eNeuro

Research Article: New Research | Neuronal Excitability

Sex differences in the alcohol-mediated modulation of BLA network states

https://doi.org/10.1523/ENEURO.0010-22.2022

Cite as: eNeuro 2022; 10.1523/ENEURO.0010-22.2022

Received: 7 January 2022
Revised: 19 May 2022
Accepted: 2 June 2022

Sex differences in the alcohol-mediated modulation of BLA network states

Alyssa DiLeo[1], Pantelis Antonodiou[2], Spencer Ha[2], Jamie L. Maguire[2]

[1]Program in Neuroscience, Graduate School of Biomedical Sciences, Tufts University, Boston, MA 02111, USA; [2]Department of Neuroscience, Tufts School of Medicine, Tufts University, Boston, MA 02111, USA

Author Contributions: AD and JM designed research; AD and SH performed research, AD and PA analyzed data; AD, PA, and JM wrote the paper.

Correspondence should be addressed to jamie.maguire@tufts.edu

Number of figures: 5 (7 extended data figures)
Number of tables: 6
Number of multimedia: 0
Number of words for abstract: 208
Number of words for significance statement: 113
Number of words for introduction: 677
Number for words for discussion: 1936

Acknowledgements: The authors would like to thank Dr. Klaus Miczek, Dr. Laverne Melón, and Dr. Leon Reijmers for thoughtful feedback and guidance on this project. Visual abstract created with BioRender.com.

Conflict of Interest: JLM serves as a member of the Scientific Advisory Board for SAGE Therapeutics, Inc. for work unrelated to this project. All other authors report no potential biomedical financial interests or conflicts of interest.

Funding Sources: Authors are supported by funding from the National Institutes of Health under award numbers F31AA028410, R01AA026256, R01NS105628, R01NS102937, R01MH128235, and P50MH122379.

Abstract

Alcohol use, reported by 85% of adults in the United States, is highly comorbid with mood disorders, like generalized anxiety disorder and major depression. The basolateral amygdala (BLA) is an area of the brain that is heavily implicated in both mood disorders and alcohol use disorder. Importantly, modulation of BLA network/oscillatory states via parvalbumin-positive (PV) GABAergic interneurons has been shown to control the behavioral expression of fear and anxiety. Further, PV interneurons express a high density of δ-subunit-containing $GABA_A$ receptors ($GABA_A$Rs), which are sensitive to low concentrations of alcohol. Therefore, we hypothesized that the effects of alcohol may modulate BLA network states that have been associated with fear and anxiety behaviors via δ-$GABA_A$Rs on PV interneurons in the BLA. Given the impact of ovarian hormones on the expression of δ-$GABA_A$Rs, we also examined the ability of alcohol to modulate local field potentials (LFPs) in the BLA from male and female C57BL/6J and *Gabrd*$^{-/-}$ mice after acute and repeated exposure to alcohol. Here, we demonstrate that acute and repeated alcohol can differentially modulate oscillatory states in male and female C57BL/6J mice, a process which involves δ-$GABA_A$Rs. This is the first study to demonstrate that alcohol is capable of altering network states implicated in both anxiety and alcohol use disorders.

Significance Statement

Alcohol use disorder and mood disorders are highly comorbid. The basolateral amygdala (BLA) is implicated in both disorders, but the mechanisms contributing to their shared pathophysiology remain uncertain. Here we demonstrate that acute and repeated alcohol exposure can alter

network oscillations in the BLA which control the behavioral expression of fear and anxiety. These data suggest that alcohol may directly influence network states associated with mood. Further, we demonstrate sex differences in alcohol's ability to modulate BLA network states, an effect involving δ-$GABA_A$ receptors, which may contribute to sex differences in alcohol intake and comorbid mood disorders. These data potentially point to a novel mechanism mediating the effects of alcohol on affective states.

