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Neuropharmacology. Author manuscript; available in PMC 2022 September 15.

Published in final edited form as:

Neuropharmacology. 2021 September 15; 196: 108702. doi:10.1016/j.neuropharm.2021.108702.

Lateral Hypothalamus-Projecting Noradrenergic Locus Coeruleus Pathway Modulates Binge-Like Ethanol Drinking in Male and Female TH-*ires*-cre Mice

Nathan W. Burnham[1,2], **Corryn N. Chaimowitz**[1], **Cortland C. Vis**[1], **Ana Paula Segantine Dornellas**[1,2], **Montserrat Navarro**[1,2], **Todd E. Thiele**[1,2]

[1]Department of Psychology & Neuroscience, University of North Carolina at Chapel Hill, NC 27599-3270

[2]The Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, NC 27599-3270

Abstract

A growing body of literature implicates noradrenergic (NE) signaling in the modulation of ethanol consumption. However, relatively few studies have detailed specific brain pathways that mediate NE-associated binge-like ethanol consumption. To begin to fill this gap in the literature, male and female C57BL6/J and TH-*ires*-cre mice underwent pharmacological and chemogenetic testing, respectively, in combination with "drinking in the dark" procedures to model binge-like consumption of ethanol or sucrose solutions. First, we showed that intraperitoneal administration of the NE reuptake inhibitor, reboxetine, blunted binge-like ethanol intake in C57BL6/J mice. Chemogenetic activation of locus coeruleus (LC) tyrosine hydroxylase (TH)-expressing neurons blunted binge-like ethanol intake regardless of sex. Chemogenetic activation of LC projections to the lateral hypothalamus (LH), a region implicated in ethanol consumption, blunted binge-like ethanol drinking without altering sucrose intake in ethanol-experienced or ethanol-naïve mice. In C57BL/6J mice, LH-targeted microinfusion of an α1- adrenergic receptor (AR) agonist blunted binge-like ethanol intake across both sexes, while LH infusion of a β-AR agonist blunted binge-like ethanol intake in females exclusively. Finally, in mice with high baseline ethanol intake both an α1- AR agonist and an α-2 AR antagonist blunted binge-like ethanol intake. The present results provide novel evidence that increased NE tone in a circuit arising from the LC and projecting to the LH reduces binge-like ethanol drinking in mice, and may represent a novel approach to treating binge or heavy drinking prior to the development of dependence.

*Address for Correspondence: Todd E. Thiele, Ph.D., Department of Psychology & Neuroscience, University of North Carolina, Davie Hall, CB#3270, Chapel Hill, NC 27599-3270, USA, Phone: 919-966-1519, Fax: 919-962-2537.

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Conflict of interest statement: The authors declare no competing financial interests. Dr. Thiele owns shares of Glauser Life Sciences, a copy the aims to develop therapeutics for mental health disorders. The work that is presented in this paper is not directly related to the scientific aims of Glauser Life Sciences.

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Keywords

Norepinephrine; Lateral Hypothalamus; Chemogenetic; Binge-Like Drinking; Drinking in the Dark; Mice

1. Introduction

Alcohol abuse poses significant health risks and economic costs to individuals around the world (Lim et al., 2012; Rehm et al., 2009). Repeated bouts of binge drinking, defined as a pattern of drinking yielding blood ethanol concentrations (BECs) achieving the U.S. legal limit of 80 mg/dl within a short (two-hour) time window (NIAAA, 2004), are thought to contribute to the development of later-life alcohol dependence (Hingson et al., 2006). Recent estimates indicate that nearly 90% of U.S. adults that drink excessively consume alcohol in the form of a binge (Esser et al., 2014). Thus, understanding the neurochemical signaling systems that modulates binge-like ethanol consumption is critical for identification of potential therapies for reducing this dangerous pattern of behavior. To this end, the 4-day “drinking-in-the-dark” (DID) paradigm and its variants are among the more popular paradigms for modeling voluntary binge-like ethanol intake in mice (Rhodes et al., 2005; Thiele and Navarro, 2014) and rats (Bell et al., 2011; Holgate et al., 2017), promoting ingestion of large quantities of ethanol and reliably generating BECs exceeding 80 mg/dl. Using DID procedures, researchers have begun to examine roles for neurochemical signaling systems and circuitry that modulate binge-like ethanol intake (Sprow and Thiele, 2012). Interestingly, there is evidence that overlapping neurochemical pathways in the brain modulate both alcohol use disorders and eating disorders (Thiele et al., 2003), suggesting that at least in some circumstances common therapeutic strategies may be identified.

Norepinephrine (NE) is centrally synthesized in brainstem nuclei, with the majority of its synthesis occurring via the locus coeruleus (LC) (Sawchenko and Swanson, 1982), and has long been implicated in ethanol ingestion (Arango et al., 1994; Gilpin and Koob, 2010; Lu et al., 1997; O’Neil et al., 2013; Rasmussen et al., 2014a; Rasmussen et al., 2009; Rasmussen et al., 2014b; Simpson et al., 2009; Verplaetse et al., 2012) as well as feeding behavior (Bello et al., 2019). In fact, the NE/dopamine (DA) reuptake inhibitor bupropion (BUP), when combined with the non-selective opioid antagonist, naltrexone (NAL), has been used successfully to treat binge eating in human (Halseth et al., 2018), and we have recently showed that BUP alone and in combination with NAL blunts binge-like ethanol intake in mice (Navarro et al., 2019). Further, in a preliminary open-label study we recently reported that BUP + NAL therapy blunts the frequency of binge ethanol drinking in humans (Walter et al., 2020). These observations suggest that blunted NE tone contributes to binge behavior, and increasing NE tone is protective. However, there is a gap in the literature on the specific NE neurocircuitry that modulates binge ethanol drinking.

Insight into the NE mechanisms involved in modulating binge behavior comes from studies that have used c-Fos immunoreactivity (IR) as a marker of neuronal activation. It has been shown that the LC is activated following voluntary binge-like ethanol consumption (Burnham and Thiele, 2017). Interestingly, i.p. injection of ethanol into rats bred for high

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versus low ethanol consumption revealed reduced ethanol-induced c-Fos IR in the LC among rats bred for high levels of intake (Thiele et al., 1997). More recently, we showed that an inbred line of mice that was selectively bred to achieve binge-like blood ethanol levels (the iHDID-1 line) failed to exhibit ethanol-induced c-Fos expression in the LC at doses that induced c-Fos expression in the control HS/Npt line (Robinson et al., 2020). These observations suggest that LC activity is triggered by ethanol, and the observation that this activity is blunted in high drinking lines of rats and mice supports a role for ethanol-induced LC activity as a protective mechanism to limit ethanol intake.

In rodents, the lateral hypothalamus (LH) has been implicated in modulation of many behaviors including ethanol consumption (Chen et al., 2013; Chen et al., 2014; Navarro et al., 2016; Sprow et al., 2016; Wayner et al., 1971) and seeking (Marchant et al., 2009; Marchant et al., 2014). Though brainstem NE nuclei innervate numerous regions (Robertson et al., 2016), the LH connects reciprocally with the LC (Jones and Moore, 1977; Papp and Palkovits, 2014) and contains rich populations of α-1, α-2, and β-adrenergic receptors (ARs) (Leibowitz et al., 1982). Thus, the LC may signal through the LH to regulate binge-like ethanol consumption. Experiments herein elucidated the role of the LC in binge-like ethanol intake. First, we show that peripheral administration of a NE reuptake inhibitor (NRI) significantly blunted binge-like ethanol consumption without altering overall motor behavior or anxiety-like behavior, consistent with our previous studies using BUP. Using excitatory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), we demonstrated that general activation the LC blunted binge-like ethanol and sucrose consumption. Specific activation of TH-expressing LC neuron projecting to the LH similarly reduced binge-like ethanol consumption without altering sucrose drinking in ethanol-experienced or ethanol-naïve mice. Finally, we show that LH-directed adrenergic receptor (AR) manipulations reduced ethanol consumption that were compound-specific, and in some cases sex-specific and dependent on baseline levels of ethanol intake. The present results provide novel evidence that increased NE tone in a circuit arising from the LC and projecting to the LH reduces binge-like ethanol drinking, and may represent a novel strategy for treating binge drinking disorders prior to the development of ethanol dependence.

2. Methods and Materials

2.1. Animals

Male and female TH-*ires*-Cre mice (Savitt et al., 2005), bred in house and backcrossed on a C57BL6/J strain, were utilized for DREADD manipulations and tracing studies. Male and female C57BL6/J mice (stock # 000664, Jackson Laboratory), 6 – 8 weeks old upon arrival were utilized for pharmacological manipulations. All mice were individually housed at least 1 week prior to testing onset with *ad libitum* access to Prolab® RMH 3000 (Purina labDiet®; St. Louis, MO) and water except where noted. The animal vivarium was maintained at 22 °C on a 12:12 h reverse light/dark cycle, with lights off at 0830 h. All protocols were conducted under National Institute of Health guidelines and were approved by the University of North Carolina Institutional Animal Care and Use Committee.

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2.2. Surgery

Mice were anesthetized via an intraperitoneal ketamine (66.7 mg/kg; Henry Schein, Dublin, OH) and xylazine (6.67 mg/kg; Henry Schein) cocktail. 0.1 mL 1% lidocaine HCl (Hospira, Inc., Lake Forest, IL) was subcutaneously applied above the skull. For chemogenetic manipulations, TH-*ires*-Cre mice received bilateral infusions of the excitatory DREADD, AAV8-hSyn-DIO-hM3d(Gq)-mcherry (6×10^{12} vg/ml), or control vector lacking the DREADD construct, AAV8-hSyn-DIO-mcherry (8×10^{12}), targeting the LC (AP: −5.40 mm, ML: ±0.85 mm, DV: −3.85mm). For all DREADD experiments, 0.3 μL virus/hemisphere was infused over five minutes, and infusion cannula were left in place for ten minutes post-infusion to discourage virus transport up the cannula tract. For pharmacological and chemogenetic pathway manipulations, 26-G cannula (Double guide cannula, 2.2mm width, 5mm below pedestal; internal: 0.5mm projection; Plastics One, Roanoke, VA) were permanently affixed to the skull, targeting the LH (AP: −1.10 mm, ML: ±1.10 mm, DV: −5.10 mm).

