Alcohol disrupts long-term potentiation at hippocampus-medium spiny neuron synapses in the medial shell of the nucleus accumbens

Ashley E. Copenhaver, Ph.D.[1], Jalane R. Campbell[1], Tara A. LeGates, Ph.D.[1,2]

[1]Department of Biological Sciences, University of Maryland, Baltimore County (UMBC)

[2]Department of Pharmacology and Physiology, University of Maryland, School of Medicine

Corresponding author:

Tara LeGates, Ph.D.

1000 Hilltop Circle, Interdisciplinary Life Sciences Building 315

Baltimore, MD 21250

tlegates@umbc.edu

Support: We would like to thank Dr. Yaohui Zhu and Kaela Befano for their assistance and Dr. Brian Mathur for his technical support and thoughtful comments on the manuscript. This work was supported by NSF IOS2402645, T32GM144876-02, the Meyerhoff program at UMBC, and start-up funds from UMBC.

ABSTRACT

Background: Chronic alcohol exposure is a major driver of alcohol use disorders (AUD), in part through its ability to induce maladaptive plasticity within neural circuits that regulate reward, motivation, and affect. Excitatory projections from the hippocampus (Hipp) to the nucleus accumbens (NAc) play a pivotal role in regulating reward-related behaviors, and this pathway serves as a key locus for establishing associations between rewarding stimuli and related contextual information. Regulation of the strength of Hipp-NAc synapses is critical for supporting these behaviors, and aberrant Hipp-NAc plasticity is associated with anhedonia and disrupted reward learning.

Methods: To examine acute ethanol effects, we used whole-cell electrophysiology to record Hipp-NAc synaptic plasticity in acute brain slices in the presence or absence of 50mM ethanol. To examine the effects of chronic ethanol administration, mice were exposed to ethanol vapor in a 3-week chronic intermittent ethanol (CIE) paradigm. Slices from ethanol and air exposed mice were used for whole-cell electrophysiology to record Hipp-NAc synaptic plasticity.

Results: Here, we demonstrate that acute ethanol application to *ex vivo* brain slices prevents long-term potentiation (LTP) at Hipp-NAc synapses, without altering presynaptic release probability. Furthermore, chronic intermittent exposure to ethanol abolishes LTP at these synapses, even during abstinence, indicating persistent synaptic dysfunction.

Conclusions: Together, our findings demonstrate that ethanol has immediate and long-lasting effects on Hipp-NAc plasticity. Given the behavioral relevance of these synapses, this work has important implications for the mechanisms underlying ethanol-dependent effects on reward processing and negative affective states associated with AUD.

KEYWORDS hippocampus, nucleus accumbens, synaptic plasticity, chronic intermittent ethanol, reward

INTRODUCTION

Alcohol exerts both immediate and long-lasting effects on motivated behaviors, cognitive function, and emotional regulation (Koob and Volkow, 2010). These effects arise primarily from alcohol's direct modulation of synaptic transmission and its influence over plasticity within key brain reward circuits (Lovinger and Abrahao, 2018). Although heightened plasticity in reward pathways may initially promote alcohol-seeking behaviors, persistent changes in circuit activation can ultimately impair the brain's ability to respond to natural rewards and trigger prolonged activation of stress-related neural systems. As a result, disruption of normal reward processing and persistent stress signaling are thought to underlie the negative emotional states frequently associated with chronic alcohol use and dependence. AUD with comorbid anhedonia (loss of pleasure derived from previously pleasurable activities) is associated with increased risk of relapse and poorer clinical outcomes, presenting a major obstacle to recovery (Garfield, Lubman and Yücel, 2014; Destoop *et al.*, 2019).

The nucleus accumbens (NAc) is a key mediator of motivated behaviors where both stress and rewarding experiences modulate synaptic function and plasticity to drive alterations in behavior (Carlezon and Thomas, 2009; Stuber, Britt and Bonci, 2012; Russo and Nestler, 2013). Excitatory input from several key brain regions (e.g., hippocampus, prefrontal cortex, thalamus, amygdala) has a crucial role in regulating NAc neuron activity to effectively encode reward-associated information and modulate motivated behaviors (O'Donnell and Grace, 1995; Goto and Grace, 2008; Calhoon and O'Donnell, 2013). Alterations in the strength and plasticity of these synapses as well as changes in excitability of NAc neurons have been associated with substance use disorders and depression, further demonstrating the key role of NAc activity in modulating motivated behaviors and emotional states (Volkow and Morales, 2015; Scofield *et al.*, 2016;

Francis and Lobo, 2017). For instance, chronic ethanol exposure enhances excitability and increases NMDAR activity in NAc neurons, consistently leading to increased volitional ethanol intake while also disrupting N-methyl-D-aspartate receptor (NMDAR)-dependent long-term depression (LTD) (Renteria *et al.*, 2017; Kircher *et al.*, 2019). This combination of effects mirrors the consequences of chronic stress, which similarly induces anhedonia and disrupts NMDAR-dependent LTD within the NAc (Lim *et al.*, 2012). Together, this suggests that ethanol and stress may converge on overlapping mechanisms within the NAc, particularly through alterations in excitatory synaptic plasticity, to promote anhedonia and dysregulated reward processing.

Projections from the hippocampus (Hipp) provide a majority of the excitatory input to the NAc, critically modulating neuronal activity and gating the flow of information through the region (O'Donnell and Grace, 1995; Wilson and Kawaguchi, 1996; Stern, Jaeger and Wilson, 1998; Goto and O'Donnell, 2001). Hipp-NAc connectivity has been tied to several behaviors related to reward and motivation in humans and rodents including positive reinforcement learning, substance abuse, positive affect, as well as spatial- and contextual-based reward learning (Floresco and Phillips, 1999; Ito *et al.*, 2008; Britt *et al.*, 2012; Davidow *et al.*, 2016; LeGates *et al.*, 2018; Sjulson *et al.*, 2018; Trouche *et al.*, 2019; Zhou *et al.*, 2019; Heller *et al.*, 2020; Huntley *et al.*, 2020; Sosa, Joo and Frank, 2020; Barnstedt, Mocellin and Remy, 2024). Our previous work revealed that activity-dependent strengthening of Hipp-NAc synapses drives reward-related behaviors while chronic stress disrupts this plasticity and induces anhedonia (LeGates *et al.*, 2018). Hipp and NAc activity also influences alcohol-related behaviors, where reduced Hipp activity causes an increase in alcohol consumption, and chemogenetic activation of Hipp-NAc synapses attenuates excessive drinking (Griffin *et al.*, 2023). Furthermore, chronic alcohol exposure induces pre- and postsynaptic changes at Hipp-NAc synapses and abolishes activity-dependent LTD (Kircher *et al.*, 2019), again highlighting the similarly powerful effects of chronic stress and chronic alcohol on synaptic function.

