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

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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 examine 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-NAc 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-NAc
98 synapses similarly in both sexes. Here, we show that both acute *in vitro* and chronic *in vivo*
99 exposure to ethanol prevents LTP at Hipp-NAc synapses in males and females. Given the link
100 between Hipp-NAc potentiation in mediating reward-related behaviors, ethanol's effects on these
101 synapses may be a key mechanism driving maladaptive reward-related behaviors observed in
102 response to alcohol use.

103

104 MATERIALS AND METHODS

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

109 Ethanol Vapor Chamber Exposure

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

concentrations were monitored daily, and air flow was adjusted to produce ethanol concentrations within 1.8-2.0% ethanol content as measured by a digital alcohol breath tester (BACTrack). After four days in the inhalation chambers, mice underwent a 72-h forced abstinence period. Whole cell electrophysiology was performed on week 4 after the 72-h abstinence period.

Brain Slice Preparation

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

Whole-Cell Recordings

Slices were placed in a submersion-type recording chamber and superfused with room-temperature aCSF (flow rate 0.5-1mL/min). Cells were visualized using a 60x water immersion objective on a Nikon Eclipse FN-1 or Scientifica microscope. Whole-cell patch-clamp recordings were performed using an Axopatch 200B amplifier (Axon Instruments, Molecular Devices) and a Digidata 1550B digitizer (Axon Instruments). Patch pipettes (4-8MΩ) were made from borosilicate glass (World Precision Instruments) using a Sutter Instruments P-97 model puller.

139 All recordings were performed in voltage-clamp conditions from MSNs in the NAc medial shell.
140 A bipolar stimulating electrode (FHC) was placed in the fornix to electrically stimulate hippocampal
141 axons while recording evoked excitatory postsynaptic currents (EPSC) (Figure 1a). Patch pipettes
142 were filled with a solution containing 130 mM K-gluconate, 5 mM KCl, 2 mM MgCl6-H2O, 10 mM
143 HEPES, 4 mM Mg-ATP, 0.3 mM Na2-GTP, 10 mM Na2-phosphocreatine, and 1 mM EGTA;
144 pH=7.3-7.4; osmolarity=285-295mOsm. EPSCs were recorded at -70mV from paired pulses
145 (100ms apart) elicited every 10s. After obtaining a stable 5-minute baseline EPSC recording, high-
146 frequency stimulation (HFS: four trains of 100Hz stimulation for 1s with 15s between trains while
147 holding the cell at -40mV) was applied, followed by a 30-minute recording of EPSCs. One cell
148 was recorded per slice. For acute ethanol experiments, aCSF containing 50mM ethanol (Sigma)
149 was washed onto slices for at least 20 minutes prior to recording, and experiments were done in
150 the presence of ethanol. We used the following exclusion criteria to eliminate unhealthy cells and
151 unreliable recordings: 1) We only proceeded with experiments on cells with series resistances
152 <10MΩ, 2) Cells were excluded if their series resistance changed by >20% (comparing the
153 resistance at the beginning and end of the experiment), 3) Cells in poor health or poor recording
154 status were excluded (i.e. cell partially or fully sealed up, a decrease in holding current >100pA
155 that is consistent with the cell dying, an increase in the variability of timing of EPSCs (jitter) post
156 HFS, and/or an increase in response failure rate to > 50%).

157 Data analysis

158 To analyze data from HFS experiments, EPSC amplitudes were normalized to the average
159 baseline amplitude. Normalized EPSC amplitudes were averaged over the last 5 minutes of the
160 recording (minutes 25-30 post HFS) to examine the direction and magnitude of plasticity. Paired
161 pulse ratio (PPR) was measured by calculating 5-minute averages of EPSC amplitudes from our
162 paired pulses ($EPSC_1$ and $EPSC_2$) and dividing $EPSC_2$ by $EPSC_1$ (ie: $EPSC_2/EPSC_1$ = PPR).
163 Statistical tests were performed using GraphPad Prism 9/10 software. A p-value of <0.05 was

considered statistically significant, and exact p-values can be found in the results section. For acute ethanol experiments, n represents the number of cells/slices. For chronic ethanol experiments, n represents the number of mice. For line graphs, points represent the mean and error bars indicate SEM. Summary data plots display one point for each cell, with each point representing the average EPSC amplitude from the last 5 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-NAc LTP

To determine whether ethanol acutely influences plasticity at Hipp-NAc synapses, we recorded HFS-induced LTP in the presence and absence of 50mM ethanol (Figure 1a). We found that, in the absence of ethanol, HFS induced LTP at Hipp-NAc synapses slices taken from male and female mice with no difference in magnitude between the sexes (Figure 1b-c), 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 1b-c; 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 1d; 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.

