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Chronic intermittent ethanol exposure reduces presynaptic dopamine neurotransmission in the mouse nucleus accumbens

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ABSTRACT

Background: Increasing evidence suggests that chronic ethanol exposure decreases dopamine (DA) neurotransmission in the nucleus accumbens (NAc), contributing to a hypodopaminergic state during withdrawal. However, few studies have investigated adaptations in presynaptic DA terminals after chronic intermittent ethanol (CIE) exposure. In monkeys and rats, chronic ethanol exposure paradigms have been shown to increase DA uptake and D2 autoreceptor sensitivity.
Methods: The current study examined the effects of ethanol on DA terminals in CIE exposed mice during two time-points after the cessation of CIE exposure. DA release and uptake were measured using fast scan cyclic voltammetry in NAc core slices from C57BL/6J mice, 0 h and 72 h following three weekly cycles (4 days of 16 h ethanol vapor/8 h room air/day + 3 days withdrawal) of CIE vapor exposure.
Results: Current results showed that DA release was reduced, uptake rates were increased, and inhibitory D2-type autoreceptor activity was augmented following CIE exposure in mice.
Conclusions: Overall, these CIE-induced adaptations in the accumbal DA system reduce DA signaling and therefore reveal several potential mechanisms contributing to a functional hypodopaminergic state during alcohol withdrawal.

1. Introduction

In the nucleus accumbens (NAc), acute administration of ethanol elevates dopamine (DA) levels (Yim et al., 1998, 2000; Tang et al., 2003), an effect that has been implicated in its rewarding properties (Wise, 2004). As with many drugs of abuse, chronic or repeated exposure to ethanol produces compensatory neuroadaptations that oppose acute drug effects, normalizing function during intoxication, but resulting in below-normal function in the absence of the drug (Koob, 2003). Following chronic ethanol exposure, the mesolimbic DA system is fundamentally hypoactive. For example, dopaminergic neurons in the VTA of ethanol-dependent rats exhibit reduced tonic and burst firing rates (Diana et al., 1993; Bailey et al., 2001; Shen, 2003; Shen et al., 2007) during withdrawal. Animals exposed to chronic ethanol also have decreased expression of tyrosine hydroxylase (TH) coupled with augmented levels of DA transporter (DAT) in the striatum (Rothblat et al., 2001; Healey et al., 2008), consistent with an attenuation in DA signaling. Together, these observations suggest that there are multiple functional changes in DA regulation that reduce signaling during ethanol withdrawal. However, to date, there have been no studies examining the changes in DA uptake in the NAc of a mouse model of ethanol dependence.

In addition to DATs, presynaptic DA signaling is also regulated by D2 autoreceptors which inhibit DA release (Phillips et al., 2002) and synthesis (Wolf and Roth, 1990) while facilitating DA uptake (Wu et al., 2002; Garris et al., 2003). Previous studies have revealed sensitization of D2 autoreceptors following chronic exposure to ethanol. For example, chronic ethanol intake in monkeys results in supersensitivity of release-regulating D2 autoreceptors (Budygin et al., 2003). In rats, chronic oral ethanol intake has been shown to increase total (pre- and post-synaptic) D2 receptor density in the NAc (Kim et al., 1997; Djouma and Lawrence, 2002; Sari et al., 2006). However, a previous study from our lab showed that 10 days of intermittent ethanol exposure (CIE) in rats did not affect functional D2 autoreceptor sensitivity (Budygin et al., 2007). In mice, sensitizing regimens of intraperitoneal ethanol injections have been shown to increase the function of D2 receptors in the NAc

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(Souza-Formigoni et al., 1999; Abrahao et al., 2012). Although there is evidence for supersensitivity of pre- and post-synaptic D2 receptors following chronic ethanol exposure, the goal of the current study, was to determine the changes specifically in presynaptic D2 autoreceptor function following repeated CIE exposure in mice.