91 Focusing on medium spiny neurons (MSNs), which represent ~95% of the neuron population
92 in the NAc, we recently showed that LTP at Hipp-MSN synapses relies on NMDAR and L-type
93 voltage gated calcium channels (L-type VGCCs) in males and females, respectively (Copenhaver
94 and LeGates, 2024). Given the previously established ability of ethanol to inhibit NMDAR function
95 within the NAc and its ability to inhibit L-type VGCCs (Lovinger, White and Weight, 1989; Zucca
96 and Valenzuela, 2010; Mah, Fleck and Lindsley, 2011; Lee, Yeh and Yeh, 2022), we sought to
97 determine whether ethanol exposure influenced activity-dependent potentiation of Hipp-MSN
98 synapses. Here, we show that both acute *in vitro* and chronic *in vivo* exposure to ethanol prevents
99 LTP at Hipp-MSN synapses in males and females. Given the link between Hipp-NAc potentiation
100 in mediating reward-related behaviors, ethanol's effects on these synapses may be a key
101 mechanism driving maladaptive reward-related behaviors observed in response to alcohol use.

102

103 **MATERIALS AND METHODS**

104 Male and female C57BL/6J mice (8-10 weeks) were group housed (2-5 mice per cage) in a
105 12:12LD cycle with food and water ad libitum. We did not track the estrous cycle in female mice.
106 All experiments were performed in accordance with the regulations set forth by the Institutional
107 Animal Care and Use Committee at the University of Maryland, Baltimore County.

108 **Ethanol Vapor Chamber Exposure**

109 Male and female mice (8-10 weeks at the start of the experiment) were placed in acrylic
110 inhalation chambers (60 × 59 × 35 cm) in their home cages and exposed to chronic intermittent
111 ethanol (CIE) vapor or air 14 hours/day (ZT11-1), 4 days a week, for a total of 3 weeks.
112 Compressed air (10–20 psi) was passed through a tube submerged in 100% ethanol to volatilize.
113 Control vapor chambers delivered only air with no ethanol vapor. Vapor chamber ethanol
114 concentrations were monitored daily, and air flow was adjusted to produce ethanol concentrations

115 within 1.8–2.0% ethanol content as measured by a digital alcohol breath tester (BACTrack). These
116 conditions produce stable blood ethanol concentrations in C57BL/6J mice ranging from 100 to
117 200 mg/dl (Becker and Lopez, 2004). After four days in the inhalation chambers, mice underwent
118 a 72-h forced abstinence period. Whole cell electrophysiology was performed on week 4 after the
119 72-h abstinence period.

120 **Brain Slice Preparation**

121 Acute parasagittal slices (lateral 0.36-0.72) containing the fornix and nucleus accumbens
122 were prepared for whole-cell patch-clamp electrophysiology. Animals were deeply anesthetized
123 with isoflurane, decapitated, and brains were quickly dissected and submerged in ice-cold,
124 bubbled (carbogen: 95% O_2/5% CO_2) N-methyl-D-glucamine (NMDG) recovery solution
125 containing the following (in mM): 93 NMDG, 2.5 KCl, 1.2 NaH_2PO_4, 11 glucose, 25 $NaHCO_3$, 1.2
126 $MgCl_2$, and 2.4 $CaCl_2$, pH=7.3-7.4, osmolarity=300-310 mOsm. Using a vibratome (VT1000S,
127 Leica Microsystems), parasagittal slices (400 μm) were cut in cold, oxygenated NMDG. Slices
128 were transferred to 32-34°C NMDG for 7-12 minutes to recover and were then transferred to
129 room-temperature artificial cerebrospinal fluid (aCSF) containing the following (in mM): 120 NaCl,
130 3 KCl, 1.0 NaH_2PO_4, 20 glucose, 25 $NaHCO_3$, 1.5 $MgCl_2 \cdot 7H_2O$, and 2.5 $CaCl_2$, pH=7.3-7.4. Slices
131 were allowed to recover for 1-hour at room-temperature before beginning electrophysiological
132 recordings.

133 **Whole-Cell Recordings**

134 Slices were placed in a submersion-type recording chamber and superfused with room-
135 temperature aCSF (flow rate 0.5-1mL/min). Cells were visualized using a 60x water immersion
136 objective on a Nikon Eclipse FN-1 or Scientifica microscope. Whole-cell patch-clamp recordings
137 were performed using an Axopatch 200B amplifier (Axon Instruments, Molecular Devices) and a

138 Digidata 1550B digitizer (Axon Instruments). Patch pipettes (4-8MΩ) were made from borosilicate
139 glass (World Precision Instruments) using a Sutter Instruments P-97 model puller.

140 All recordings were performed in voltage-clamp conditions from MSNs in the NAc medial shell.
141 A bipolar stimulating electrode (FHC) was placed in the fornix to electrically stimulate hippocampal
142 axons while recording evoked excitatory postsynaptic currents (EPSC). Patch pipettes were filled
143 with a solution containing 130 mM K-gluconate, 5 mM KCl, 2 mM MgCl6-H2O, 10 mM HEPES, 4
144 mM Mg-ATP, 0.3 mM Na2-GTP, 10 mM Na2-phosphocreatine, and 1 mM EGTA; pH=7.3-7.4;
145 osmolarity=285-295mOsm. EPSCs were recorded from paired pulses (100ms apart) elicited
146 every 10s. After obtaining a stable 5-minute baseline EPSC recording, high-frequency stimulation
147 (HFS: four trains of 100Hz stimulation for 1s with 15s between trains while holding the cell at -
148 40mV) was applied, followed by a 30-minute recording of EPSCs. For acute ethanol experiments,
149 aCSF containing 50mM ethanol (Sigma) was washed onto slices for at least 20 minutes prior to
150 recording, and experiments were done in the presence of ethanol. We used the following
151 exclusion criteria to eliminate unhealthy cells and unreliable recordings: 1) We only proceeded
152 with experiments on cells with series resistances <10MΩ, 2) Cells were excluded if their series
153 resistance changed by >20% (comparing the resistance at the beginning and end of the
154 experiment), 3) Cells in poor health or poor recording status were excluded (i.e. cell partially or
155 fully sealed up, a decrease in holding current >100pA that is consistent with the cell dying, an
156 increase in jitter post HFS, and/or an increase in response failure rate to > 50%).

