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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