A CIE vapor exposure paradigm for mice was developed by Becker and colleagues as a means to quantify behavioral and biochemical changes associated with chronic ethanol exposure (Becker and Lopez, 2004; Lopez and Becker, 2005, Griffin et al., 2009). For example, in C57 mice, this CIE exposure/withdrawal paradigm produces: (1) behavioral sensitization to ethanol (Zapata et al., 2006), (2) handling-induced seizures during withdrawal (Becker and Hale, 1993; Metten et al., 2010), (3) increased ethanol drinking (Becker and Lopez, 2004; Lopez and Becker, 2005; Griffin et al., 2009) and (4) blunted DA responses to ethanol during withdrawal (Zapata and Shippenberg, 2006; Zapata et al., 2006). Over the past decade, the ethanol field has used C57 mice as a model of dependence to study the neurobiological and behavioral effects of chronic exposure to ethanol due to their propensity to voluntarily consume large amounts of ethanol (Yoneyama et al., 2008). However, while the behavioral effects of CIE are well documented, studies investigating the neurobiological underpinnings of these behavioral manifestations are limited, especially in the NAc (Zapata and Shippenberg, 2006; Zapata et al., 2006; Jeanes et al., 2011; Griffin et al., 2014).

In the present study, *ex vivo* fast-scan cyclic voltammetry was used to examine the effects of repeated CIE exposure on presynaptic DA release and uptake in the core of the NAc, as well as changes in the functional sensitivity of D2 autoreceptors, at two different times after the cessation of CIE exposure (0 h and 72 h time-points). The primary goal of these experiments was to identify CIE-induced changes in presynaptic DA signaling that may underlie reduced DA system function in the NAc during withdrawal in C57 mice.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (8–12 weeks; Jackson Laboratories, Bar Harbor, ME) were used for all experiments. Animals were housed in groups of three to four per cage with food and water *ad libitum* (12-h light-dark cycle). Experimental protocols adhered to National Institutes of Health Animal Care guidelines and were approved by the Wake Forest University Institutional Animal Care and Use Committee.

2.2. CIE and withdrawal

The general design of the repeated ethanol exposure and withdrawal paradigm was adapted from Becker and colleagues (Becker, 1994; Becker et al., 1997) with the following modifications. Mice were assigned to either control or ethanol exposure groups. The ethanol group underwent 16 h of continuous ethanol vapor exposure followed by 8 h off in room air each day for four days, followed by three days of abstinence (1 cycle; Fig. 1A). This procedure was repeated three times for a total of 3 cycles. A loading dose of 1.0 g/kg ethanol (20% w/v, CIE group) or saline (Air group) and the alcohol dehydrogenase inhibitor, pyrazole (85 mg/kg) in 0.9% saline was administered i.p. prior to entering the ethanol vapor or air inhalation chamber. Ethanol vapor was delivered to the ethanol inhalation chamber by volatilizing 190 proof ethanol and mixing the ethanol vapor with fresh air at a rate of 10 L/min. The concentration of ethanol in the chamber was monitored at the completion of each cycle by taking a 2 mL air sample through a port in the chamber door.

2.3. Blood ethanol concentrations

Blood ethanol concentrations (BEC) were measured at the end of each cycle to ensure physiologically and behaviorally relevant BECs (Griffin et al., 2009). Blood samples were collected from the mice using the submandibular vein punch immediately after removal from the ethanol vapor chamber. Blood samples (5 μL) were mixed with 0.38 N trichloroacetic acid (10 μL) and centrifuged at 12,000 g. The supernatant was used in a spectrophotometric enzymatic assay (229-29; Sekisui Diagnostics LLC, Lexington, MA). The mean BEC for the mice exposed to three cycles of the ethanol vapor was 185.1 ± 11.3 mg/dl (mean ± SEM; Fig. 1B).