157 **Data analysis**

158 Statistical tests were performed using GraphPad Prism 9/10 software. A p-value of <0.05 was
159 considered statistically significant, and exact p-values can be found in the figure captions. For line
160 graphs, points represent the mean and error bars indicate SEM. Summary data plots display one
161 point for each cell, with each point representing the average EPSC amplitude from the last 5
162 minutes of recording. For bar graphs, the bar represents the mean and error bars indicate SEM

with individual data points overlayed. Statistical significance ($p<0.05$) was assessed by Two-way ANOVA (sex x treatment) with Šídák's multiple comparisons. A three-way ANOVA (sex x treatment x time) was used to analyze PPR data before and after HFS.

RESULTS

Acute ethanol application to *ex vivo* slices prevents Hipp-MSN LTP

To determine whether ethanol acutely influences plasticity at Hipp-MSN synapses, we recorded HFS-induced LTP in the presence and absence of 50mM ethanol. We found that, in the absence of ethanol, HFS induced LTP at Hipp-MSN synapses slices taken from male and female mice with no difference in magnitude between the sexes (Figure 1a-b), similar to our previous published results (LeGates *et al.*, 2018; Copenhaver and LeGates, 2024). However, pretreatment with 50mM ethanol prevented LTP induction in slices taken from males and females (Figure 1a-b; Sex x Treatment Interaction: $F_{(1,16)} = 0.1512$, $p=0.7025$; Main effect of Sex: $F_{(1,16)} = 0.01263$, $p=0.9119$; Main effect of treatment: $F_{(1,16)} = 19.24$, $p=0.0005$; Šídák's multiple comparisons post hoc: $p_{(\text{Male:control vs EtOH})} = 0.0175$, $p_{(\text{Female:control vs EtOH})} = 0.0107$). We observed no difference in paired pulse ratio (PPR) at baseline (Figure 1c; Sex x Treatment Interaction: $F_{(1,16)} = 0.003732$, $p=0.9520$; Main effect of Sex: $F_{(1,16)} = 1.066$, $p=0.3173$; Main effect of treatment: $F_{(1,16)} = 0.2307$, $p=0.6375$) suggesting that application of ethanol did not alter basal release probability. Furthermore, no change in PPR was observed following HFS irrespective of whether ethanol was present (Figure 1a, bottom; Time x Sex x Treatment Interaction: $F_{(2.590, 41.45)} = 0.5932$, $p=0.5992$; Time x Treatment Interaction: $F_{(2.590,41.45)} = 1.814$, $p=0.1661$; Time x Sex Interaction: $F_{(2.590, 41.45)} = 0.3843$, $p=0.7359$), suggesting that HFS did not elicit changes in presynaptic neurotransmitter release probability.

187 **CIE disrupts Hipp-MSN LTP**

188 To determine whether chronic exposure to ethanol leads to changes in Hipp-MSN plasticity,
189 we used CIE to ethanol vapor for controlled delivery and dosing of ethanol over a 3-week period.
190 Control mice were exposed to air. Following at least 72 hours of forced abstinence, slices from
191 CIE-exposed mice demonstrated a deficit in HFS-induced LTP relative to air-exposed controls,
192 which showed typical LTP (Figure 2a-b; Sex x Treatment Interaction: $F_{(1,20)} = 0.03737$, p=0.8487;
193 Main effect of Sex: $F_{(1,20)} = 0.1118$, p=0.7415; Main effect of treatment: $F_{(1,20)} = 12.53$, p=0.002;
194 Šídák's multiple comparisons post hoc: $p_{(\text{Male:control vs EtOH})} = 0.0444$, $p_{(\text{Female:control vs EtOH})} = 0.0390$).
195 This effect was consistent in both male and female mice. Comparison of baseline PPRs revealed
196 no difference between CIE mice and air exposed controls (Figure 2c; Sex x Treatment Interaction:
197 $F_{(1,20)} = 0.2254$, p=0.6401; Main effect of Sex: $F_{(1,20)} = 0.0008649$, p=0.9768; Main effect of
198 treatment: $F_{(1,20)} = 1.146$, p=0.2971), suggesting that CIE did not induce changes in release
199 probability. Similar to the data shown in Figure 1, HFS did not induce changes in PPR regardless
200 of ethanol exposure or sex (Figure 2a bottom; Time x Sex x Treatment Interaction: $F_{(6,20)} = 1.600$,
201 p=0.1529; Time x Treatment Interaction: $F_{(6,20)} = 0.6492$, p=0.6906; Time x Sex Interaction: $F_{(6,20)}$
202 = 1.067, p=3862), demonstrating a lack of effect on presynaptic function.

203

204 **DISCUSSION**

205 Our data demonstrate that acute application of ethanol to *ex vivo* brain slices disrupts HFS-
206 induced LTP at Hipp-MSN synapses. We also found that chronic exposure to ethanol *in vivo*
207 interfered with HFS-induced LTP at Hipp-MSN synapses, an effect observed after a 72-hour
208 abstinence period when ethanol was no longer present. Neither treatment led to a change in basal
209 release probability, suggesting that ethanol may be acting postsynaptically to interfere with LTP.
210 Given the previously described role for Hipp-NAc synaptic strength in mediating reward-related

211 behaviors, ethanol's impact on Hipp-MSN plasticity has important implications for understanding
212 the neurobiological basis for disrupted reward processing and negative mood states associated
213 with alcohol use disorders.

214 **Alcohol influences affective states and cognitive function**

215 Acute alcohol intoxication and persistent alcohol consumption have extensive effects on
216 cognitive processing, executive function, and memory, while also influencing affective state and
217 emotional processing. Many of these alcohol-dependent changes in behavior are closely linked
218 to altered activity within key brain regions such as the Hipp and NAc. (Zorumski, Mennerick and
219 Izumi, 2014; Ewin *et al.*, 2019; Van Skike, Goodlett and Matthews, 2019; Bach *et al.*, 2021).
220 Hippocampal input to the NAc plays a central role in mediating reward-based learning and
221 memory, as well as regulating affective state. Furthermore, the bidirectional regulation of Hipp-
222 NAc connectivity is a critical regulator of reward-related behaviors and has been implicated in
223 substance use and depression (Davidow *et al.*, 2016; LeGates *et al.*, 2018; Sjulson *et al.*, 2018;
224 Trouche *et al.*, 2019; Heller *et al.*, 2020; Huntley *et al.*, 2020; Griffin *et al.*, 2023). We previously
225 showed that LTP of Hipp-NAc synapses facilitates reward-related behaviors while disruptions in
226 LTP is associated with anhedonia (LeGates *et al.*, 2018). Here, we show that both acute and
227 chronic ethanol exposure interfere with this behaviorally relevant LTP, mirroring the immediate
228 and lasting effects of ethanol on synaptic plasticity observed within the Hipp and at other synapses
229 in the NAc.