188 Furthermore, no change in PPR was observed following HFS irrespective of whether ethanol was
189 present (Figure 1b, bottom; Time x Sex x Treatment Interaction: $F_{(2.590, 41.45)} = 0.5932$, p=0.5992;
190 Time x Treatment Interaction: $F_{(2.590,41.45)} = 1.814$, p=0.1661; Time x Sex Interaction: $F_{(2.590, 41.45)} =$
191 0.3843, p=0.7359), suggesting that HFS did not elicit changes in presynaptic neurotransmitter
192 release probability.

193 CIE disrupts Hipp-NAc LTP

194 To determine whether chronic exposure to ethanol leads to changes in Hipp-NAc plasticity,
195 we used CIE to ethanol vapor for controlled delivery and dosing of ethanol over a 3-week period
196 (Figure 2a). Control mice were exposed to air. Following at least 72 hours of forced abstinence,
197 slices from CIE-exposed mice demonstrated a deficit in HFS-induced LTP relative to air-exposed
198 controls, which showed typical LTP (Figure 2b-c; Sex x Treatment Interaction: $F_{(1,20)} = 0.03737$,
199 p=0.8487; Main effect of Sex: $F_{(1,20)} = 0.1118$, p=0.7415; Main effect of treatment: $F_{(1,20)} = 12.53$,
200 p=0.002; Šídák's multiple comparisons post hoc: $p_{(\text{Male:control vs EtOH})} = 0.0444$, $p_{(\text{Female:control vs EtOH})} =$
201 0.0390). This effect was consistent in both male and female mice. Comparison of baseline PPRs
202 revealed no difference between CIE mice and air exposed controls (Figure 2d; Sex x Treatment
203 Interaction: $F_{(1,20)} = 0.2254$, p=0.6401; Main effect of Sex: $F_{(1,20)} = 0.0008649$, p=0.9768; Main
204 effect of treatment: $F_{(1,20)} = 1.146$, p=0.2971), suggesting that CIE did not induce changes in
205 release probability. Similar to the data shown in Figure 1, HFS did not induce changes in PPR
206 regardless of ethanol exposure or sex (Figure 2b bottom; Time x Sex x Treatment Interaction:
207 $F_{(6,20)} = 1.600$, p=0.1529; Time x Treatment Interaction: $F_{(6,20)} = 0.6492$, p=0.6906; Time x Sex
208 Interaction: $F_{(6,20)} = 1.067$, p=3862), demonstrating a lack of effect on presynaptic function.

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212 DISCUSSION

213 Our data demonstrate that acute application of ethanol to *ex vivo* brain slices disrupts HFS-
214 induced LTP at Hipp-NAc synapses. We also found that chronic exposure to ethanol *in vivo*
215 interfered with HFS-induced LTP at Hipp-NAc synapses, an effect observed after a 72-hour
216 abstinence period when ethanol was no longer present. Neither treatment led to a change in basal
217 release probability, suggesting that ethanol may be acting postsynaptically to interfere with LTP.
218 Given the previously described role for Hipp-NAc synaptic strength in mediating reward-related
219 behaviors, ethanol's impact on Hipp-NAc plasticity has important implications for understanding
220 the neurobiological basis for disrupted reward processing and negative mood states associated
221 with alcohol use disorders.