2.3. Drinking in the dark paradigm (DID)

The DID procedure is commonly used to model voluntary binge-like ethanol consumption in mice (Albrechet-Souza et al., 2015; McCall et al., 2013; Patkar et al., 2016; Rinker et al., 2016). Thorough procedural details have been described elsewhere (Thiele et al., 2014). Briefly, DID is a four-day procedure wherein standard mouse water bottles were removed and replaced with a 10 ml pipette containing 20% (v/v) ethanol beginning 3 hours into the dark cycle, and mice were permitted two-hours of ethanol access. Ethanol bottles were removed, and water was returned after the two-hour test each day. On the fourth day (test day) experimental manipulation occurred approximately 30-min before ethanol access and at the conclusion of testing approximately 10 mL of tail blood were acquired from each mouse and processed as described previously (Burnham and Thiele, 2017). Mice undergoing DID procedures commonly achieve blood ethanol concentrations (BECs) exceeding the NIAAA-defined 80 mg/dl threshold for a binge episode (Thiele et al., 2014). Each four-day testing period constituted a binge episode or cycle.

2.4. Open-Field Testing (OFT)

OFT was used to assess whether changes in consumption were potentially influenced by compound-driven alterations in locomotor and/or anxiety-like behavior. In OFT, mice were pretreated with either drug or vehicle 20 minutes prior to placement in the center of the testing chamber (42cm × 42 cm × 30cm; catalog #71-SFAC; Omnitech Electronics, Inc., Columbus, OH). Doses used for OFT were identical to doses used in DID testing except where otherwise noted in figures. Testing began 3-h into the dark cycle, and for the duration of a 2-h test period, animal movements were tracked using VersaMax (AccuScan Instruments, Inc., Columbus, OH) and quantified using VersaDat Version 4.00 (AccuScan Instruments, Inc.). Differences in total distance traveled and time spent in center of test chamber defined drug-elicited effects in locomotor activity and anxiety-like behavior (Simon et al., 1994), respectively.

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2.5. DREADD and TH-ires-Cre mouse verification

Functional validation of DREADDs was performed via examination of c-Fos activity. Specifically, hM3Dq-expressing mice were pretreated with either vehicle or CNO (i.p.) 90 min prior to sacrifice. To validate the Cre line, immunohistochemistry for TH expression (green; see immunohistochemistry below) and viral vector expression (mCherry tag) images were acquired and subsequently overlayed in Adobe Photoshop. Given previous research indicating back-metabolism of CNO to clozapine (Manvich et al., 2018; Raper et al., 2017), utilization of a DREADD-deficient control vector was critical to determining whether effects of CNO were specific to DREADD-expressing constructs. Across our chemogenetic studies, CNO (3.0 mg/kg i.p. or 900 pmol microinfusions) failed to alter behavior relative to vehicle in control-DREADD cohorts, suggesting behavioral alterations occurring post-CNO exposure in hM3Dq-transfected mice were products of anticipated compound and virus interaction. Indeed, it has been shown that 3.0 – 3.5 mg/kg CNO doses fail to alter behavior in the absence of a DREADD construct (Jendryka et al., 2019; Mazzone et al., 2018; Vardy et al., 2015)

2.6. Immunohistochemistry

At the completion of the study, mice were overdosed with 0.1 mL i.p. ketamine/xylazine (6.67 mg/0.1 mL; 0.67 mg/01. mL; in 0.9% saline) and transcardially perfused with 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS (pH = 7.4). Brains were collected and processed according to previously established protocols (Burnham and Thiele, 2017). Rabbit anti-TH (1:20k; catalog #AB1542, EMD Millipore, Billerica, MA) and rabbit anti-c-Fos (1:1k; catalog #226003, Synaptic Systems, Goettingen, Germany) primary antibodies and a 488-conjugated goat anti-Rb (1:500; catalog #111-545-144, Jackson Immunoresearch, West Grove, PA) secondary antibody were utilized where indicated. Virus and cannulae placements were determined via fluorescent and bright field microscopes, respectively.

2.6. Experimental Design

2.6.1. Experiment 1: Effects of Systemic Administration of a Peripherally Bioavailable NRI on Binge-Like Ethanol Intake—

All mice were habituated to handling and gentle restraint for several minutes prior to ethanol access on days 1–3 of DID procedures. On day 4, the selective NRI, reboxetine mesylate (Tocris, catalog #1982), was dissolved in 0.9% saline and administered intraperitoneally 30-min prior to testing onset on the fourth day of the DID cycle. Each animal received vehicle (0.9% saline) or 10 mg/kg reboxetine (10 ml/kg body weight) in a Latin square design over 2-DID cycles. Vehicle or drug administration order was determined based on mouse drinking pattern observed on days 1 – 3 of the first DID cycle such that average baseline consumption between vehicle and drug groups were approximately equal (n=21: 11 female, 10 male). After 1-week of recovery from ethanol testing, mice underwent additional DID testing using the same Latin square design described above but with 3% sucrose in place of 20% ethanol to determine if drug effects on behavior were specific to ethanol consumption, followed by OFT as described above. Dosing based upon previous literature in C57BL6/J mice (Roni and Rahman, 2015).

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2.6.2. Experiment 2: Effects of Chemogenetic Activation of LC on Binge-Like Ethanol Intake—Following 6-weeks of recovery/virus transduction, mice underwent 2-consecutive DID ethanol cycles wherein vehicle (saline+DMSO) or 3.0 mg/kg clozapine-N-oxide (CNO; in 0.5% DMSO+saline solution, injected intraperitoneally (i.p) at 5.0 ml/kg; National Institute of Health, Lot# 13626-76, SAF# 27709) was administered 30-min prior to ethanol access on test days. On days 1–3 of DID procedures mice were habituated to handling and gentle restraint for several minutes prior to ethanol access. Vehicle or CNO administration order was determined based on mouse drinking pattern observed on days 1 – 3 of the first DID cycle such that average baseline consumption between vehicle and CNO groups were approximately equal, and one group was randomly selected to receive CNO week 1. Over 2-DID cycles each mouse experienced vehicle and CNO in a counterbalanced Latin square design (hM3Dq n=15, control viral vector n=8). CNO dosage was based upon previous literature (Navarro et al., 2016; Vardy et al., 2015). After 1-week of recovery from ethanol testing, mice underwent additional DID testing using the same Latin square design described above but with 3% sucrose in place of 20% ethanol to determine if drug effects on behavior were specific to ethanol consumption.

2.6.3. Experiment 3: Effects of Chemogenetic Activating the LC ➔ LH TH+ Pathway on Binge-Like Ethanol Intake—Following 6-weeks of recovery/virus transduction, mice underwent 2-consecutive DID ethanol cycles wherein vehicle (saline+1% DMSO; 0.3 μl/1min infusion/hemisphere) or CNO (900 pmol/0.3 μl/1 min infusion/hemisphere; dose based on our recent work (Rinker et al., 2016)) was infused bi-laterally into LH-targeted cannulae 30 min prior to ethanol access on test days. On days 1–3 of DID procedures mice were habituated to handling, gentle restraint, and cannulae head-cap manipulations for several minutes prior to ethanol access. As above, vehicle or CNO administration order was determined based on mouse drinking pattern observed on days 1 – 3 of the first DID cycle such that average baseline consumption between vehicle and CNO groups were approximately equal, and one group was randomly selected to receive CNO week 1. Over 2-DID cycles each mouse experienced vehicle and CNO in a counterbalanced Latin square design (hM3Dq n=10, control viral vector n=6). After 1-week of recovery from ethanol testing, mice underwent additional DID testing using the same Latin square design described above but with 3% sucrose in place of 20% ethanol to determine if drug effects on behavior were specific to ethanol consumption, followed by OFT as described above.

2.6.4. Experiment 4: Effects of LH α1 AR Agonism on Binge-Like Ethanol Intake—The selective α1 AR agonist, phenylephrine (PHEN; catalog # 2838, R&D Systems, Minneapolis, MN), was dissolved in 0.9% saline and injected bilaterally into the LH 15 – 25 minutes prior to the start of testing on DID day 4. Each animal received vehicle (0.9% saline/0.3 μl/hemisphere over 1 min) and PHEN (20 nmol/0.3μl/hemisphere over 1 minute) on DID day 4 in a Latin square design over 2-DID cycles. On days 1–3 of DID procedures mice were habituated to handling, gentle restraint, and cannulae head-cap manipulations for several minutes prior to ethanol access. Vehicle or drug administration order was determined based on mouse drinking pattern observed on days 1 – 3 of the first DID cycle such that average baseline consumption between vehicle and drug groups were approximately equal, and one group was randomly selected to receive CNO week 1 (n=17:

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9 female, 8 male). After 1-week of recovery from ethanol testing, mice underwent additional DID testing using the same Latin square design described above but with 3% sucrose in place of 20% ethanol to determine if drug effects on behavior were specific to ethanol consumption. Dosing was based upon previous literature (Kochenborger et al., 2012; Mansur et al., 2010, 2011).