230 **Alcohol-dependent effects on synaptic function**

231 The ability of alcohol to exert effects across several temporal domains is likely due to its ability
232 to impact synaptic function and plasticity in several brain regions that regulate cognitive function
233 and emotional state such as the Hipp and NAc. At intoxicating concentrations (~ 40–60 mM),
234 ethanol can reversibly inhibit both NMDAR responses and LTP in the Hipp (Chandler, Harris and

235 Crews, 1998). However, the ethanol-mediated inhibition of NMDARs is partial at these
236 concentrations, and partial inhibition by selective NMDAR antagonists does not fully block LTP
237 (Schummers, Bentz and Browning, 1997; Izumi *et al.*, 2005). This would suggest that blockade of
238 NMDARs may not completely explain ethanol's effect on LTP and may involve alterations of
239 additional pathways. For instance, ethanol has also been found to block NMDAR-independent
240 LTP that instead relies on L-type VGCCs (Izumi *et al.*, 2005). Chronic ethanol, especially with
241 binge-like or high-dose patterns, consistently diminishes the ability of hippocampal neurons to
242 elicit LTP, an effect that can persist for days to months after ethanol withdrawal, though some
243 recovery is possible with prolonged abstinence (Roberto *et al.*, 2002). This is thought to be driven
244 by alterations in NMDAR-mediated responses and receptor expression as well as altered
245 $GABA_A$ receptor function, leading to an overall disruption in the balance required for normal
246 synaptic plasticity (Nelson, Ur and Gruol, 2005). Depending on the exposure model, chronic
247 ethanol can also induce changes in signaling pathways (like MAPK) and neurotrophins (BDNF,
248 NGF) as well as neuron loss and morphological alterations in the Hipp (Stragier *et al.*, 2015).

249 In the NAc shell, ethanol dose-dependently inhibits NMDAR-dependent LTD through a
250 mechanism involving GluN2B-containing NMDARs and Dopamine-1 receptor signaling while
251 chronic ethanol reduces NMDAR function and depresses NMDAR-dependent LTP in the NAc
252 (Jeanes, Buske and Morrisett, 2014). Post-withdrawal, GluN2B and mGluR1 expression
253 increases, altering synaptic signaling (Renteria *et al.*, 2017). Chronic intermittent ethanol (CIE)
254 exposure followed by withdrawal has time-dependent effects on synaptic plasticity: 24-hours after
255 the last ethanol exposure LTD converts to NMDAR-dependent LTP while 72-hours of abstinence
256 abolishes both LTD and LTP (Renteria *et al.*, 2017). These neuroadaptations promote aberrant
257 learning, reinforcing alcohol-seeking behaviors and contributing to dependence.

258 Our data demonstrate that acute ethanol treatment applied to slices and CIE *in vivo* prevent
259 LTP at Hipp-MSN synapses. The absence of ethanol during recordings from slices of CIE-

260 exposed mice suggests that ethanol has both acute and longer lasting effects on plasticity that
261 persist into abstinence. We previously found that the mechanisms underlying Hipp-MSN plasticity
262 are sex-specific where LTP in males is NMDAR-dependent while in females, LTP is dependent
263 on L-type VGCCs. Ethanol's ability to prevent LTP in both sexes may therefore be due to
264 combined effects on NMDARs and L-type VGCCs, in line with prior observations in the Hipp
265 (Chandler, Harris and Crews, 1998; Izumi *et al.*, 2005). Previous work also found that acute and
266 chronic ethanol treatment can interfere with NMDAR-dependent LTD of Hipp-MSN synapses
267 (Kircher *et al.*, 2019), providing further support that ethanol may be altering NMDAR function at
268 Hipp-NAc synapses. We did not observe ethanol-induced changes in release probability as
269 measured by PPR (Figure 1c and 2c). This differs from previous work where chronic ethanol
270 exposure through vapor and two-bottle choice induced presynaptic changes increasing Hipp input
271 to the NAc as well as postsynaptic changes involving preferential insertion of Ca^{2+}-permeable α-
272 amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) (Kircher *et al.*, 2019).
273 The variations in results between the two studies may be due to the differences in the length of
274 exposure time (3 weeks versus ~10 weeks) or the method of administration involving CIE and
275 two-bottle choice.

276 **Stress and alcohol**

277 The ethanol-induced effects on Hipp-NAc LTP observed in this study closely resemble those
278 we reported previously in response to chronic stress (LeGates *et al.*, 2018). In many respects,
279 responses to alcohol closely resemble stress responses. For instance, both acute alcohol or
280 stress exposure activate the hypothalamic-pituitary-adrenal (HPA) axis, whereas repeated or
281 chronic exposure leads to dysregulation of HPA axis function (Richardson *et al.*, 2008; Stephens
282 and Wand, 2012). Chronic stress is a common precipitator of several reward-related psychiatric
283 disorders including depression and substance use disorders, and there is a high rate of
284 comorbidity between depression and AUD that is associated with greater severity and poorer

outcomes (McHugh and Weiss, 2019). Stress-induced changes in excitatory synaptic function within key circuits involved in reward and emotion regulation are thought to be a crucial factor underlying the pathophysiology of depression (Thompson *et al.*, 2015), and there is evidence to suggest that drugs of misuse can elicit changes in circuit function similar to those observed in response to stress (Saal *et al.*, 2003). These overlapping neurobiological responses highlight the potential importance of interactions between stress, alcohol, and neural circuit function in depression and AUD pathophysiology, though further research is needed to fully delineate mechanisms and relevant circuits.