2.4. Tissue preparation

Immediately upon completion of the vapor exposure (or following 72 h of abstinence), mice were sacrificed by decapitation and brains were rapidly removed and transferred into ice-cold, pre-oxygenated (95% O_2/5% CO_2) artificial cerebral spinal fluid (aCSF) consisting of (in mM): NaCl (126), KCl (2.5), NaH_2PO_4 (1.2), $CaCl_2$ (2.4), $MgCl_2$ (1.2), $NaHCO_3$ (25), glucose (11), L-ascorbic acid (0.4) and pH was adjusted to 7.4. The brain was sectioned into 400 μm-thick coronal slices containing the striatum with a vibrating tissue slicer (Leica VT1000S, Vashaw Scientific, Norcross, GA) and transferred to a submersion recording chamber perfused at 1 ml/min at 32 °C with oxygenated aCSF.

2.5. Fast scan cyclic voltammetry

Following an equilibration period (30 min), a carbon fiber microelectrode (approximately 150 μM length, 7 μM radius) and a bipolar stimulating electrode (Plastics One, Roanoke, VA) were placed in close proximity to each other (approximately 100 μM apart) into the NAc core. DA was evoked by a single, rectangular, electrical pulse (350 μA, 4 ms) applied every 5 min. Changes in current were monitored every 100 ms using fast-scan cyclic voltammetry (Kennedy et al., 1992) by applying a triangular waveform (−0.4 to +1.2 to −0.4 V vs. Ag/AgCl, 400 V/s). One slice was used per animal per experiment. Once the extracellular DA response was stable (variation between peak height data did not exceed 10%), quinpirole, a D2/D3 autoreceptor agonist, was added cumulatively to the bath (0.1–1.0 μM). Immediately following the completion of each experiment, recording electrodes were calibrated by recording their response (in current; nA) to 3 μM DA in aCSF using a flow-injection system. Baseline measures were made using averaged data from all slices, with statistical outliers removed. For autoreceptor studies, only data with signals that allowed modeling through the entire cumulative dose curve were included.

To determine kinetic parameters, evoked levels of DA were modeled using Michaelis–Menten kinetics, as a balance between release and uptake (Wightman

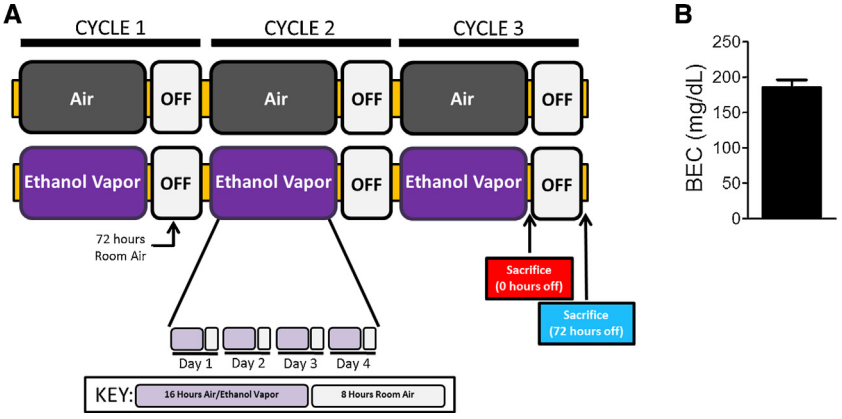

Fig. 1. Chronic intermittent ethanol (CIE) exposure paradigm and blood ethanol concentrations (BECs). (A) CIE experimental procedure. Mice were exposed to four "cycles" of CIE, each consisting of ethanol vapor or room air inhalation for 16 h/day with 8 h of abstinence for four days, followed by 72 h of room air exposure. Cycles were repeated three times. Mice were sacrificed either immediately after cessation of third cycle (0 h time-point) or at 72 h after cessation of the third cycle (72 h time-point). (B) Blood ethanol concentrations (BEC) were evaluated to ensure that physiologically and behaviorally relevant BECs were achieved and maintained throughout the experiment. Mean BEC were 185.1 ± 11.3 mg/dl.