222 Alcohol influences affective states and cognitive function

223 Acute alcohol intoxication and persistent alcohol consumption have extensive effects on
224 cognitive processing, executive function, and memory, while also influencing affective state and
225 emotion. Chronic alcohol use contributes to the emergence of negative affective symptoms,
226 including depression-related features such as anhedonia and sleep disturbances (McHugh and
227 Weiss, 2019). Additionally, individuals with co-occurring alcohol use disorder (AUD) and major
228 depressive disorder (MDD) are at elevated risk for relapse and tend to exhibit more severe
229 symptoms (Garfield, Lubman and Yücel, 2014; Destoop *et al.*, 2019). These behavioral results
230 suggest key interplay between AUD and MDD that may contribute to the development and
231 severity of these disorders.

232 Bidirectional regulation of the strength of Hipp-NAc connectivity plays a central role in
233 mediating reward-based learning and memory, as well as regulating affective state and has been
234 implicated in substance use and depression (Davidow *et al.*, 2016; LeGates *et al.*, 2018; Sjulson
235 *et al.*, 2018; Trouche *et al.*, 2019; Heller *et al.*, 2020; Huntley *et al.*, 2020; Griffin *et al.*, 2023). We

236 previously showed that LTP of Hipp-NAc synapses facilitates reward-related behaviors while
237 disruptions in LTP is associated with anhedonia (LeGates *et al.*, 2018). Here, we show that both
238 acute and chronic ethanol exposure also interferes with LTP at these synapses, mirroring the
239 immediate and lasting effects of ethanol on synaptic plasticity previously observed within the Hipp
240 and at other synapses in the NAc. Given the role of Hipp-NAc synapses in mediating reward-
241 related behaviors, regulating affective state, and enduring alterations in response to ethanol, this
242 connection may represent a key site of information convergence, providing an opportunity to
243 identify mechanistic relationships among alcohol, mood, and cognitive processes.

244 Alcohol-dependent effects on synaptic function and plasticity

245 Alcohol has a wide range of effects on the brain, but a key factor that may underlie the ability
246 of alcohol to influence cognitive function and emotional state across several temporal domains is
247 its ability to impact synaptic function and plasticity in several relevant brain regions including the
248 Hipp and NAc (Zorumski, Mennerick and Izumi, 2014; Ewin *et al.*, 2019; Van Skike, Goodlett and
249 Matthews, 2019; Bach, Morgan, *et al.*, 2021). At intoxicating concentrations (~ 40–60 mM), acute
250 ethanol application can reversibly inhibit both NMDAR responses and LTP in the Hipp (Lovinger,
251 White and Weight, 1990; Zorumski, Mennerick and Izumi, 2014) as well as NMDAR-dependent
252 LTD of Hipp-NAc synapses (Kircher *et al.*, 2019). However, ethanol-mediated inhibition of
253 NMDARs is partial at these concentrations, and partial inhibition by selective NMDAR antagonists
254 does not fully block LTP (Schummers, Bentz and Browning, 1997; Izumi *et al.*, 2005). This would
255 suggest that NMDAR blockade does not completely explain ethanol's effect on LTP, implicating
256 potential involvement of additional signaling pathways. In line with this, ethanol has also been
257 found to block a form of NMDAR-independent LTP that is dependent on L-type VGCCs (Izumi *et*
258 *al.*, 2005). We previously demonstrated Hipp-NAc LTP is mediated by sex-specific mechanisms:
259 in males, LTP is NMDAR-dependent while in females, LTP is NMDAR-independent and instead
260 relies on L-type VGCCs (Copenhaver and LeGates, 2024). Dual-effects of ethanol on NMDARs

261 and L-type VGCCs, as observed in the Hipp (Chandler, Harris and Crews, 1998; Izumi *et al.*,
262 2005), may therefore explain the deficits in LTP we observed in both males and females at Hipp-
263 NAc synapses.

264 Chronic ethanol, especially with binge-like or high-dose patterns, consistently diminishes the
265 ability of hippocampal neurons to elicit LTP, an effect that can persist for days to months after
266 ethanol withdrawal (Roberto *et al.*, 2002; Zorumski, Mennerick and Izumi, 2014). Similar effects
267 on plasticity have been observed in the NAc shell where CIE-induced disruption of NMDAR-
268 dependent LTD persists for two weeks following the last exposure (Renteria, Buske and Morrisett,
269 2018). Changes in glutamatergic and GABAergic receptor function and expression, which can
270 lead to overall disruptions in excitation/inhibition balance, are thought to be key factors mediating
271 the effects of chronic ethanol on synaptic plasticity (Nelson, Ur and Gruol, 2005; Qiang, Denny
272 and Ticku, 2007; Obara *et al.*, 2009). Additionally, chronic ethanol exposure induces
273 hyperexcitability, neuron loss, and morphological alterations in the Hipp (Stragier *et al.*, 2015;
274 Bach, Ewin, *et al.*, 2021), which may serve as additional factors that ultimately contribute to
275 alterations in synaptic plasticity and produce long-lasting changes that persist into abstinence.