Hipp-NAc synapses are critical mediators of reward-related behaviors that are both stress and alcohol sensitive (LeGates *et al.*, 2018; Kircher *et al.*, 2019; Griffin *et al.*, 2023; Lucantonio *et al.*, 2025). Activation and potentiation of these synapses is rewarding and attenuates excessive alcohol consumption (Britt *et al.*, 2012; LeGates *et al.*, 2018; Griffin *et al.*, 2023) while chronic stress as well as acute and chronic alcohol exposure disrupt activity-dependent synaptic plasticity and is associated with anhedonia (LeGates *et al.*, 2018; Kircher *et al.*, 2019) (and Figures 1 and 2). Together, this underscores the crucial interplay between Hipp-NAc circuit function and motivated behaviors, highlighting how alcohol's influence can rapidly alter behavior and induce lasting disruptions that contribute to dependence and comorbid mental health disorders. Together, these insights position the Hipp-NAc pathway as a critical synaptic locus underlying alcohol dependence and depression, and a promising target for therapeutic intervention.

References

Bach, E.C. *et al.* (2021) ‘Chronic Ethanol Exposures Leads to a Negative Affective State in Female Rats That Is Accompanied by a Paradoxical Decrease in Ventral Hippocampus Excitability’, *Frontiers in Neuroscience*, 15. Available at: https://www.frontiersin.org/articles/10.3389/fnins.2021.669075 (Accessed: 28 November 2023).

Barnstedt, O., Mocellin, P. and Remy, S. (2024) ‘A hippocampus-accumbens code guides goal-directed appetitive behavior’, *Nature Communications*, 15(1), p. 3196. Available at: https://doi.org/10.1038/s41467-024-47361-x.

Becker, H.C. and Lopez, M.F. (2004) ‘Increased ethanol drinking after repeated chronic ethanol exposure and withdrawal experience in C57BL/6 mice’, *Alcoholism, Clinical and Experimental Research*, 28(12), pp. 1829–1838. Available at: https://doi.org/10.1097/01.alc.0000149977.95306.3a.

Britt, J.P. *et al.* (2012) ‘Synaptic and Behavioral Profile of Multiple Glutamatergic Inputs to the Nucleus Accumbens’, *Neuron*, 76, pp. 790–803.

Calhoon, G.G. and O’Donnell, P. (2013) ‘Closing the Gate in the Limbic Striatum: Prefrontal Suppression of Hippocampal and Thalamic Inputs’, *Neuron*, 78(1), pp. 181–190. Available at: https://doi.org/10.1016/j.neuron.2013.01.032.

Carlezon, W.A. and Thomas, M.J. (2009) ‘Biological substrates of reward and aversion: A nucleus accumbens activity hypothesis’, *Neuropharmacology*, 56(SUPPL. 1), pp. 122–132. Available at: https://doi.org/10.1016/j.neuropharm.2008.06.075.

Chandler, L.J., Harris, R.A. and Crews, F.T. (1998) ‘Ethanol tolerance and synaptic plasticity’, *Trends in Pharmacological Sciences*, 19(12), pp. 491–495. Available at: https://doi.org/10.1016/s0165-6147(98)01268-1.

Copenhaver, A.E. and LeGates, T.A. (2024) ‘Sex-Specific Mechanisms Underlie Long-Term Potentiation at Hippocampus→Medium Spiny Neuron Synapses in the Medial Shell of the Nucleus Accumbens’, *Journal of Neuroscience*, 44(27). Available at: https://doi.org/10.1523/JNEUROSCI.0100-24.2024.

Davidow, J.Y. *et al.* (2016) ‘An Upside to Reward Sensitivity: The Hippocampus Supports Enhanced Reinforcement Learning in Adolescence’, *Neuron*, 92(1), pp. 93–99. Available at: https://doi.org/10.1016/j.neuron.2016.08.031.

Destoop, M. *et al.* (2019) ‘Addiction, Anhedonia, and Comorbid Mood Disorder. A Narrative Review’, *Frontiers in Psychiatry*, 10, p. 311. Available at: https://doi.org/10.3389/fpsyt.2019.00311.

Ewin, S.E. *et al.* (2019) ‘Chronic Intermittent Ethanol Exposure Selectively Increases Synaptic Excitability in the Ventral Domain of the Rat Hippocampus’, *Neuroscience*, 398, pp. 144–157. Available at: https://doi.org/10.1016/j.neuroscience.2018.11.028.

Floresco, S.B. and Phillips, A.G. (1999) ‘Dopamine and hippocampal input to the nucleus accumbens play an essential role in the search for food in an unpredictable environment’, *Psychobiology*, 27(2), pp. 277–286. Available at: https://doi.org/10.3758/BF03332121.

Francis, T.C. and Lobo, M.K. (2017) ‘Emerging Role for Nucleus Accumbens Medium Spiny Neuron Subtypes in Depression’, *Biological Psychiatry*, 81(8), pp. 645–653. Available at: https://doi.org/10.1016/j.biopsych.2016.09.007.

Garfield, J.B.B., Lubman, D.I. and Yücel, M. (2014) ‘Anhedonia in substance use disorders: A systematic review of its nature, course and clinical correlates’, *Australian & New Zealand Journal of Psychiatry*, 48(1), pp. 36–51. Available at: https://doi.org/10.1177/0004867413508455.

Goto, Y. and Grace, A.A. (2008) ‘Limbic and cortical information processing in the nucleus accumbens’, *Trends in Neurosciences*, 31(11), pp. 552–558. Available at: https://doi.org/10.1016/j.tins.2008.08.002.

Goto, Y. and O’Donnell, P. (2001) ‘Network synchrony in the nucleus accumbens in vivo’, *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 21(12), pp. 4498–4504. Available at: https://doi.org/10.1523/JNEUROSCI.21-12-04498.2001.

Griffin, W.C. *et al.* (2023) ‘Alcohol dependence and the ventral hippocampal influence on alcohol drinking in male mice’, *Alcohol*, 106, pp. 44–54. Available at: https://doi.org/10.1016/j.alcohol.2022.10.004.

Heller, A.S. *et al.* (2020) ‘Association between real-world experiential diversity and positive affect relates to hippocampal-striatal functional connectivity.’, *Nature neuroscience*, 23(7), pp. 800–804. Available at: https://doi.org/10.1038/s41593-020-0636-4.

Huntley, E.D. *et al.* (2020) ‘Adolescent substance use and functional connectivity between the ventral striatum and hippocampus’, *Behavioural Brain Research*, 390, p. 112678. Available at: https://doi.org/10.1016/j.bbr.2020.112678.

Ito, R. *et al.* (2008) ‘Functional interaction between the hippocampus and nucleus accumbens shell is necessary for the acquisition of appetitive spatial context conditioning’, *Journal of Neuroscience*, 28(27), pp. 6950–6959. Available at: https://doi.org/10.1523/JNEUROSCI.1615-08.2008.