2.6.5. Experiment 5: Effects of LH β AR Agonism on Binge-Like Ethanol Intake—

The β agonist, xamoterol hemifumarate (XAM; product # ab145957, Abcam, Cambridge, MA), was dissolved in 0.9% saline and injected bilaterally into the LH 15 – 25 minutes prior to testing onset on DID day 4. Each mouse received vehicle (0.9% saline/0.5 μl/hemisphere over 1 min) and xamoterol (0.5 μg/0.5 μl/hemisphere over 1 minute) in a Latin square design over 2-DID cycles. On days 1–3 of DID procedures mice were habituated to handling, gentle restraint, and cannulae head-cap manipulations for several minutes prior to ethanol access. Vehicle or drug administration order was determined based on mouse drinking pattern observed on days 1 – 3 of the first DID cycle such that average baseline consumption between vehicle and drug groups were approximately equal, and one group was randomly selected to receive CNO week 1 (n=13: 6 female, 7 male). After 1-week of recovery from ethanol testing, mice underwent additional DID testing using the same Latin square design described above but with 3% sucrose in place of 20% ethanol to determine if drug effects on behavior were specific to ethanol consumption. Dosing was based upon previous literature (Torkaman-Boutorabi et al., 2014).

2.6.6. Experiment 6: Effects of LH α2 Antagonism on Binge-Like Ethanol Intake—

The selective α2 antagonist, atipamezole (catalog # A9611, Sigma-Aldrich), was dissolved in a 25% DMSO, 75% dH_20 solution and was injected bilaterally into the LH 15 – 25 minutes prior to the start of testing on DID day 4. On days 1–3 of DID procedures mice were habituated to handling, gentle restraint, and cannulae head-cap manipulations for several minutes prior to ethanol access. Vehicle or drug administration order was determined based on mouse drinking pattern observed on days 1 – 3 of the first DID cycle such that average baseline consumption between vehicle and drug groups were approximately equal. Each animal received vehicle (25% DMSO, 75% dH_20 solution/0.3 μl/hemisphere/1 minute), 1.5 μg (in 0.3 μl/hemisphere over 1 minute), and 15 μg (in 0.3 μl/hemisphere over 1 minute) in a Latin square design over 3-DID cycles. Vehicle or drug administration order was determined based on mouse drinking pattern observed on days 1 – 3 of the first DID cycle such that average baseline consumption between vehicle and drug groups were approximately equal, and one group was randomly selected to receive CNO week 1 (n=21: 11 female, 10 male). After 1-week of recovery from ethanol testing, mice underwent additional DID testing using a similar Latin square design described above but with 3% sucrose in place of 20% ethanol to determine if drug effects on behavior were specific to ethanol consumption. In this study, mice were given vehicle or the 15 μg dose of atipamezole. Dosing was based upon previous literature in rats (Ostock et al., 2015).

2.7. Statistical Analyses

For DREADD manipulations, three-way ANOVA analysis failed to detect a significant effect of treatment order, so data were collapsed and analyzed via two-way ANOVA.

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Two-way ANOVA failed to detect significant effect of sex, so data were collapsed across sexes and analyzed via paired *t*-tests (treatment; total consumption and BECs). For pharmacological manipulations, repeated-measures ANOVAs (sex × treatment) were used to assess total consumption and BECs, respectively. When significant main effects or interactions were obtained, Bonferroni-corrected *t*-tests were performed such that statistical significance was accepted based on the formula of $\alpha = 0.05/n$ where n = the number of comparisons conducted. All other manipulations utilized paired *t*-tests (when comparing pre/post treatment within animals) or independent *t*-tests (when comparing animals in different conditions), and analyses were performed using GraphPad Prism 7 (La Jolla, CA) or SPSS 25 (IBM Analytics, Armonk, New York). Findings are presented as mean ± standard error of the mean (SEM) and considered significant if $p < 0.05$ (two-tailed).

3. Results

3.1. Experiment 1: Effects of Systemic Administration of a Peripherally Bioavailable NRI on Binge-Like Ethanol Intake

Data from this experiment are presented in Fig. 1. A repeated-measures ANOVA (sex × treatment) failed to reveal a main effect of sex or sex by treatment interaction, so data from males and females were collapsed. Paired t-test for total ethanol intake revealed a significant reduction in intake following 10 mg/kg reboxetine pretreatment (Fig. 1A) [*t* test: $t(20)=4.199$, $p<0.001$]. 10 mg/kg reboxetine similarly blunted BECs (Fig. 1B) [*t* test: $t(20)=3.473$, $p=0.002$]. Fig. 1C shows a reboxetine-driven reduction in sucrose consumption [*t* test: $t(20)=4.451$, $p=0.002$]. Unpaired *t*-tests failed to find significant differences in total distance traveled (Fig. 1D) [$t(12)=1.116$, $p=0.29$] or time spent in chamber center in the first 5 minutes of testing (Fig. 1E) [$t(12)=0.335$, $p=0.74$]. Since we hypothesized that binge-induced activation of NE is protective against ethanol intake, we hypothesized that mice with high ethanol intake would be more sensitive to reductions in ethanol intake following reboxetine administration. To this end, we performed a median-split of mice based on their consumption during vehicle infusion, and re-analyzed consumption data with a two-way, 2 × 2 (treatment: vehicle versus reboxetine × drinker: low drinkers versus high drinking) ANOVA (Fig. 1F). We found a significant main effects of treatment [$F(1, 19)=22.31$, $p<0.001$], drinker [$F(1, 19)=17.17$, $p=0.001$], and treatment × drinker interaction [$F(1, 19)=7.81$, $p=0.012$]. Bonferroni corrected t-tests indicated a significant differences in binge-like ethanol intake between vehicle and reboxetine in high drinkers [*t*-test $t(10)=4.92$, $p=0.002$], but not in levels of binge-like ethanol intake between vehicle and reboxetine in low drinkers [*t*-test $t(9)=1.54$, $p=0.16$].

3.2. Experiment 2: Effects of Chemogenetic Activation of LC on Binge-Like Ethanol Intake

Data from this experiment are presented in Fig. 2. As seen in Fig. 2A, among TH-*ires*-cre mice expressing AAV8-hSyn-DIO-hM3D(Gq)-mcherry virus in the LC, 3.0 mg/kg i.p. CNO administration blunted binge-like ethanol intake [*t*-test $t(14)=3.34$, $p=0.005$]. As seen in Fig. 2B, BECs (n=10; 5 blood samples were lost due to mechanical error) are blunted following CNO administration relative to vehicle treatment [*t* test $t(9)=2.811$, $p=0.02$]. Conversely, in TH-*ires*-cre mice expressing AAV8-hSyn-DIO-mcherry in the LC, i.p. CNO failed to

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alter ethanol consumption as shown in Fig. 2C [*t*-test $t(7)=0.06$, $p=0.96$] and associated BECs shown in Fig. 2D (n=6; 2 blood samples were lost due to mechanical error) [*t*-test $t(5)=0.54$, $p=0.61$]. A previous report has shown that chemogenetic activation of the LC complex induces an anxiogenic-like effect as measured via OFT in a CNO dose-dependent manner (Sciolino et al., 2016). The induction of anxiety-like behavior could be a potential explanation for blunted ethanol consumption stemming from LC activation, as a general reduction of consummatory behavior may have been secondary to increased anxiety. To test this possibility, we assessed sucrose consumption. As seen in Fig. 2E, CNO pretreatment in a randomly selected subset of TH-*ires*-cre mice expressing AAV8-hSyn-DIO-hM3D(Gq)-mcherry virus in the LC also blunted intake of a 3% sucrose solution [*t* test $t(10)=3.818$, $p=0.003$], suggesting that chemogenetic activation of TH+ LC neurons may nonspecifically blunt consumption, at least in mice with histories of binge-like ethanol intake. As seen in Fig. 2F, this effect is not likely a product of CNO given that CNO pretreatment in TH-*ires*-cre mice expressing AAV8-hSyn-DIO-mcherry in the LC failed to alter consumption of 3% sucrose [*t* test $t(5)=0.086$, $p=0.93$]. Representative photomicrographs show hM3Dq DREADD expression (red; Fig. 2G), TH IHC (green; Fig. 2H), hM3Dq DREADD/TH IHC overlay (yellow; Fig. 2I). Approximate spread of DREADD expression in the LC is shown in Fig. 2J. To verify hM3Dq DREADD function, a randomly-selected subset of mice was pretreated with 3.0 mg/kg CNO or vehicle prior to sacrifice, and neuronal activation determined via c-Fos immunohistochemistry (Suppl. Fig. 1A–H). 3.0 mg/kg CNO pretreatment significantly increased c-Fos expression in mice expressing hM3Dq in the LC relative to vehicle pretreated controls [*t* test $t(6)=7.174$, $p<0.001$]. CNO pretreatment had no effect in mice expressing the empty vector control [*t* test $t(7)=0.52$, $p=0.619$]. As above, we performed a follow-up assessment of ethanol consumption data by performing a median-split of mice based on their consumption during vehicle infusion, and we then re-analyzed consumption data with a two-way, 2 × 2 (treatment: vehicle versus CNO × drinker: low drinkers versus high drinking) ANOVA (Fig. 2K). We found a significant main effects of treatment [$F(1, 13)=13.76$, $p=0.003$], drinker [$F(1, 13)=7.97$, $p<0.014$], and treatment × drinker interaction [$F(1, 13)=5.25$, $p=0.039$]. Bonferroni corrected t-tests indicated a significant differences in binge-like ethanol intake between vehicle and CNO in high drinkers [*t*-test $t(7)=3.36$, $p=0.012$], but not in levels of binge-like ethanol intake between vehicle and CNO in low drinkers [*t*-test $t(6)=2.03$, $p=0.088$].