Keywords: alcohol use disorder; basolateral amygdala; local field potentials; network states; oscillations; sex differences; GABA; extrasynaptic receptors

Introduction

Alcohol is the most widely used drug in the United States, with approximately 85% of adults reporting alcohol use in their lifetime. Despite this high rate of use, only about 5% will go on to develop an alcohol use disorder while most adults continue to drink without reaching this diagnostic criterion (SAMHSA, 2020). The transition from first drink to alcohol dependence is encouraged by both the positive and negative reinforcing effects of alcohol, each with corresponding neurobiological frameworks (Gilpin and Koob, 2008). Comorbid mood disorders, such as major depression and anxiety disorders, contribute to the reinforcing effects of alcohol by pushing individuals to drink to relieve tension in high stress or high anxiety situations (Kushner et al., 2011). The basolateral amygdala (BLA) has been identified as a brain region contributing to both alcohol use disorder and anxiety disorders (Silberman et al., 2009; Tye et al., 2011; Agoglia and Herman, 2018).

Accumulating evidence demonstrates a critical role for oscillatory states in the BLA in modulating fear and anxiety-like behaviors (Likhtik et al., 2013; Stujenske et al., 2014; Davis et

al., 2017; Antonoudiou et al., 2021). However, the impact of alcohol on these network states has not been explored. Network oscillations within and between brain areas represent a mechanism for the transition between brain and behavioral states. Specifically, particular oscillation frequencies within and between the BLA and mPFC are associated with either a fear (3-6 Hz) or safety (6-12 Hz) state (Davis et al., 2017). This circuit, along with other regions like the hippocampus, has also been shown to contribute to high and low anxiety states in mice (Likhtik et al., 2013).

It is well established that the anxiolytic properties of alcohol can motivate consumption and contribute to the high comorbidity of alcohol use disorders and mood disorders (Thomas et al., 2003; Smith and Randall, 2012; Mason et al., 2018). However, it is unclear how alcohol impacts network states underlying modulation of anxiety states. Here we examine the ability of acute, low dose alcohol to modulate BLA network activity in alcohol naïve mice, using local field potentials (LFPs) to measure network oscillations in the BLA in male and female C57BL/6J mice during acute and repeated exposures to alcohol.

The generation of oscillations is thought to involve the ability of GABAergic interneurons, particularly parvalbumin (PV) expressing interneurons, to synchronize populations of principal neurons (Bartos et al., 2007; Sohal et al., 2009; Fuchs et al., 2017). Somatic-targeting, fast-spiking PV interneurons exert powerful control over a large network of excitatory principal cells, and as such, are capable of generating and synchronizing oscillations to orchestrate network communication (Mcdonald, 1992; Bocchio and Capogna, 2014). There is a critical role for PV interneurons in oscillation generation within the BLA both *ex vivo* and *in vivo*, where PV interneurons can shift oscillatory frequencies and drive behavioral states (Antonoudiou et al., 2021; Ozawa et al., 2020; Davis et al., 2017).

PV interneurons in the BLA express a high density of extrasynaptic δ subunit-containing $GABA_ARs$, which are uniquely sensitive to alcohol and play a role in regulating both alcohol consumption and anxiety-like behaviors, including anxiety associated with alcohol withdrawal (Glykys et al., 2007; Melón et al., 2018; Antonoudiou et al., 2021). Tonic inhibition mediated by δ-$GABA_ARs$ has been shown to control hippocampal oscillations (Mann and Mody, 2010; Pavlov et al., 2014) and loss of the δ subunit in PV interneurons alters γ oscillations in the CA3 region of the hippocampus (Ferando and Mody, 2013, 2014). Given the evidence that PV interneurons, which modulate oscillations in the BLA, have a high density of δ subunit expression, we further hypothesized that alcohol acts through δ-$GABA_ARs$ in the BLA to modulate oscillations associated with the network communication of fear and anxiety. To test this, we examined the ability of alcohol to alter oscillatory states in the BLA of male and female *Gabrd*$^{-/-}$ mice. Our findings suggest that the ability of alcohol to modulate network states involves δ subunit-containing $GABA_ARs$. We conclude that alcohol can modulate BLA oscillatory states in a sex-specific manner, a process which, in part, involves δ subunit-containing $GABA_ARs$.