370 Izumi, Y. *et al.* (2005) ‘Acute effects of ethanol on hippocampal long-term potentiation and long-
371 term depression are mediated by different mechanisms’, *Neuroscience*, 136(2), pp. 509–517.
372 Available at: https://doi.org/10.1016/j.neuroscience.2005.08.002.

373 Jeanes, Z.M., Buske, T.R. and Morrisett, R.A. (2014) ‘Cell type-specific synaptic encoding of
374 ethanol exposure in the nucleus accumbens shell’, *Neuroscience*, 277, pp. 184–195. Available
375 at: https://doi.org/10.1016/j.neuroscience.2014.06.063.

376 Kircher, D.M. *et al.* (2019) ‘Ethanol Experience Enhances Glutamatergic Ventral Hippocampal
377 Inputs to D1 Receptor-Expressing Medium Spiny Neurons in the Nucleus Accumbens Shell’,
378 *Journal of Neuroscience*, 39(13), pp. 2459–2469. Available at:
379 https://doi.org/10.1523/JNEUROSCI.3051-18.2019.

380 Koob, G.F. and Volkow, N.D. (2010) ‘Neurocircuitry of Addiction’, *Neuropsychopharmacology*,
381 35(1), pp. 217–238. Available at: https://doi.org/10.1038/npp.2009.110.

382 Lee, S.M., Yeh, P.W.L. and Yeh, H.H. (2022) ‘L-Type Calcium Channels Contribute to Ethanol-
383 Induced Aberrant Tangential Migration of Primordial Cortical GABAergic Interneurons in the
384 Embryonic Medial Prefrontal Cortex’, *eNeuro*, 9(1). Available at:
385 https://doi.org/10.1523/ENEURO.0359-21.2021.

386 LeGates, T.A. *et al.* (2018) ‘Reward behaviour is regulated by the strength of hippocampus–
387 nucleus accumbens synapses’, *Nature*, 564(7735), pp. 258–262. Available at:
388 https://doi.org/10.1038/s41586-018-0740-8.

389 Lim, B.K. *et al.* (2012) ‘Anhedonia requires MC4R-mediated synaptic adaptations in nucleus
390 accumbens’, *Nature*, 487(7406), pp. 183–189. Available at: https://doi.org/10.1038/nature11160.

Lovinger, D.M. and Abrahao, K.P. (2018) ‘Synaptic plasticity mechanisms common to learning and alcohol use disorder’, *Learning & Memory (Cold Spring Harbor, N.Y.)*, 25(9), pp. 425–434. Available at: https://doi.org/10.1101/lm.046722.117.

Lovinger, D.M., White, G. and Weight, F.F. (1989) ‘Ethanol Inhibits NMDA-Activated Ion Current in Hippocampal Neurons’, *Science*, 243(4899), pp. 1721–1724.

Lucantonio, F. *et al.* (2025) ‘Ketamine rescues anhedonia by cell-type- and input-specific adaptations in the nucleus accumbens’, *Neuron*, p. S0896627325001394. Available at: https://doi.org/10.1016/j.neuron.2025.02.021.

Mah, S.J., Fleck, M.W. and Lindsley, T.A. (2011) ‘ETHANOL ALTERS CALCIUM SIGNALING IN AXONAL GROWTH CONES’, *Neuroscience*, 189, pp. 384–396. Available at: https://doi.org/10.1016/j.neuroscience.2011.05.042.

McHugh, R.K. and Weiss, R.D. (2019) ‘Alcohol Use Disorder and Depressive Disorders’, *Alcohol Research : Current Reviews*, 40(1), p. arcr.v40.1.01. Available at: https://doi.org/10.35946/arcr.v40.1.01.

Nelson, T.E., Ur, C.L. and Gruol, D.L. (2005) ‘Chronic intermittent ethanol exposure enhances NMDA-receptor-mediated synaptic responses and NMDA receptor expression in hippocampal CA1 region’, *Brain Research*, 1048(1), pp. 69–79. Available at: https://doi.org/10.1016/j.brainres.2005.04.041.

O’Donnell, P. and Grace, A.A. (1995) ‘Synaptic interactions among excitatory afferents to nucleus accumbens neurons: Hippocampal gating of prefrontal cortical input’, *Journal of Neuroscience*, 15(5 I), pp. 3622–3639. Available at: https://doi.org/10.1523/jneurosci.15-05-03622.1995.

413 Renteria, R. *et al.* (2017) ‘Selective alterations of NMDAR function and plasticity in D1 and D2
414 medium spiny neurons in the nucleus accumbens shell following chronic intermittent ethanol
415 exposure’, *Neuropharmacology*, 112(Pt A), pp. 164–171. Available at:
416 https://doi.org/10.1016/j.neuropharm.2016.03.004.

417 Richardson, H.N. *et al.* (2008) ‘Alcohol self-administration acutely stimulates the hypothalamic-
418 pituitary-adrenal axis, but alcohol dependence leads to a dampened neuroendocrine state’, *The*
419 *European Journal of Neuroscience*, 28(8), pp. 1641–1653. Available at:
420 https://doi.org/10.1111/j.1460-9568.2008.06455.x.

421 Roberto, M. *et al.* (2002) ‘Long-Term Potentiation in the Rat Hippocampus Is Reversibly
422 Depressed by Chronic Intermittent Ethanol Exposure’, *Journal of Neurophysiology*, 87(5), pp.
423 2385–2397. Available at: https://doi.org/10.1152/jn.2002.87.5.2385.

424 Russo, S.J. and Nestler, E.J. (2013) ‘The brain reward circuitry in mood disorders’, *Nature*
425 *Reviews Neuroscience*, 14(9), pp. 609–625. Available at: https://doi.org/10.1038/nrn3381.

426 Saal, D. *et al.* (2003) ‘Drugs of Abuse and Stress Trigger a Common Synaptic Adaptation in
427 Dopamine Neurons’, *Neuron*, 37(4), pp. 577–582. Available at: https://doi.org/10.1016/S0896-
428 6273(03)00021-7.

429 Schummers, J., Bentz, S. and Browning, M.D. (1997) ‘Ethanol’s inhibition of LTP may not be
430 mediated solely via direct effects on the NMDA receptor’, *Alcoholism, Clinical and Experimental*
431 *Research*, 21(3), pp. 404–408. Available at: https://doi.org/10.1111/j.1530-0277.1997.tb03783.x.

