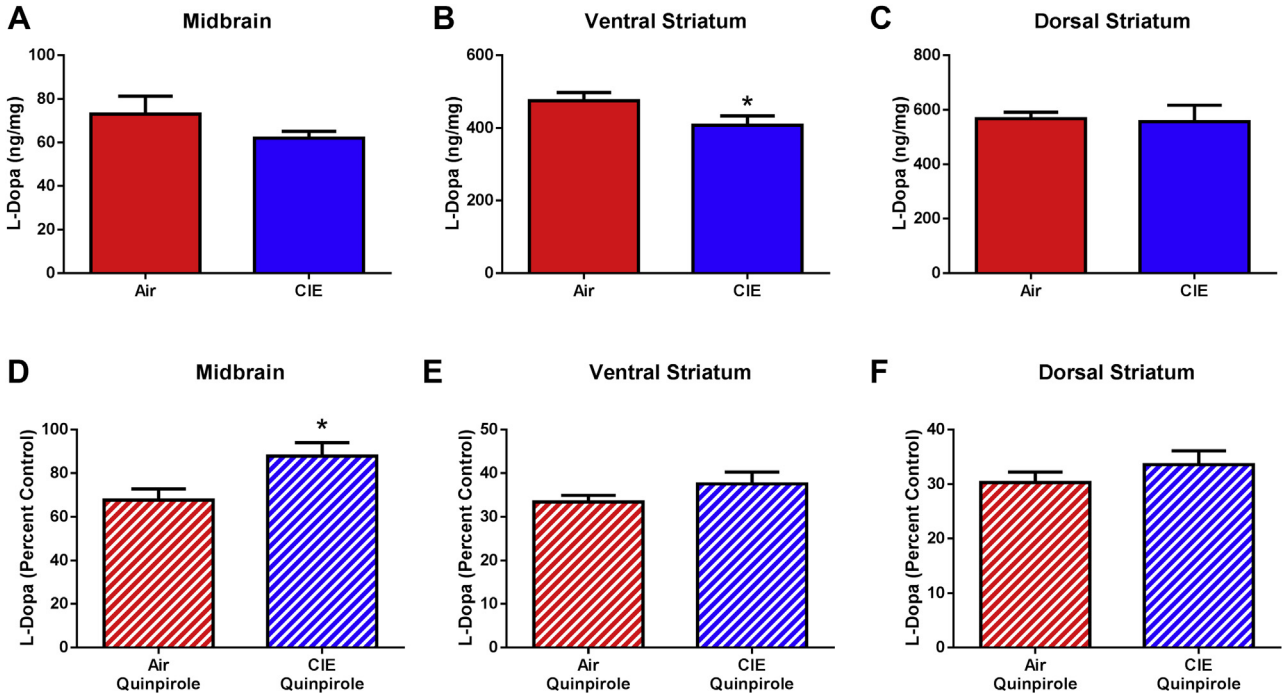

Fig. 4. Two cycles of ethanol exposure and withdrawal decreased dopamine synthesis in the ventral striatum. (A) Baseline dopamine synthesis is unchanged by two cycles of ethanol exposure and withdrawal in the midbrain of C57 mice. (B) Dopamine synthesis is reduced by two cycles of ethanol exposure and withdrawal in the ventral striatum, and unchanged in the dorsal striatum (C). (D) Quinpirole-induced inhibition of dopamine synthesis is decreased in the midbrain of ethanol-exposed mice as compared to air-exposed controls. In contrast, quinpirole-induced decreases in dopamine synthesis are unaffected by two cycles of ethanol exposure and withdrawal in the ventral (E) and dorsal (F) striatum. $^{*}p < 0.05$.