3.3. Experiment 3: Effects of Chemogenetic Activating the LC ➔ LH TH+ Pathway on Binge-Like Ethanol Intake

Given the reciprocal signaling between the LC and LH regions detailed in literature as well as LC➔LH tracing in TH-*ires*-cre mice (Suppl. Fig. 2A–D) and results from NE nuclei chemogenetic activation, we sought to manipulate LC ➔ LH NE neurocircuitry to determine the role of the pathway in modulating binge-like ethanol consumption. Results from this experiment are presented in Fig. 3. As seen in Fig. 3A, relative to vehicle injection days, LH-targeted CNO microinfusion blunted binge-like ethanol intake (n=10) [*t* test: $t(9)=3.61$, $p=0.006$]. There were also blunted BECs (Fig. 3B) [$t(9)=2.801$, $p=0.02$] in TH-*ires*-cre mice expressing hM3Dq DREADD in the LC. Conversely, in TH-*ires*-cre mice expressing AAV8-hSyn-DIO-mcherry, LH-targeted CNO microinfusions failed to alter ethanol intake (Fig. 3C) [*t* test: $t(5)=0.134$, $p=0.90$] or associated BECs (Fig. 3D; 1 sample

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was lost due to mechanical error) [$t(4)=0.544$, $p=0.62$]. As is evident in Fig. 3E, CNO failed to alter intake of a 3% sucrose solution in mice expressing the hM3Dq DREADD in the LC [*t* test: $t(9)=0.119$, $p=0.91$] or mice expressing the empty vector control in the LC (Fig. 3F) [*t* test: $t(4)=0.75$, $p=0.50$] relative to vehicle. In a separate experiment, chemogenetic activation of the LC ➔ LH TH+ pathway also failed to blunt sucrose consumption in ethanol-naïve mice (Suppl. Fig. 3A–B).

Separate, ethanol-naïve TH-*ires*-cre mice were utilized OFT experiments. Chemogenetic activation of the LC➔LH pathway increased distance travelled in the first 5 minutes of testing [$t(5)=2.977$, $p=0.03$] (Fig. 3G, inset) but did not alter distance travelled over the entire two-hour test [treatment: $F(1,5)=1.021$, $p=0.36$; time: $F(23,115)=18.08$, $p<0.01$; interaction: $F(23,115)=1.17$, $p=0.29$] (Fig. 3G). In mice expressing the empty vector control, CNO microinfusion did not alter distance travelled in the first 5 minutes of testing [$t(7)=0.386$, $p=0.71$] (Fig. 3H, inset) or distance travelled over the entire two-hour test (treatment: $F(1,7)=0.14$, $p=0.71$; time: $F(23,161)=21.16$, $p<0.01$; interaction: $F(23,161)=0.68$, $p=0.86$] (Fig. 3H). LC➔LH projection activation increased time spent in OFT chamber center in the first 5 minutes of testing [$t(12)=3.718$, $p=0.003$] (Fig. 3I, inset) but did not yield a significant effect across the duration of the two-hour test [treatment: $F(1,5)=0.75$, $p=0.42$; time: $F(23,115)=0.83$, $p<0.69$; interaction: $F(23,115)=0.70$, $p=0.84$] (Fig. 3I). CNO did not alter center time in the first 5 minutes of testing in control mice [$t(7)=0.888$, $p=0.40$] (Fig. 3J, inset) or over the two-hour test [treatment: $F(1,7)=0.20$, $p=0.66$; time: $F(23,161)=1.09$, $p=0.36$; interaction: $F(23,161)=1.63$, $p=0.04$] (Fig. 3J). Representative DREADD virus expression in the LC, and cannula placements within the LH are presented in Figs. 3K and 3L, respectively. As above, we performed a follow-up assessment of ethanol consumption data by performing a median-split of mice based on their consumption during vehicle infusion, and we then re-analyzed consumption data with a two-way, 2 × 2 (treatment: vehicle versus CNO × drinker: low drinkers versus high drinking) ANOVA (Fig. 3M). We found significant main effects of treatment [$F(1, 8)=16.02$, $p=0.004$], drinker [$F(1, 8)=12.57$, $p=0.008$], and but the treatment × drinker interaction effect was not significant [$F(1, 8)=3.09$, $p=0.12$].

3.4. Experiment 4: Effects of LH α1 AR Agonism on Binge-Like Ethanol Intake

Data from each of the pharmacological experiments are presented in Fig. 4. A two-way ANOVA failed to detect an effect of sex on any data set, so data from males and females were collapsed. Paired *t*-tests were used to assess total consumption. Relative to vehicle injections, 20 nmol PHEN/hemisphere blunted total consumption (Fig. 4A) [*t* test: $t(16)=2.391$, $p=0.03$] but not associated BECs (Fig. 4B) [$t(16)=1.03$, $p=0.32$]. Following 2 weeks of ethanol DID testing, mice experienced 2 consecutive weeks, in a Latin square design, of 3% sucrose testing to determine if 20 nmol PHEN effects were specific to ethanol. Relative to vehicle, 20 nmol PHEN failed to alter total intake of a 3% sucrose solution (Fig. 4C) (n=15; 2 animals had clogged cannulae) [*t* test: $t(14)=0.826$, $p=0.42$]. Approximate cannula placements are denoted in Fig. 4D. As above, we performed a follow-up assessment of ethanol consumption data by performing a median-split of mice based on their consumption during vehicle infusion, and we then re-analyzed consumption data with a two-way, 2 × 2 (treatment: vehicle versus PHEN × drinker: low drinkers

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versus high drinking) ANOVA (Fig. 4E). We found significant effects of treatment [F(1, 15)=8.38, p=0.011], drinker [F(1, 15)=8.72, p<0.01], and treatment × drinker interaction [F(1, 15)=11.97, p=0.004]. Bonferroni corrected t-tests indicated a significant differences in binge-like ethanol intake between vehicle and PHEN in high drinkers [t-test t(8)=4.90, p=0.001], but not in levels of binge-like ethanol intake between vehicle and PHEN in low drinkers [t-test t(7)=−0.37, p=0.73].

3.5. Experiment 5: Effects of LH β AR Agonism on Binge-Like Ethanol Intake

Repeated-measures two-way ANOVA (sex × treatment) revealed a significant interaction of sex × treatment [F(1,11)=7.796, p=0.018], with 0.5 μg XAM/hemisphere significantly reducing total binge-like ethanol consumption in females (Fig. 5A). Main effects of sex [F(1,11)=2.546, p=0.139] and treatment [F(1,11)=2.309, p=0.239] were not detected. XAM similarly blunted BECs in females but not males (Fig. 5B) [treatment: F(1,11)=0.462, p=0.51; sex: F(1,11)=0.421, p=0.53; interaction: F(1,11)=10.56, p<0.01]. However, 0.5 μg/hemisphere XAM failed to similarly alter sucrose consumption (Fig. 5C), as we failed to detect significant main effects [sex: F(1,11)=1.188, p=0.299; treatment: F(1,11)=1.02, p=0.334] or a sex × treatment interaction [F(1,11)=0.008, p=0.932]. Approximate cannula placements are denoted in Fig. 5D. As above, we performed a follow-up assessment of ethanol consumption data by performing a median-split of mice based on their consumption during vehicle infusion, and we then re-analyzed consumption data with a two-way, 2 × 2 (treatment: vehicle versus XAM × drinker: low drinkers versus high drinking) ANOVA (Fig. 5E). While the treatment effect was not significant [F(1, 11)=1.13, p=0.311], drinker [F(1, 11)=13.04, p=0.004], and the treatment × drinker interaction [F(1, 11)=6.43, p=0.028] were both statistically significant. Bonferroni corrected t-tests failed to reveal significant differences in binge-like ethanol intake between vehicle and XAM in high drinkers [t-test t(6)=2.42, p=0.052], as well as binge-like ethanol intake between vehicle and XAM in low drinkers [t-test t(5)=−1.15, p=0.302].

3.6. Experiment 6: Effects of LH α2 Antagonism on Binge-Like Ethanol Intake

A repeated-measures two-way ANOVA (treatment × sex) revealed a significant interaction of sex × dose [F(2, 38)=3.245, p=0.05] on total ethanol consumption but failed to reveal significant main effects of sex [F(1,19)=0.985, p=0.33] or dose [F(2,38)=0.16, p=0.85]. However, Bonferroni post-hoc testing failed to reveal group differences, thus the sexes are collapsed in the Fig. 6A. Further, we failed to detect significant differences in BECs (Fig. 6B) [treatment: F(2,34)=0.399, p=0.67; sex: F(1,17)=0.354, p=0.56; interaction: F(2,34)=0.722, p=0.49]. Animals were repurposed for 3% sucrose testing to determine specificity of drug effects, comparing vehicle and 1.50 μg atipamazole/hemisphere treatments. Fig. 6C shows results from a repeated-measures ANOVA (treatment × sex), where 1.50 μg atipamezole(APZ)/hemisphere microinfusion revealed an interaction of drug and sex on intake of a 3% sucrose solution (1 female was lost between studies due to clogged cannula) [treatment: F(1,18)=0.987, p=0.33; sex: F(1,18)=0.007, p=0.93; interaction: F(1,18)=7.451, p=0.014], but Bonferroni post-hoc testing failed to reveal group differences, thus the sexes are collapsed in Fig. 6C. Approximate cannula placements are defined in Fig. 6D. As above, we performed a follow-up assessment of ethanol consumption data by performing a median-split of mice based on their consumption during vehicle

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infusion, and we then re-analyzed consumption data with a two-way, 3 × 2 (treatment: vehicle versus APZ × drinker: low drinkers versus high drinking) ANOVA (Fig. 5E). While the treatment effect was not significant [$F(2, 38)=0.38$, $p=0.152$], drinker [$F(1, 19)=8.84$, $p=0.008$], and the treatment × drinker interaction [$F(2, 38)=6.33$, $p=0.004$] were both statistically significant. Bonferroni corrected t-tests indicated a non-significant reduction of binge-like ethanol intake following the 1.5 μg dose [t-test $t(10)=2.3$, $p=0.045$] but a significant reduction following the 15 μg dose [t-test $t(10)=3.24$, $p=0.009$] of APZ relative to vehicle treatment in the high drinkers. Conversely, in the low drinkers, relative to vehicle treatment the 1.5 μg dose of APZ increased ethanol intake [t-test $t(9)=-3.13$, $p=0.012$] while the and 15 μg dose of APZ did not significantly alter intake [t-test $t(9)=-1.91$, $p=0.089$].