432 Scofield, M.D. *et al.* (2016) ‘The nucleus accumbens: Mechanisms of addiction across drug
433 classes reflect the importance of glutamate homeostasis’, *Pharmacological Reviews*, 68(3), pp.
434 816–871. Available at: https://doi.org/10.1124/pr.116.012484.

Sjulson, L. *et al.* (2018) ‘Cocaine Place Conditioning Strengthens Location-Specific Hippocampal Coupling to the Nucleus Accumbens’, *Neuron*, 98(5), pp. 926-934.e5. Available at: https://doi.org/10.1016/j.neuron.2018.04.015.

Sosa, M., Joo, H.R. and Frank, L.M. (2020) ‘Dorsal and Ventral Hippocampal Sharp-Wave Ripples Activate Distinct Nucleus Accumbens Networks’, *Neuron*, 105(4), pp. 725-741.e8. Available at: https://doi.org/10.1016/j.neuron.2019.11.022.

Stephens, M.A.C. and Wand, G. (2012) ‘Stress and the HPA axis: role of glucocorticoids in alcohol dependence’, *Alcohol Research: Current Reviews*, 34(4), pp. 468–483.

Stern, E.A., Jaeger, D. and Wilson, C.J. (1998) ‘Membrane potential synchrony of simultaneously recorded striatal spiny neuronsin vivo’, *Nature*, 394(6692), pp. 475–478. Available at: https://doi.org/10.1038/28848.

Stragier, E. *et al.* (2015) ‘Brain plasticity and cognitive functions after ethanol consumption in C57BL/6J mice’, *Translational Psychiatry*, 5(12), pp. e696–e696. Available at: https://doi.org/10.1038/tp.2015.183.

Stuber, G.D., Britt, J.P. and Bonci, A. (2012) ‘Optogenetic modulation of neural circuits that underlie reward seeking’, *Biological Psychiatry*, 71(12), pp. 1061–1067. Available at: https://doi.org/10.1016/j.biopsych.2011.11.010.

Thompson, S.M. *et al.* (2015) ‘An excitatory synapse hypothesis of depression’, *Trends in Neurosciences*, 38(5), pp. 279–294. Available at: https://doi.org/10.1016/j.tins.2015.03.003.

Trouche, S. *et al.* (2019) ‘A Hippocampus-Accumbens Tripartite Neuronal Motif Guides Appetitive Memory in Space’, *Cell*, 176(6), pp. 1393-1406.e16. Available at: https://doi.org/10.1016/j.cell.2018.12.037.

Van Skike, C.E., Goodlett, C. and Matthews, D.B. (2019) ‘Acute alcohol and cognition: Remembering what it causes us to forget’, *Alcohol*, 79, pp. 105–125. Available at: https://doi.org/10.1016/j.alcohol.2019.03.006.

Volkow, N.D. and Morales, M. (2015) ‘The Brain on Drugs: From Reward to Addiction’, *Cell*, 162(4), pp. 712–725. Available at: https://doi.org/10.1016/j.cell.2015.07.046.

Wilson, C.J. and Kawaguchi, Y. (1996) ‘The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons’, *Journal of Neuroscience*, 16(7), pp. 2397–2410. Available at: https://doi.org/10.1523/jneurosci.16-07-02397.1996.

Zhou, Y. *et al.* (2019) ‘A ventral CA1 to nucleus accumbens core engram circuit mediates conditioned place preference for cocaine’, *Nature Neuroscience*, 22(12), pp. 1986–1999. Available at: https://doi.org/10.1038/s41593-019-0524-y.

Zorumski, C.F., Mennerick, S. and Izumi, Y. (2014) ‘Acute and Chronic Effects of Ethanol on Learning-Related Synaptic Plasticity’, *Alcohol (Fayetteville, N.Y.)*, 48(1), pp. 1–17. Available at: https://doi.org/10.1016/j.alcohol.2013.09.045.

Zucca, S. and Valenzuela, C.F. (2010) ‘Low Concentrations of Alcohol Inhibit BDNF-Dependent GABAergic Plasticity via L-type Ca2+ Channel Inhibition in Developing CA3 Hippocampal Pyramidal Neurons’, *The Journal of Neuroscience*, 30(19), pp. 6776–6781. Available at: https://doi.org/10.1523/JNEUROSCI.5405-09.2010.

477 **Figure Legends**

478 **Figure 1: Ethanol interferes with LTP at Hipp-MSN synapses** a) Pretreatment of slices with
479 50mM ethanol prevents HFS-induced LTP at Hipp-MSN synapses in males and females (top).
480 Paired-pulse ratios were not altered by HFS or ethanol (bottom). b) Summary data from the last
481 5 minutes of recording with representative traces below. c) No differences in paired-pulse ratios
482 recorded in the presence and absence of ethanol with representative traces shown below. Scale
483 bar for representative traces is 20pA/10ms. * $p<0.05$, Scale bar for representative traces =
484 20pA/10ms.

485 **Figure 2: Chronic intermittent ethanol exposure disrupts LTP at Hipp-MSN synapses.** a)
486 Slices taken from male and female mice exposed to CIE show disrupted LTP as compared to air-
487 exposed controls (top). Paired-pulse ratios were not altered by HFS or ethanol (bottom). b)
488 Summary data from the last 5 minutes of recording with representative traces below. c) No
489 differences in paired-pulse ratios between cells recorded from air- and CIE-exposed mice with
490 representative traces shown below. * $p<0.05$, Scale bar for representative traces is 20pA/10ms.