ventral striatum of C57 mice, autoreceptor regulation of dopamine synthesis was greater than in DBA mice. We have shown previously that increased autoreceptor sensitivity in the nucleus accumbens, a part of the ventral striatum, is concomitant with ethanol exposure and excessive ethanol drinking (Budygin et al., 2003; Karkhanis et al., 2015). We have hypothesized that this increased inhibitory feedback onto dopamine terminals may result in a hypo-dopaminergic state (Budygin et al., 2003; Karkhanis et al., 2015; Siciliano, Calipari, Yorgason, Lovinger, et al., 2016) which has been linked to anhedonia (Kokkinidis & McCarter, 1990) and may cause increased ethanol intake to alleviate these symptoms (Koob, 2013). Thus, heightened autoreceptor control of dopamine at baseline in this region, as shown in the current study, could contribute to greater ethanol drinking in C57 mice (Belknap et al., 1993; Meliska et al., 1995; Mittleman et al., 2003; Yoneyama et al., 2008). It is also possible that dampened inhibitory autoreceptor feedback in DBA mice may be a factor in increased locomotor-activating effects of ethanol (Rose et al., 2013), and increased ethanol-induced locomotor sensitization in DBA mice as compared to C57 mice (Phillips et al., 1994). Because differences in drinking between C57 and DBA mice have been suggested to stem at least in part from a disparate perceived taste of ethanol (Blizard, 2007; McCool & Chappell, 2012), further work will be needed to elucidate the contribution of autoreceptor regulation of dopamine synthesis to differential ethanol drinking behaviors.

We have shown previously that dopamine release, as measured with fast-scan cyclic voltammetry, is reduced following three (Karkhanis et al., 2015) or five (Rose et al., 2015) cycles of CIE in the nucleus accumbens of C57 mice. Further, CIE concomitantly increases autoreceptor regulation of dopamine release (Karkhanis et al., 2015). We show here that biosynthesis of dopamine is decreased following two cycles of CIE, which suggests that

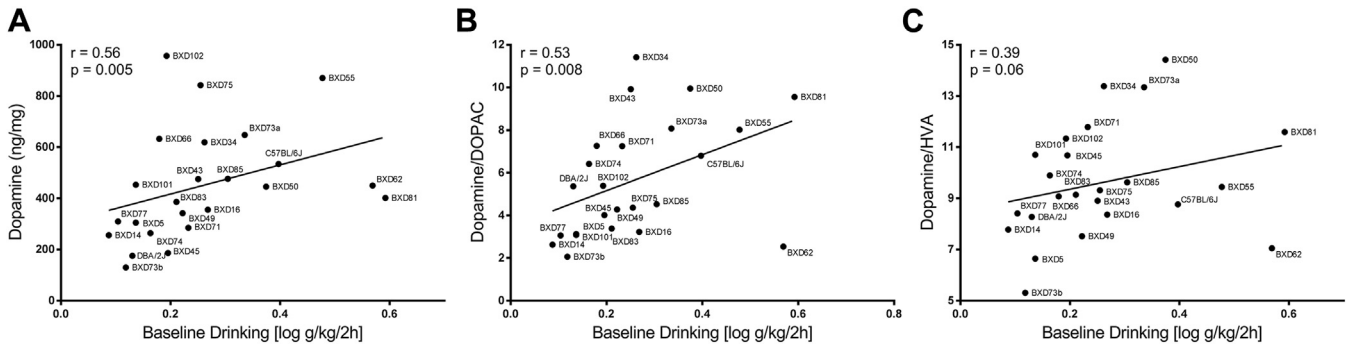

Fig. 5. Low dopamine activity is associated with high ethanol intake across BXD strains. (A) Dopamine tissue content is positively correlated with ethanol intake during a 2-bottle choice test. (B) High dopamine/DOPAC ratio, indicative of low dopamine activity, is associated with high ethanol intake during a 2-bottle choice test. (C) Similarly, there was a trend toward a correlation between dopamine/HVA ratio and drinking.

decreased exocytotic release may stem from chronic ethanol-induced inhibition of tyrosine hydroxylase activity. Ethanol increases dopamine synthesis and basal dopamine levels acutely (Carlsson & Lindqvist, 1973; Imperato & Di Chiara, 1986). Thus, decreases after chronic ethanol may be a compensatory mechanism. In agreement with this hypothesis, we observed ethanol-induced decreases in dopamine synthesis only in the ventral striatum, where acute ethanol produced a greater increase in dopamine levels, as compared to more dorsal striatal regions (Imperato & Di Chiara, 1986). Importantly, dopamine release is greatly attenuated in human alcoholics, and this effect is most prominent in the ventral striatum (Volkow et al., 2007).