Materials and Methods

Animals

Adult male and female C57BL/6J mice, aged 8-12 weeks old, were purchased from The Jackson Laboratory (stock #000664) and group housed in temperature and humidity-controlled housing rooms on a 12-hour light-dark cycle (lights on at 7AM) with ad libitum food and water. Animals were handled according to protocols and procedures approved by the Tufts University Institutional Animal Care and Use Committee (IACUC). Female mice are maintained in an

acyclic state without exposure to males. Global *Gabrd*$^{-/-}$ knockout mice were bred in house (Mihalek et al., 1999, 2001). Mice were single housed and habituated to new cages for 24 hours before the start of experiments.

Stereotaxic Surgery

All mice undergoing surgery were anesthetized with ketamine/xylazine (90 mg/kg and 5-10 mg/kg, respectively, i.p.) and treated with sustained release buprenorphine (0.5-1.0 mg/kg, s.c.). A lengthwise incision was made to expose the skull and a unilateral craniotomy was performed to lower a depth electrode (PFA-coated stainless-steel wire, A-M systems) into the BLA (AP -1.50 mm, ML 3.30 mm, DV -5 mm), affixed to a head mount (Pinnacle #8201) with stainless steel screws as ground, reference, and frontal cortex EEG (AP +0.75 mm, ML ± 0.3 mm, DV -2.1 mm) electrodes. EMG wires were positioned in the neck muscles.

LFP Recordings

LFP recordings were performed in male and female C57BL/6J and *Gabrd*$^{-/-}$ mice after a week of recovery from implant surgery. LFP recordings were acquired using Lab Chart software (AD Instruments) collected at 4 KHz and amplified 100X. Spectral analysis was performed in MATLAB (Antonoudiou et al., 2021) using MatWAND (https://github.com/pantelisantonoudiou/MatWAND) which utilizes the fast Fourier transform similar to previous reports (Kruse and Eckhorn, 1996; Frigo and Johnson, 1998; Pape et al., 1998; Freeman et al., 2000). Briefly, recordings were divided into 5 second overlapping segments and the power spectral density for a range of frequencies was obtained (Oppenheim et al., 1999). LFP power was quantified as power area.

111 *Acute & Repeated Alcohol Exposure*

112 Mice were habituated to new cages with ad libitum food and water for 24 hours before starting
113 the experimental paradigm. All injections were performed 2-3 hours into the light cycle (on at 7
114 AM) at the same time each day across all cohorts. A dose-response of four doses (0.5, 1.0, 1.5,
115 and 2.0 g/kg i.p.) was completed in a group of male C57BL/6J mice to determine the appropriate
116 dose to use in our experiments. The acute exposure consisted of a 60-minute baseline period
117 followed by a saline injection (0.9% NaCl i.p.) and a subsequent 1 g/kg ethanol injection (20%
118 v/v i.p.). The repeated exposure consisted of a 60-minute baseline period followed by an i.p.
119 injection of either saline or 1 g/kg ethanol (20% v/v) for five consecutive days. For the females,
120 the acute ethanol exposure was calculated from the first day of the repeated exposure paradigm.

121 *Blood Ethanol Concentration (BEC) Measurements*

122 Blood from the submandibular vein was collected from a separate cohort of male and female
123 C57BL/6J mice 15 minutes after exposure to alcohol (1 g/kg i.p.) on days 1, 2, and 5 of repeated
124 alcohol exposure. Blood was spun down at 1.8xg for 15 minutes at 4°C and serum was stored at -
125 80°C until BEC measurements were performed using the BioAssay Systems EnzyChrom Ethanol
126 Assay Kit (ECET-100) according to the manufacturing protocol. Measurements are reported in
127 mg/dl.