4. Discussion

This series of studies sought to uncover the roles of the LC and its projection to the LH in modulation of voluntary binge-like ethanol consumption. First, we first showed that systemic administration of the NRI, reboxetine, blunted binge-like ethanol intake in both sexes without altering locomotor or anxiety-like behavior. These findings are consistent with our previous observation that the NE and dopamine re-uptake inhibitor, BUP, significantly blunts binge-like ethanol drinking mice (Navarro et al., 2019). Next, we showed that general chemogenetic activation of NE neurons in the LC blunted binge-like ethanol, but also reduced and sucrose intake suggesting non-specific effects on consummatory behavior. These non-specific effects on behavior may be secondary to the increased anxiety-like behavior, which is also triggered by general chemogenetic activation of these neurons (Sciolino et al., 2016). On the other hand, chemogenetic activation specifically of the NE LC➔LH projection blunted binge-like ethanol consumption without altering sucrose consumption in both ethanol experienced and ethanol naïve mice. Interestingly, while general activation of the NE neurons in the LC is anxiogenic (Sciolino et al., 2016), we found that activation of NE LC ➔ LH was anxiolytic, reflected as increased center time during the first 5-minutes in the OFT. There was also an increase in distance traveled during the first 5-minute of the OFT following activation of the NE LC ➔ LH pathway, though this transient increase in locomotor activity is unlikely to have impacted the 2-hour ethanol intake test, reinforced by the lack of an effect of this manipulation on 2-hour sucrose drinking. In our pharmacological studies with C57BL/6J mice, at the doses tested we found that LH infusion of the α1 AR agonist, PHEN, blunted binge-like ethanol intake independent of sex and without altering sucrose intake, the β1 AR agonist, XAM, blunted ethanol intake without altering sucrose intake in female mice, and the a high dose of the α2 AR antagonist, ATP, blunted binge-like ethanol intake in mice with high baseline ethanol intake but a low dose of ATP increased ethanol intake in mice with low baseline ethanol intake.

NE binds to α2 ARs to decrease NE release from presynaptic terminals (Aghajanian and VanderMaelen, 1982; Starke et al., 1974) and engages post-synaptic Gi-signaling cascades (Wang et al., 2007) while engagement of postsynaptic Gq-coupled α1 ARs (Piascik and Perez, 2001) and Gs-coupled β ARs (Molinoff, 1984) increase neuronal activity. Our findings that elevated NE signaling via systemic NRI, LH-localized α1 AR activation and α2 AR blockade, chemogenetic activation of NE neurons in the LC or LC➔LH projection,

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blunts binge-like ethanol intake appears incongruent with literature showing reduced ethanol intake following systemic administration of drugs that *blunt*, rather than increase, NE signaling. For example, the α2 AR agonist, clonidine (Rasmussen et al., 2014a), the α1 AR antagonist, prazosin (Froehlich et al., 2013; Rasmussen et al., 2009; Verplaetse et al., 2012), or the β AR antagonist, propranolol (Verplaetse and Czachowski, 2015) all reduce ethanol intake. There are at least two possible explanations for these differences. First, systemic administration of AR-selective pharmacological compounds detailed in the literature inherently modifies signaling throughout the central and peripheral nervous systems, so altered intake patterns resulting from systemic administration reflect a net effect of system-wide receptor interactions. However, our chemogenetic and pharmacological manipulations were brain region- and circuit-specific and suggest that increasing NE tone in these regions is protective against excessive consumption. One caveat to this reasoning is our finding that systemic treatment with the NRI, reboxetine, blunted binge-like ethanol intake, though the actions of NRIs are mechanistically distinct from AR agonists or antagonists, as they increase global NE availability for all ARs (Dostert et al., 1997). Secondly, previous studies showing that reduced NE tone blunts ethanol intake utilized rodent strains bred for high levels of ethanol consumption (Froehlich et al., 2013; O'Neil et al., 2013; Rasmussen et al., 2014a; Rasmussen et al., 2009; Rasmussen et al., 2014b; Verplaetse and Czachowski, 2015; Verplaetse et al., 2012), ethanol-dependent rats (Gilpin and Koob, 2010), or alcohol-dependent humans (Simpson et al., 2009) while we studied non-dependent C57BL6/J and TH-*ires*-cre mice. Thus, the contributions of NE signaling may differ between subjects that are ethanol-dependent or selectively bred for high ethanol intake versus non-dependent binge drinking subjects. Overall, our data suggest that treatments that increase NE tone may have therapeutic value for treating binge or heavy alcohol abuse, prior to the development of dependence. Following the development of ethanol dependence, reducing NE tone may be protective, particularly against the adverse symptoms of withdrawal, such as anxiety, which drive high ethanol intake through negative reinforcement (Koob and Le Moal, 2001).

We previously found that ethanol-induced c-Fos expression in the LC was significantly lower among rats selectively bred for high ethanol intake relative to low ethanol drinking lines (Thiele et al., 1997), and more recently we found that an inbred line of mice that was selectively bred to achieve binge-like blood ethanol levels (the iHDID-1 line) failed to exhibit ethanol-induced c-Fos expression in the LC at doses that induced c-Fos expression in the control HS/Npt line (Robinson et al., 2020). Together these results suggest that the LC may be part of a mechanism that protects against ethanol intake and which is compromised in high drinking lines. Additionally, high-alcohol drinking (HAD) and alcohol-preferring (P) rats each demonstrate fewer NE transporter sites within the LC relative to low-alcohol drinking (LAD) and alcohol non-preferring (NP) rats, possibly as a compensatory mechanism for reduced NE release (Hwang et al., 2000). Thus, dysregulated NE activity in the LC may contribute to a pattern of high ethanol consumption observed in ethanol-preferring and high drinking rodent lines. Consistent with our chemogenetic manipulations, increasing or decreasing NE activity via LC-targeted microinfusion of substance P or clonidine, respectively, suppresses or increases voluntary ethanol consumption in rats (West et al., 2015). Collectively, these data suggest that ethanol-induced activation of NE signaling in the LC may represent a protective "braking" mechanism against high ethanol intake.

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This conclusion is bolstered by our observations with chemogenetic and pharmacological manipulations that increased NE/AR signaling was most effective in blunting binge-like ethanol intake in mice with higher levels of ethanol intake in most cases. We speculate that this signaling becomes blunted over repeated ethanol use, which in turns leaves individuals vulnerable to increased levels of drinking and ultimately dependence. Ongoing research within our lab is exploring this possibility.

Activation of the NE LC➔LH pathway blunted binge-like ethanol intake without altering the consumption of sucrose in ethanol-experience or ethanol naïve-mice. These observations suggest that the effects of activating this pathway are specific to binge-like ethanol intake. Similarly, our pharmacological manipulations blunted binge-like ethanol intake without altering sucrose intake. It is important to keep in mind that the LC and other NE nuclei (Ahlskog, 1974; Redmond et al., 1977; Rinaman, 2003; Rinaman et al., 1998; Roman et al., 2016; Sahakian et al., 1983; Schreihofer et al., 1997; Uemura et al., 1997) and the LH (Jennings et al., 2013; Navarro et al., 2016; Stamatakis et al., 2016) as well as LH ARs (Ferrari et al., 1990; Leibowitz, 1970) have been implicated in consummatory behavior encompassing ethanol as well as biologically significant rewards, such as food. What is more, the LH is critical for reward processing, as rodents will work for stimulation of the LH (Hoebel and Teitelbaum, 1962; Jennings et al., 2015; Margules and Olds, 1962). Interestingly, pharmacotherapies that increase NE tone are used to treat disorder of excessive eating (Carbone et al., 2020; Greenway et al., 2009; Greenway et al., 2010). And as noted below, we are currently investigating similar approaches to treating binge drinking prior to dependence (Navarro et al., 2019; Walter et al., 2020), and we are more thoroughly examining the potential role of the NE+ LC ➔ LH circuit in modulating the consumption of various natural rewards in ethanol-naïve subjects.

The LH is a neurochemically diverse region, producing GABA, glutamate, orexin/hypocretin, dynorphin, neurotensin, galanin, and cocaine and amphetamine-regulated transcript, each of which has been implicated in the consumption of ethanol (Olney et al., 2015; Salinas et al., 2014; Sprow et al., 2016). Future investigations aimed at identifying LH postsynaptic contacts of NE axons stemming from the LC will help unpack the mechanisms by which NE LC➔LH neurons, and AR signaling in the LH, modulate binge-like ethanol intake. Interestingly, we have previously shown that chemogenetic manipulation of GABAergic neurons in the LH modulates binge-like ethanol intake (Navarro et al., 2016), making innervation of LH GABAergic neurons by NE neurons arising from the LC as an important future research direction.

Utilization of both sexes in our studies was critical given literature implicating estrogen in modulation of NE synthesis and LH AR signaling (Alfinito et al., 2009; Etgen et al., 2001; Lubbers et al., 2010; Selmanoff et al., 1976; Vathy and Etgen, 1988), and sex differences in LC structure (Guillamon et al., 1988; Luque et al., 1992; Pinos et al., 2001). Our pharmacological manipulations within the LH revealed sex-specific effects of the β AR agonist, which may stem from estrogen modulation of NE synthesis and LH AR signaling as noted above. However, any sex differences in chemogenetic manipulations of the NE LC➔LH projection were likely masked given that LC NE activation likely influences all ARs. Further, additional sex effects may have surfaced had we examined full dose-response

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curves in our pharmacology studies. Future research is needed to more clearly establish potential sex differences in the role that AR signaling in the LH plays in modulating binge-like ethanol intake.