In contrast to Karkhanis et al. (2015), we found no effect of CIE on striatal autoreceptor sensitivity, and instead saw a decrease in sensitivity in the midbrain of C57 mice following two cycles of ethanol vapor. This disparity may indicate that autoreceptor regulation of dopamine synthesis and dopamine release are differentially affected by ethanol exposure, whereby autoreceptor effects on release are augmented and effects on synthesis are unchanged or decreased, depending on region. A second possibility is that the ethanol effects on autoreceptor sensitivity are not present until after at least three cycles of CIE, as shown previously (Karkhanis et al., 2015). Accordingly, it has been suggested that ethanol effects on autoreceptor sensitivity are exposure length-dependent, as there is no change in autoreceptor sensitivity after 6 months of voluntary ethanol self-administration in nonhuman primates (Siciliano, Calipri, Yorgason, Mateo, et al., 2016), but sensitivity is increased after 12 months (Siciliano, Calipari, Yorgason, Lovinger, et al., 2016) or 18 months (Budygin et al., 2003) of daily ethanol drinking. Future work will be aimed at determining the effects of prior ethanol exposure on autoreceptor regulation of dopamine synthesis in DBA mice.

In addition to ethanol-induced changes in dopamine synthesis in C57 mice, we examined dopamine tissue content across BXD mouse lines, which are genetically well-annotated recombinant inbred mice bred from a C57 × DBA cross. Tyrosine hydroxylase activity is positively linked to ongoing dopaminergic activity, and newly synthesized dopamine is a main component of the readily releasable pool of dopamine (Dadalko et al., 2015; Pruett & Salvatore, 2013; Salvatore, 2014). On the other hand, tissue content of dopamine has been suggested to negatively correlate with activity, with more storage in tissue indicating that there is less usage of dopamine overall. Many studies have compared the ratio of dopamine to DOPAC or HVA in tissue to estimate dopamine system activity, where higher dopamine numbers relative to metabolites are taken to indicate lower activity (Church, Adams, & Wyss, 2014; Sanghera et al., 1990). We find that, indeed, striatal dopamine tissue content is positively correlated with pre-CIE drinking, which suggests that there may be a causal link between these two variables. Further, dopamine/DOPAC ratios were positively correlated with drinking across the mouse strains, suggesting that differential dopamine system activity across strains may be a predictor of excessive ethanol intake. Thus we hypothesize that decreased dopaminergic activity, indicated by decreased synthesis and increased dopamine tissue content, is linked to lower ongoing dopamine system activity and leads to greater drinking behavior in an attempt to combat anhedonia associated with low dopamine function.

The findings reported here demonstrate a role for autoregulation of dopamine synthesis in the neurochemical disparities that may underlie differential responsiveness to ethanol between C57 and DBA mice. Further, these data highlight changes in dopamine synthesis rate as a neurochemical adaptation induced by chronic ethanol exposure and withdrawal, and suggest that changes in dopamine synthesis may contribute to ethanol-induced alterations to dopamine release which have been previously linked to excessive drinking behaviors. Ethanol-induced decreases in dopamine synthesis add to growing evidence from the clinical and preclinical literature indicating that hypofunction of dopamine neurons is a critical component in the development of aberrant drinking behaviors (Rose et al., 2015; Siciliano, Calipari, Yorgason, Lovinger, et al., 2016; Volkow et al., 1996, 2007). In further support of this hypothesis, we found that across BXD mouse strains, there is a strong association between drinking and low dopamine activity. Together, these data expand on the dopaminergic basis of the acute and chronic actions of ethanol which converge to drive aberrant ethanol drinking and other ethanol-related behaviors.

Acknowledgments

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