276 Our data demonstrate that chronic ethanol exposure disrupts LTP at Hipp-NAc synapses
277 (Figure 2b-c). The absence of ethanol during recordings from slices of CIE-exposed mice
278 indicates that ethanol produces long-lasting alterations in synaptic plasticity that persist into
279 abstinence, consistent with previously reported effects described above. The plasticity deficits we
280 observed are also supported by recent work demonstrating chronic ethanol exposure induces
281 disruption to NMDAR-dependent LTD of Hipp-NAc synapses (Kircher *et al.*, 2019). This effect
282 coincided with synaptic alterations consistent with increased presynaptic release probability and
283 postsynaptic incorporation of Ca^{2+}-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic
284 acid receptors (AMPARs), implicating both presynaptic and postsynaptic mechanisms in ethanol-
285 related plasticity disruptions (Kircher *et al.*, 2019). In contrast, we found no evidence of ethanol-

286 induced changes in release probability, as measured by paired-pulse ratio (PPR) (Figure 1d and
287 2d) suggesting ethanol is likely acting primarily through postsynaptic mechanisms to interfere with
288 LTP. The differences in experimental outcomes may also be due to differences in study design
289 including the length of exposure time (3 weeks versus ~10 weeks), the method of administration
290 in the previous study involving CIE and two-bottle choice, or the length of the abstinence period
291 (72 versus 24 hours).

292 As discussed above, the mechanisms underlying LTP at Hipp-NAc synapses are sex-specific,
293 so while previously described alterations in NMDAR function and expression may explain the
294 effects of ethanol on LTP in males, this does not fully explain the LTP deficits we observed in
295 females. A considerable amount of the previous work that has provided the mechanistic insight
296 into ethanol's influence on synaptic function and plasticity has relied on data collected from males.
297 Growing evidence of sex differences in synaptic function suggests that focusing on only one sex
298 may obscure key molecular mechanisms underlying alcohol's effects. These synaptic differences
299 may be hidden, producing similar behaviors across sexes, or manifest overtly in the well-
300 documented disparities between males and females in alcohol sensitivity and vulnerability to AUD
301 (Erol and Karpyak, 2015; Flores-Bonilla and Richardson, 2020). Taken together, the ability of
302 ethanol to disrupt plasticity of synapses that rely on inherent sex-specific mechanisms highlights
303 the complexity of mechanistically dissecting its effects on synaptic function. These findings also
304 point to the potential for sex-specific synaptic adaptations that may contribute to differential
305 vulnerability and dependence.

306 Stress and alcohol

307 The ethanol-induced effects on Hipp-NAc LTP observed in this study closely resemble those
308 we reported previously in response to chronic stress (LeGates *et al.*, 2018). In many respects,
309 responses to alcohol closely resemble stress responses. For instance, both acute alcohol or
310 stress exposure activate the hypothalamic-pituitary-adrenal (HPA) axis, whereas repeated or

311 chronic exposure leads to dysregulation of HPA axis function (Richardson *et al.*, 2008; Stephens
312 and Wand, 2012). Chronic stress is a common precipitator of several reward-related psychiatric
313 disorders including depression and substance use disorders, and there is a high rate of
314 comorbidity between depression and AUD that is associated with greater severity and poorer
315 outcomes (McHugh and Weiss, 2019). Stress-induced changes in excitatory synaptic function
316 within key circuits involved in reward and emotion regulation are thought to be a crucial factor
317 underlying the pathophysiology of depression (Thompson *et al.*, 2015), and there is evidence to
318 suggest that drugs of misuse can elicit changes in circuit function similar to those observed in
319 response to stress (Saal *et al.*, 2003). These overlapping neurobiological responses highlight the
320 potential importance of interactions between stress, alcohol, and neural circuit function in
321 depression and AUD pathophysiology, though further research is needed to fully delineate
322 mechanisms and relevant circuits.