Importantly, while we did remove animals with cannulae placements that were determined to have missed the LH (see Suppl. Fig. 4 for photomicrograph of cannulae placement histology), one caveat worth noting is the possibility that during *in vivo* pharmacological studies compounds may have moved beyond the LH and impacted AR signaling in adjacent brain regions. While we cannot completely rule out this possibility, we did note that TH+ fibers arising from the LC and innervating the hypothalamus appeared to be specific to LH innervation, and were not evident in nearby regions such as the dorsal zona incerta (ZI; see Suppl. Fig. 5). Thus, we are confident in the specificity of the TH+ LC ➔ LH circuit in the modulating of binge-like ethanol intake. A second caveat is that since TH+ neurons of the LC have been shown to co-express glutamate (Fung et al., 1994), we cannot definitively conclude that chemogenetic activation of TH+ neurons in the LC or LC ➔ LH circuit blunted binge-like ethanol intake exclusively by stimulation of NE release, and thus we must temper this conclusion. That being said, we did find that treatment with the NRI reboxetine, and that LH-infusion of the high dose of the α2 AR antagonist APZ, blunted binge-like ethanol intake in mice with high baseline levels of ethanol intake, presumably by increase synaptic levels of NE and thus implicating endogenous NE signaling. The latter observation specifically points to a role for endogenous NE signaling in the LH in the modulation of binge-like ethanol intake.

5. Conclusions

Though well-established as playing a critical role in depression and anxiety disorders (Montoya et al., 2016), a growing body of literature is unpacking a role for central NE signaling in the modulation of drug and alcohol intake. Our results indicate that elevated NE signaling in the NE LC➔LH circuit plays protective roles against binge-like ethanol intake. We recently reported that the dopamine/norepinephrine reuptake inhibitor, bupropion, blunts binge-like ethanol intake in mice (Navarro et al., 2019), and reduced the frequency of binge alcohol drinking in a phase II clinical trial study (Walter et al., 2020). These observations are consistent with our current finding that the NRI, reboxetine, blunted binge-like ethanol drinking in mice. Taken together, these results suggest potential therapeutic value for drugs that increase NE tone, such as reboxetine and bupropion, in minimizing binge-like ethanol drinking in humans prior to the development of ethanol dependence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

We thank Rhiannon Thomas, Timothy Gilliam, Sophie Bendrath, and Sonia Sabater for their expert assistance with the present projects. This research was funded by NIH grants AA013573, AA022048, & AA025809.

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Highlights

- Peripheral administration of the NE reuptake inhibitor, reboxetine, blunts binge-like ethanol intake, suggesting a potential therapeutic target.
- Chemogenetic activation of the locus coeruleus blunts binge-like ethanol intake.
- Chemogenetic activation of a norepinephrine circuit from the LC to lateral hypothalamus (LH) selectively blunts binge-like ethanol intake but not sucrose drinking.
- Pharmacological manipulation of adrenergic receptor in the LH selectively blunts binge-like ethanol intake.

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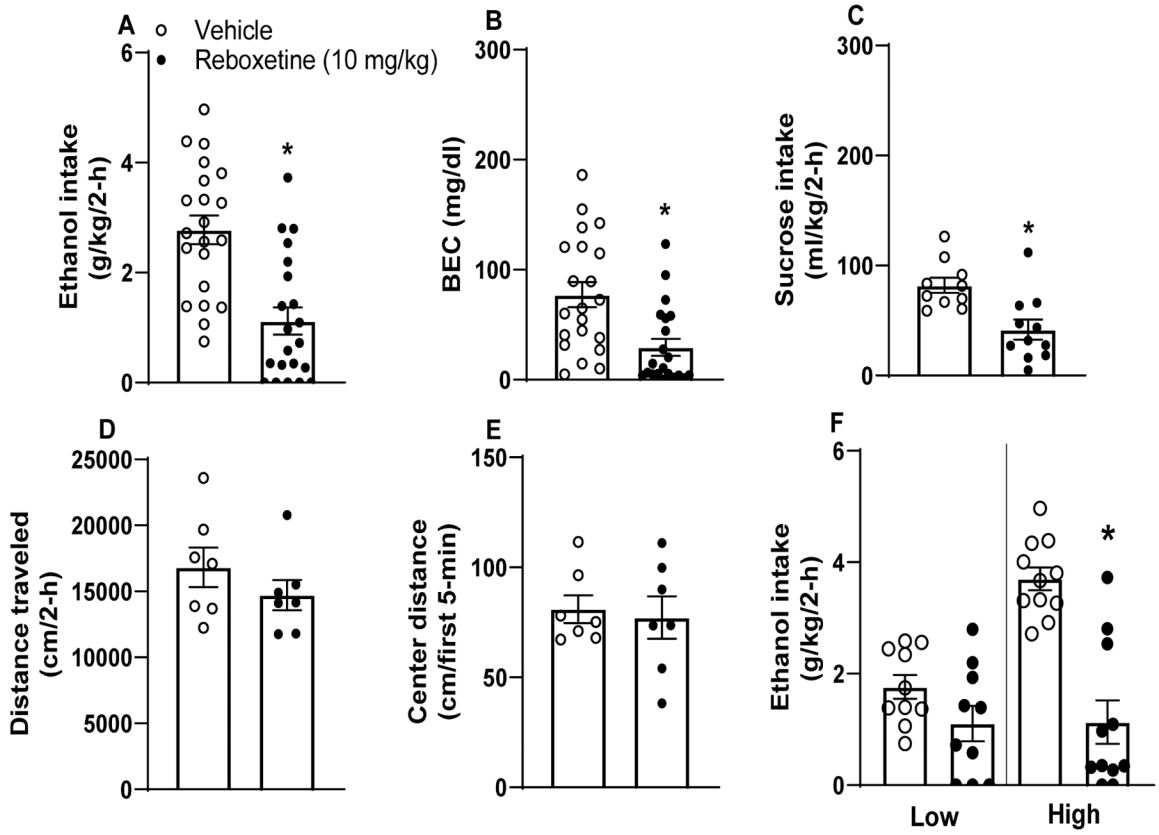

Figure 1. Systemic administration of reboxetine, a noradrenaline reuptake inhibitor, blunts binge-like ethanol intake in C57BL6/J mice.

10 mg/kg i.p. reboxetine blunted binge-like ethanol (*A; n*=21) intake and BECs (*B; n*=21). Following ethanol testing, these mice underwent sucrose testing wherein 10 mg/kg reboxetine blunted intake of a 3% sucrose solution (*C; n*=19). Alterations in intake patterns were not secondary to altered locomotor or anxiety-like states, as 10 mg/kg reboxetine pretreatment prior to OFT failed to alter total distance traveled (*D; n*=7) and center time (*E; n*=7) relative to vehicle-injected cohorts (*n*=7). Data collapsed across males and females due to absence of sex effects. (*F*) Further analyses of ethanol intake data indicated that reboxetine blunted intake in mice with High baseline ethanol intake (upper 50% of a median-split) and not in mice with Low baseline intake (lower 50% of a median-split). values represent mean ± SEM. * indicates $p<0.05$ in paired *t*-test.

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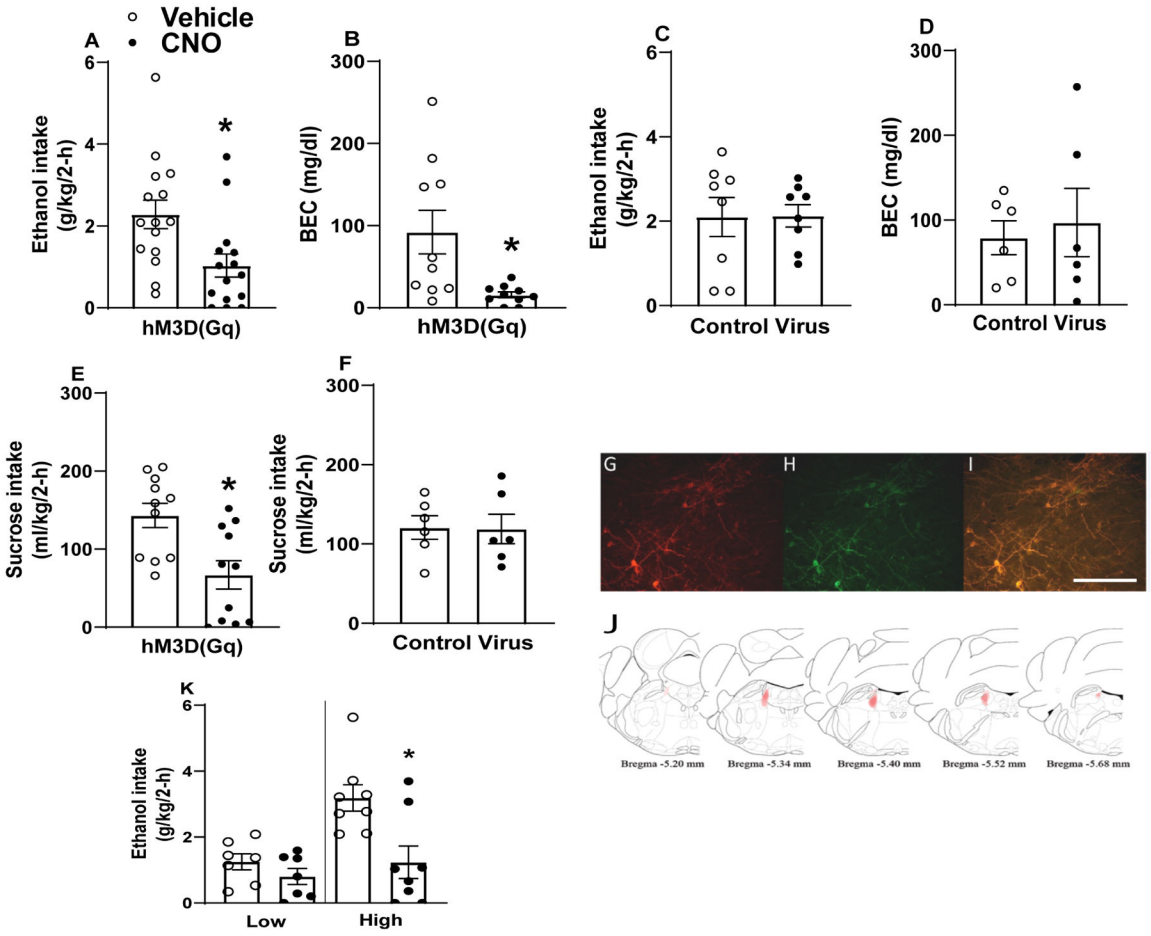

Figure 2. Chemogenetic activation of the LC blunts binge-like ethanol intake in TH-*ires*-cre mice.