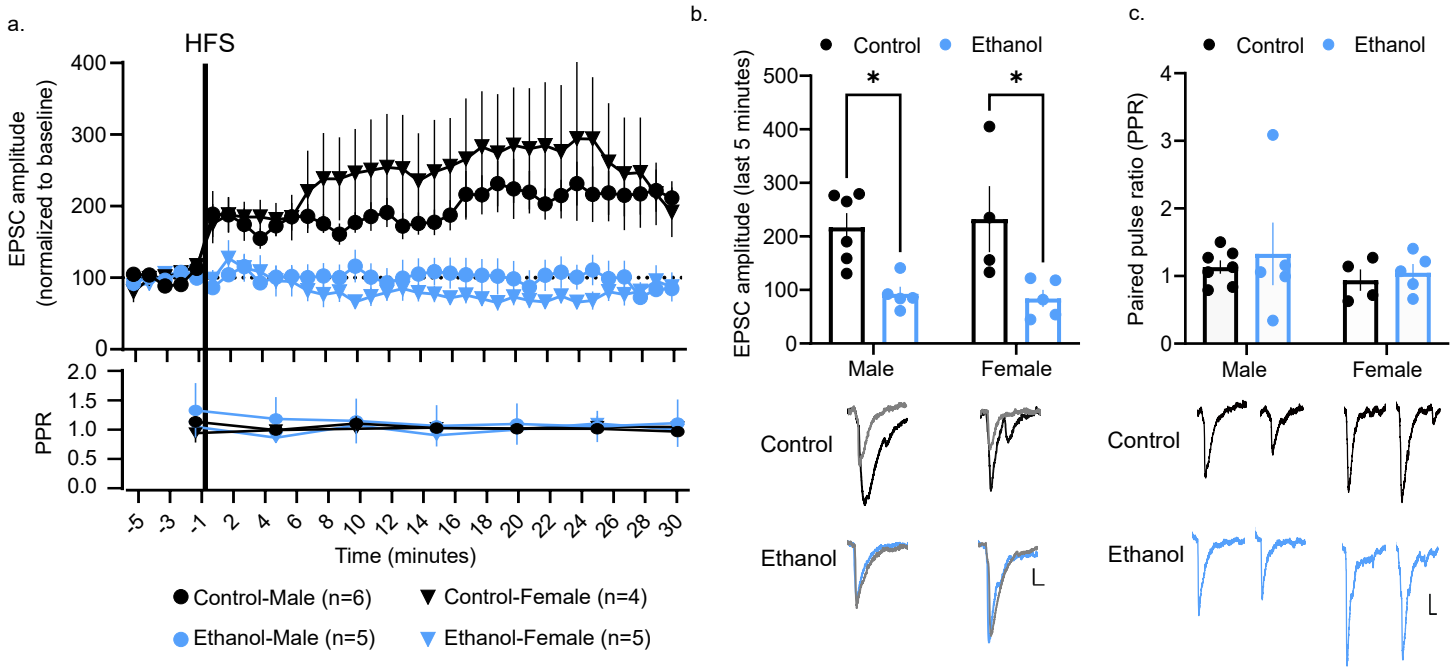
a.
HFS
EPSC amplitude (normalized to baseline)
400
300
200
100
0
PPR
2.0
1.5
1.0
0.5
0.0
-5
-3
-1
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Time (minutes)
Control-Male (n=6)
Control-Female (n=4)
Ethanol-Male (n=5)
Ethanol-Female (n=5)
b.
Control
Ethanol
EPSC amplitude (last 5 minutes)
500
400
300
200
100
0
*
*
Male
Female
Control
Ethanol
c.
Control
Ethanol
Paired pulse ratio (PPR)
4
3
2
1
0
Male
Female
Control
Ethanol

Figure 1

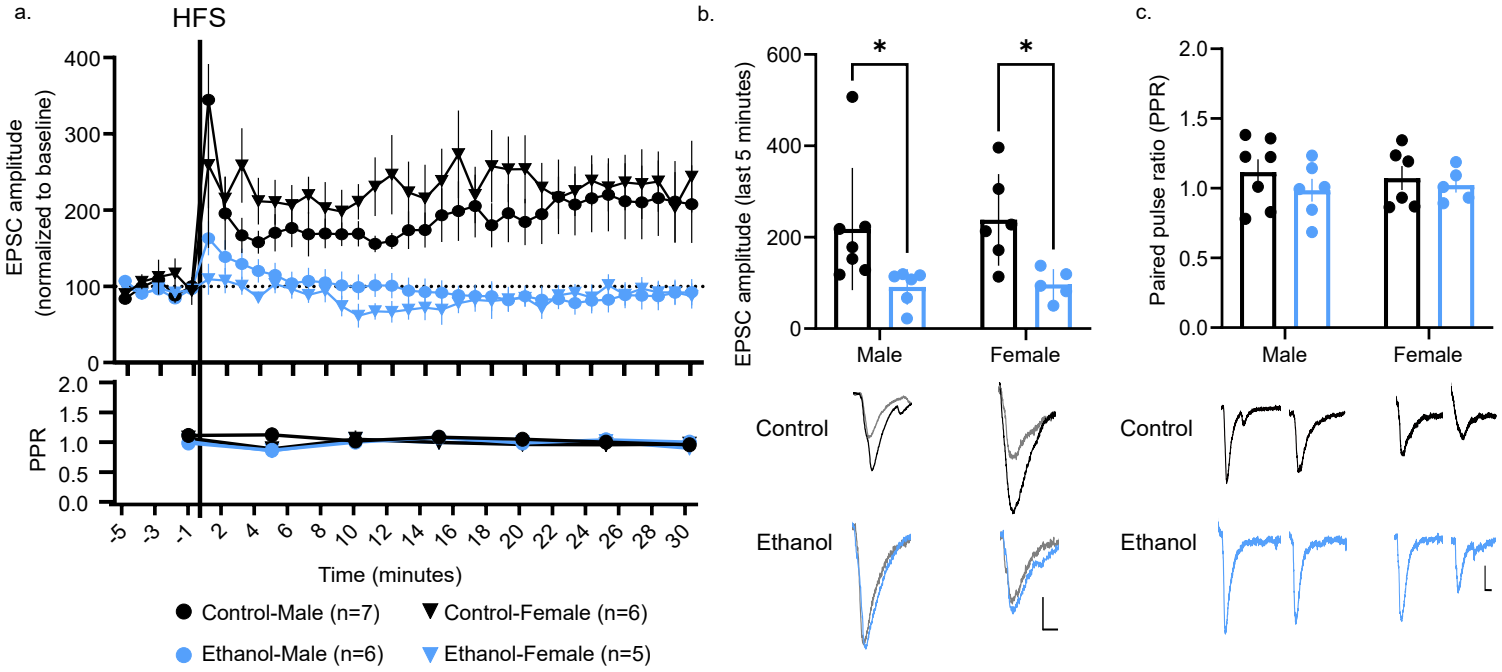
a.
HFS
EPSC amplitude
(normalized to baseline)
400
300
200
100
0
PPR
2.0
1.5
1.0
0.5
0.0
-5
-3
-1
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Time (minutes)
Control-Male (n=7)
Control-Female (n=6)
Ethanol-Male (n=6)
Ethanol-Female (n=5)
b.
EPSC amplitude (last 5 minutes)
600
400
200
0
*
*
Male
Female
Control
Ethanol
c.
Paired pulse ratio (PPR)
2.0
1.5
1.0
0.5
0.0
Male
Female
Control
Ethanol

Figure 2