323 Hipp-NAc synapses are critical mediators of reward-related behaviors that are both stress and
324 alcohol sensitive (LeGates *et al.*, 2018; Kircher *et al.*, 2019; Griffin *et al.*, 2023; Lucantonio *et al.*,
325 2025). Activation and potentiation of these synapses is rewarding and attenuates excessive
326 alcohol consumption (Britt *et al.*, 2012; LeGates *et al.*, 2018; Griffin *et al.*, 2023) while chronic
327 stress as well as acute and chronic alcohol exposure disrupt activity-dependent synaptic plasticity
328 and is associated with anhedonia (LeGates *et al.*, 2018; Kircher *et al.*, 2019) (and Figures 1 and
329 2). Together, this underscores the crucial interplay between Hipp-NAc circuit function and
330 motivated behaviors, highlighting how alcohol's influence can rapidly alter behavior and induce
331 lasting disruptions that contribute to dependence and comorbid mental health disorders. Together,
332 these insights position the Hipp-NAc pathway as a critical synaptic locus underlying alcohol
333 dependence and depression, and a promising target for therapeutic intervention.

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522 Figure Legends

523 Figure 1: Ethanol interferes with LTP at Hipp-NAc synapses a) Schematic of recording strategy
524 with stimulating electrode in the fornix and recording electrode in the NAc medial shell to record
525 Hipp-evoked EPSCs from MSNs. The illustrated parasagittal section represents a slice from
526 lateral +0.48mm. ac-anterior commissure f-fornix. b) Pretreatment of slices with 50mM ethanol
527 prevents HFS-induced LTP at Hipp-NAc synapses in males and females (top). Paired-pulse ratios
528 were not altered by HFS or ethanol (bottom). c) Summary data from the last 5 minutes of recording
529 with representative traces below. Gray traces indicate baseline and black/blue indicate post-HFS.
530 d) No differences in paired-pulse ratios recorded in the presence and absence of ethanol with
531 representative traces shown below. n= $6_{\text{control male}}$, $4_{\text{control female}}$, $5_{\text{ethanol male}}$, $5_{\text{ethanol female}}$ cells. Scale
532 bar for representative traces is 20pA/10ms. * $p<0.05$, Scale bar for representative traces =
533 20pA/10ms.

534 Figure 2: Chronic intermittent ethanol exposure disrupts LTP at Hipp-NAc synapses. a) Schematic
535 of experimental design to record Hipp-evoked EPSCs in the NAc shell of mice exposed to CIE or
536 air. ac-anterior commissure f-fornix. b) Slices taken from male and female mice exposed to CIE
537 show disrupted LTP as compared to air-exposed controls (top). Paired-pulse ratios were not
538 altered by HFS or ethanol (bottom). c) Summary data from the last 5 minutes of recording with
539 representative traces below. Gray traces indicate baseline and black/blue indicate post-HFS. d)
540 No differences in paired-pulse ratios between cells recorded from air- and CIE-exposed mice with
541 representative traces shown below. n= $7_{\text{control male}}$, $6_{\text{control female}}$, $6_{\text{ethanol male}}$, $5_{\text{ethanol female}}$ mice * $p<0.05$,
542 Scale bar for representative traces is 20pA/10ms.