I.p. CNO, but not vehicle, blunts binge-like ethanol intake in TH-*ires*-cre mice expressing hM3Dq DREADD (*A; n*=15) but not control AAV (*C; n*=8). I.p. CNO, but not vehicle, blunts BECs in TH-*ires*-cre mice expressing hM3Dq DREADD (*B; n*=10) but not control AAV (*D; n*=6). I.p. CNO, but not vehicle, blunts intake of a 3% sucrose solution in TH-*ires*-cre mice expressing hM3Dq DREADD (*E; n*=11) but not control AAV (*F; n*=6). Representative photomicrographs of hM3Dq DREADD virus (red, *G*), tyrosine hydroxylase (TH) immunohistochemistry (green, *H*), and hM3Dq/TH coexpression (yellow, *I*). For zoomed out images of DREADD expression in the LC please see Suppl. Fig. 1. Heat map of hM3Dq DREADD virus spread, with darker areas indicating similar virus expression across mice and lighter areas indicating less-common expression patterns (*J*). (*K*) Further analyses of ethanol intake data indicated that chemogenetic activation of LC TH+ neurons blunted intake in mice with High baseline ethanol intake (upper 50% of a median-split) and not in mice with Low baseline intake (lower 50% of a median-split). All values indicate mean ± SEM. Data collapsed across sexes due to absence of statistically significant sex differences. * indicates $p < 0.05$. Scale bar = 100 μm.

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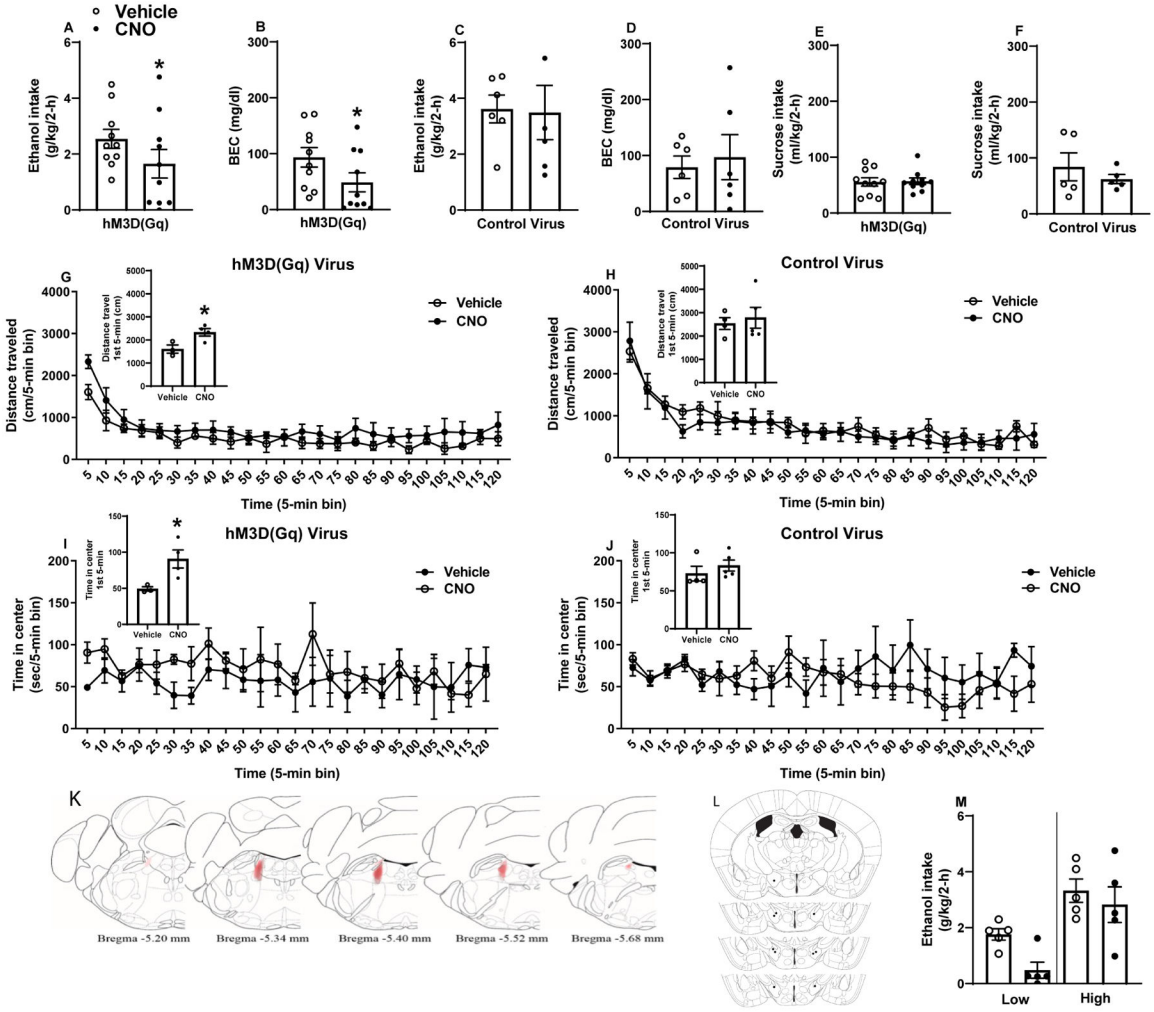

Figure 3. Chemogenetic activation of the LC ➔ LH pathway blunts binge-like ethanol intake in TH-*ires*-cre mice.

LH-targeted CNO, but not vehicle, blunts binge-like ethanol intake in TH-*ires*-cre mice expressing hM3Dq DREADD (*A; n*=10) but not control AAV (*C; n*=6) in LC. LH-targeted CNO, but not vehicle, blunts BECs in TH-*ires*-cre mice expressing hM3Dq DREADD (*B; n*=10) but not control AAV (*D; n*=5) in LH. In TH-*ires*-cre mice with a history of ethanol consumption in the DID paradigm, LH-targeted CNO failed to alter intake of a 3% sucrose solution in mice expressing hM3Dq DREADD (*E; n*=10) or control AAV (*F; n*=5) in the LC. In the OFT, LH-targeted CNO failed to alter total distance travelled in the 2-h test regardless of virus condition (*G* & *H*), though distance travelled in the first 5 minutes of testing as increased among mice expressing hM3Dq (*G inset*). Pathway activation in hM3D(Gq) treated mice increased time spent in chamber center during the first 5 minutes of testing (*I inset*), but we failed to observe a significant main effect of pathway activation on center time across the 2-h test (*I*). CNO alone failed to alter center time in control virus treated mice (*J*). Heat map of hM3Dq DREADD virus spread, with darker areas indicating similar virus expression across mice and lighter areas indicating less-common expression patterns (*K*). Approximate cannula placements at various anterior-posterior positions within

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the LH indicated by black dots (*L*). (*M*) Further analyses of ethanol intake data indicated that chemogenetic activation of LC TH+ neurons blunted intake in mice with no significant differences between mice with High baseline ethanol intake (upper 50% of a median-split) versus mice with Low baseline intake (lower 50% of a median-split). Data collapsed across sexes due to absence of statistically significant sex differences across experiments. All values indicate mean ± SEM. * indicates $p<0.05$ in paired *t*-test.

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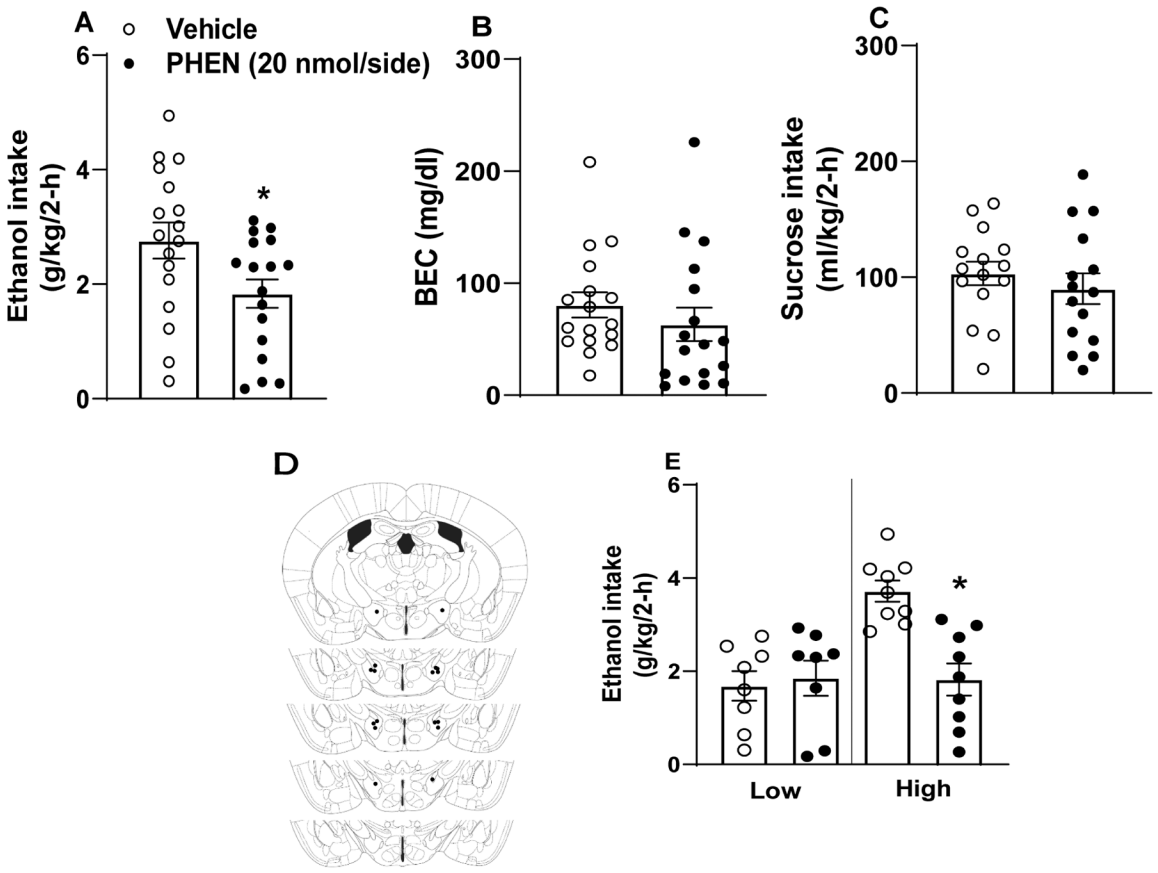