543

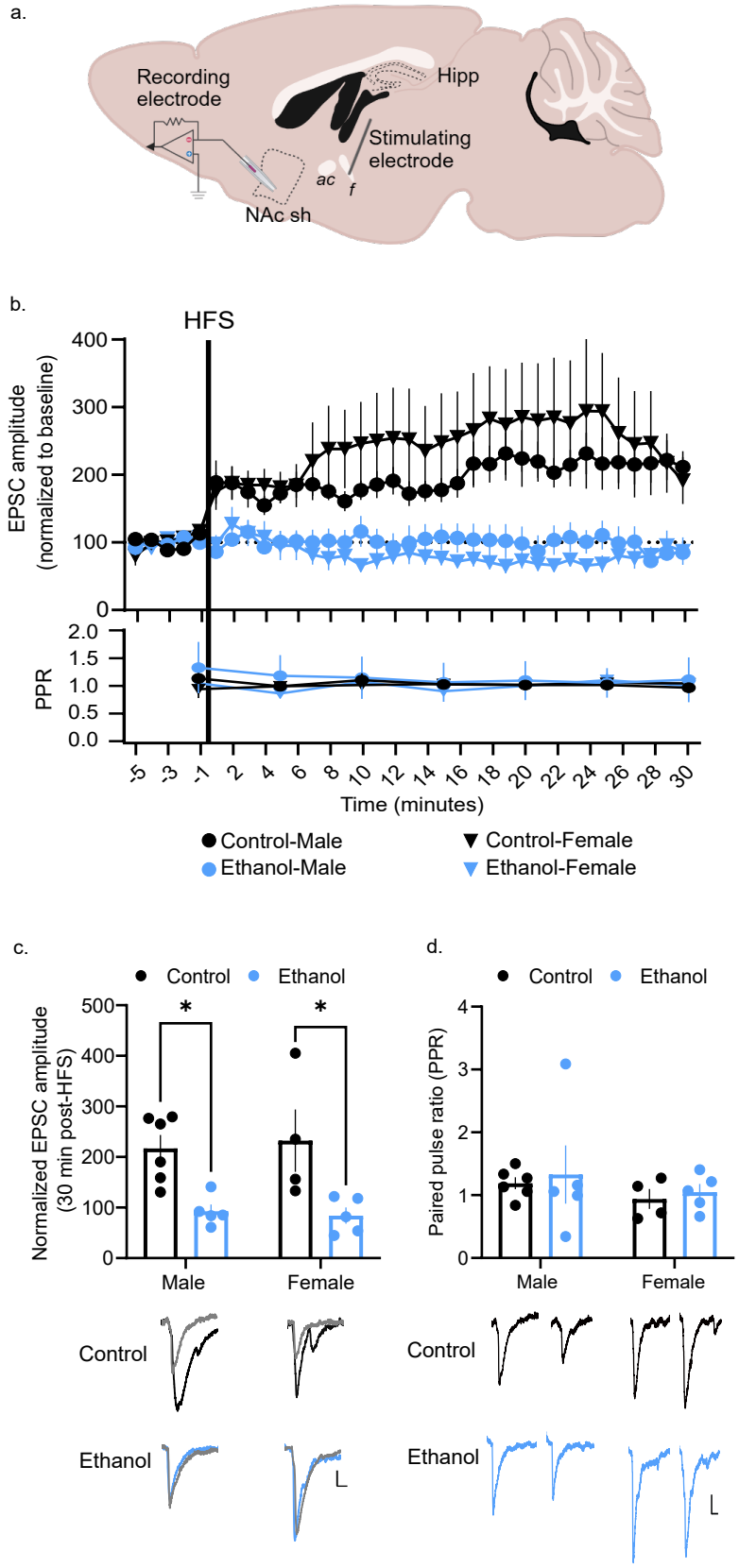
a.
Recording electrode
Hipp
Stimulating electrode
ac
f
NAc sh
b.
HFS
EPSC amplitude (normalized to baseline)
400
300
200
100
0
PPR
2.0
1.5
1.0
0.5
0.0
-5
-3
-1
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Time (minutes)
Control-Male
Control-Female
Ethanol-Male
Ethanol-Female
c.
Control
Ethanol
Normalized EPSC amplitude (30 min post-HFS)
500
400
300
200
100
0
*
*
Male
Female
Control
Ethanol
L
d.
Control
Ethanol
Paired pulse ratio (PPR)
4
3
2
1
0
Male
Female
Control
Ethanol
L