Figure 4. LH α1 adrenergic receptor activation blunts binge-like ethanol intake in C57BL6/J mice.

α1 agonism via LH-targeted phenylephrine (PHEN) blunted total ethanol intake (*A; n*=17) but not overall BECs (*B; n*=17) in the DID paradigm while failing to alter intake of a 3% sucrose solution (*C; n*=15). Male and female data are collapsed as we failed to observe an interaction of sex and drug during LH α1 manipulation. Approximate cannula placements (*D*). (*E*) Further analyses of ethanol intake data indicated that PHEN blunted intake in mice with High baseline ethanol intake (upper 50% of a median-split) and not in mice with Low baseline intake (lower 50% of a median-split). All values represent mean ± SEM. * represents $p<0.05$ in paired *t*-test.

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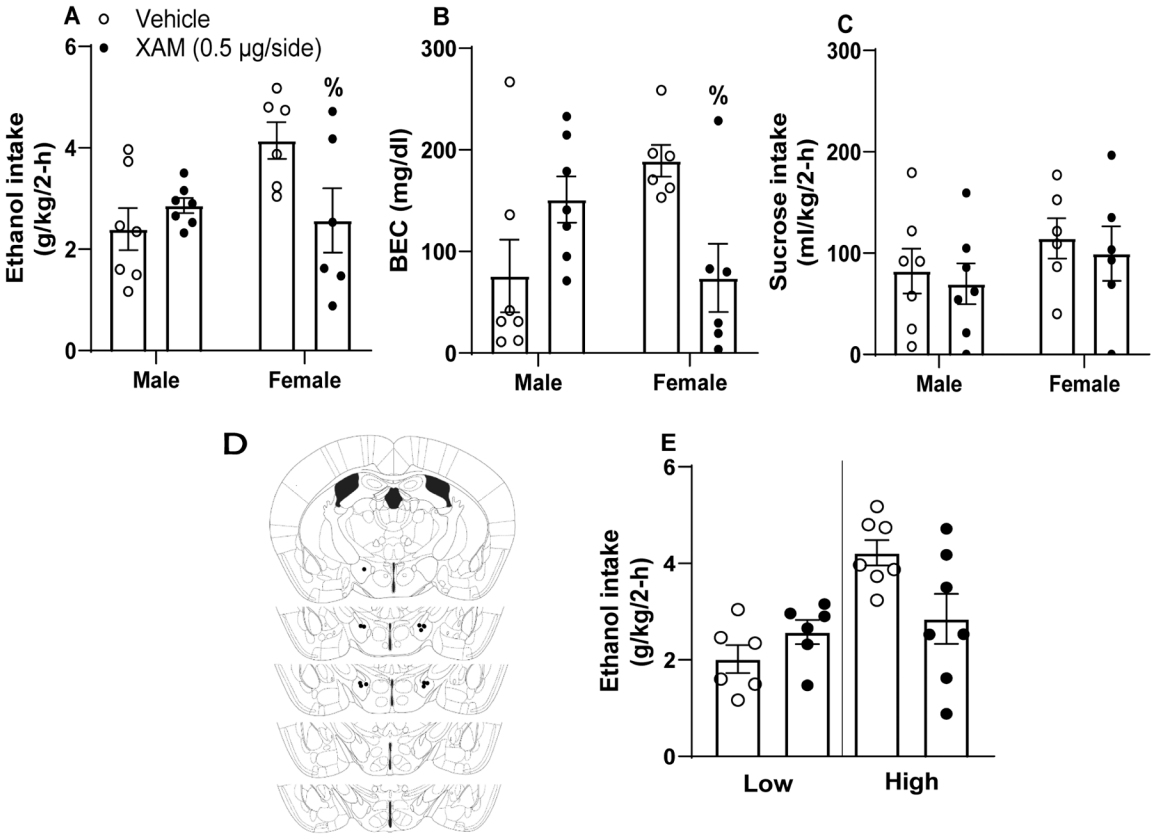

Figure 5. LH β adrenergic receptor activation blunts binge-like ethanol intake in female C57BL6/J mice.

β agonism via xamoterol hemifumerate (XAM) blunted binge-like ethanol intake (A) and BECs (B) in females exclusively without altering sucrose intake (C*; n*=6 female, 7 male). Approximate cannula placements within the LH (*D*). (*E*) Further analyses of ethanol intake data indicated, when collapsed across sexes, no significant differences between vehicle and XAM treatment in mice with High baseline ethanol intake (upper 50% of a median-split) or mice with Low baseline intake (lower 50% of a median-split). All values represent mean ± SEM. % represents significant interaction following two-way ANOVA as determined via Bonferroni post-hoc testing.

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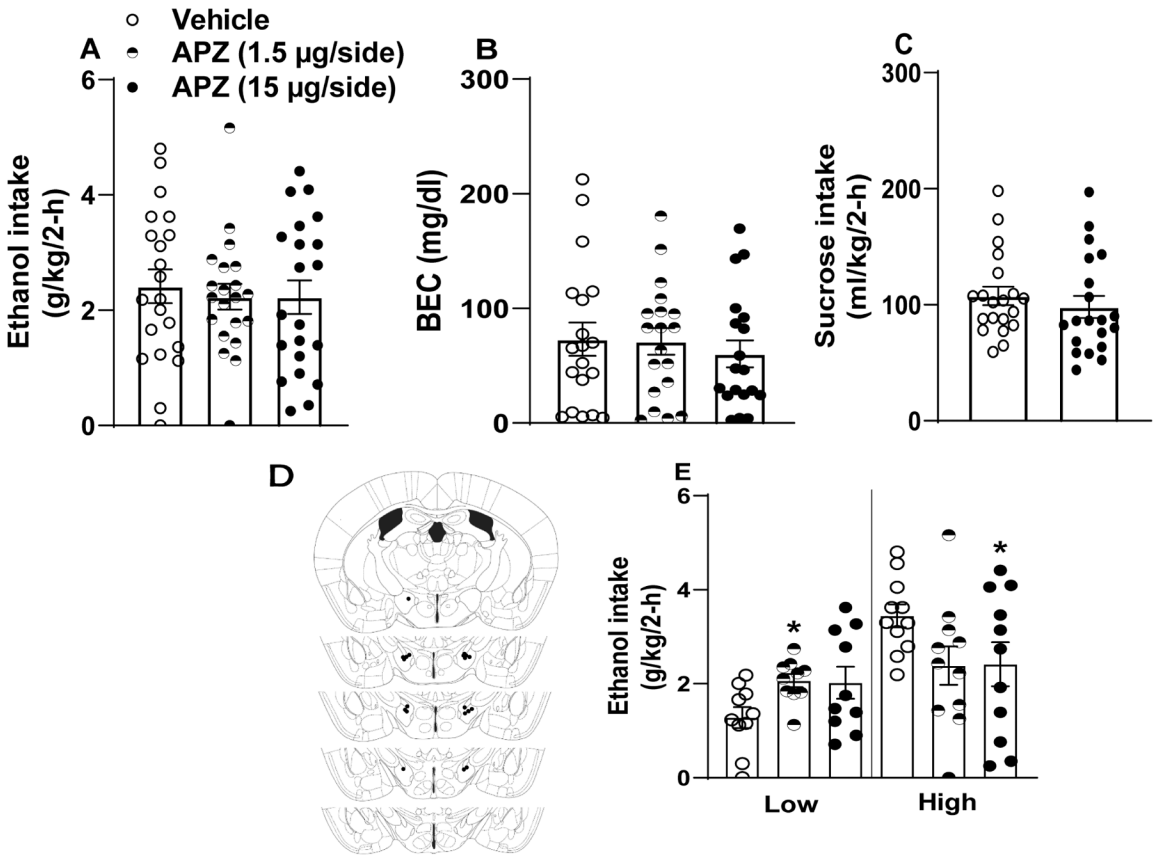

Figure 6. LH α2 adrenergic receptor blockade fails to blunt binge-like ethanol intake in C57BL6/J mice.

α2 antagonism via APZ failed to significantly alter total ethanol intake (*A; n*=21), BECs (*B*; n=21), or intake of a 3% sucrose solution (C; *n*=20). Approximate cannula placements within the LH (*D*). (*E*) Further analyses of ethanol intake data indicated that the high dose of APZ blunted intake in mice with High baseline ethanol intake (upper 50% of a median-split) and that the low dose of APZ increased ethanol intake in mice with Low baseline intake (lower 50% of a median-split). All values represent mean ± SEM.