Figure 1

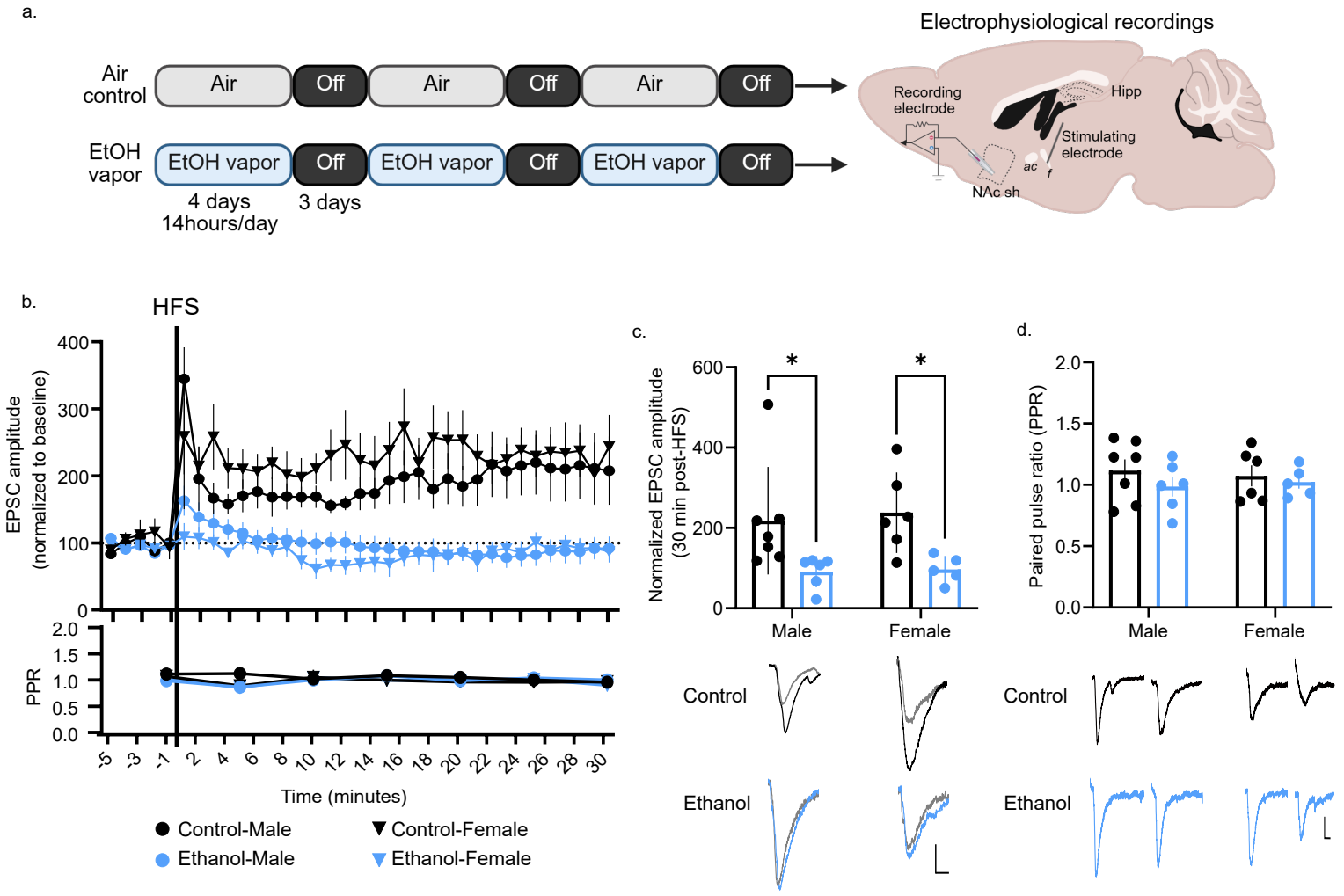
a.
Electrophysiological recordings
Air control
Air
Off
Air
Off
Air
Off
EtOH vapor
EtOH vapor
Off
EtOH vapor
Off
EtOH vapor
Off
4 days
14hours/day
3 days
Recording electrode
Hipp
Stimulating electrode
ac
f
NAc sh
b.
HFS
EPSC amplitude (normalized to baseline)
PPR
Time (minutes)
Control-Male
Control-Female
Ethanol-Male
Ethanol-Female
c.
Normalized EPSC amplitude (30 min post-HFS)
Male
Female
Control
Ethanol
d.
Paired pulse ratio (PPR)
Male
Female
Control
Ethanol

Figure 2