128 *Immunohistochemistry*

129 Immunohistochemistry was performed as previously reported (Melón et al., 2018) in a separate
130 cohort of C57BL/6J mice 30 minutes following repeated exposure to vehicle or alcohol for five
131 days. Mice were anesthetized with isoflurane, transcradially perfused with 0.9% saline and 4%

paraformaldehyde (PFA), the brains were rapidly excised, fixed in 4% PFA overnight, and subsequently cryoprotected in 10% and 30% sucrose. The brains were then flash frozen using isopentane and stored at -80°C until cryosectioning. Free floating 40 µm coronal slices were co-stained for PV and δ using universal antigen retrieval buffer (R&D systems CTS015) and primary antibodies against δ-$GABA_AR$ (1:100, Phosphosolutions 868A-GDN) and PV (1:1000, Sigma P3088) for 72 hours at 4°C. The slices were then incubated with a biotinylated goat anti-rabbit (1:1000, Vector Laboratories BA1000) and Alexa-Fluor 647 conjugated goat anti-mouse (1:200, ThermoFisher Scientific A28181) for two hours at room temperature and streptavidin conjugated Alexa-Fluor 488 (1:200, ThermoFisher Scientific S32354) for two hours at room temperature. Slices were mounted and cover slipped with antifade hard set mounting medium with DAPI (Vectashield H1500). Fluorescent labeling in the BLA was imaged on a Nikon A1R confocal microscope and z-stacks were acquired using a 20X objective. Camera settings were kept consistent across samples and cohorts. The images were analyzed using Image J software by outlining PV-positive interneurons using the ROI manager and measuring the integrated density of PV and δ expression on the outlined PV-positive interneurons. Each cell was considered its own data point within each animal.

Statistical Analysis

Data were analyzed using Prism 8 software (GraphPad) and MatWAND in MATLAB (Mathworks). To ensure a consistent time period for analysis across cohorts, we analyzed the first 40 minutes of baseline and the first 35 minutes of each injection period. Repeated measures two-way ANOVAs were performed to detect significance of frequency, treatment, sex, or genotype. A Greenhouse-Geisser correction was applied where necessary. A mixed effects model was used if values were missing across days. A post-hoc Šídák's multiple comparisons test

was performed to identify significant differences of specific frequency ranges. ANOVA results are reported in Extended Data Table 1-1, 2-1, and 3-1. Multiple comparisons are reported in Extended Data Table 1-2, 2-2, and 3-2. *P* values < than 0.05 were considered significant. All *n* values for each treatment group are shown in the figure legends.

Results

Alcohol modulates BLA network states

To characterize the effect of acute alcohol on BLA oscillations in wild type mice, we recorded LFPs in the BLA of C57BL/6J mice in response to either a vehicle (0.9% saline i.p.) or alcohol (1 g/kg i.p.) injection (Figure 1A, B). We found that vehicle injections in male C57BL/6J mice significantly decreased high θ power (6-12 Hz) ($p = 0.0117$, 95% C.I = [0.04658, 0.4029]), while increasing the low γ (40-70 Hz) ($p = 0.0027$, 95% C.I = [-0.9799, -0.2066]), and high γ power (80-120 Hz) ($p = 0.0103$, 95% C.I = [-0.6491, -0.08079]) as compared to baseline (Figure 1-1A). However, we did not find any difference between the two vehicle injections, indicating there was no sensitization or adaptation to the second injection. We have previously observed the impact of vehicle injections on oscillatory states in the BLA (Antonoudiou et al., 2021), which likely reflects the network response to the stress of the injection. Therefore, all results are compared to the first vehicle injection within the treatment paradigm.

We performed a dose-response examining changes in network activity in response to four different doses of alcohol (0.5, 1.0, 1.5, 2.0 g/kg i.p.; Figure 1-2). These experiments determined that the 1.0 g/kg dose was capable of significantly altering relevant BLA network state frequencies without producing lethargy or sedation in the mice (6-12 Hz: $p = 0.030$, 95% C.I = [0.024, 0.450; Figure 1-2A-C). Therefore, we chose this concentration for our experiments

throughout this study. In response to alcohol treatment in male C57BL/6J mice, the power in the β frequency range (15-30 Hz) is decreased compared to vehicle (p = 0.034, 95% C.I = [0.01033, 0.2925]; Figure 1C, E, G; Figure 1-1C). This indicates that alcohol can modulate specific oscillatory frequencies within the BLA that are implicated both in addiction and mood disorders (Jurado-Barba et al., 2020).

Alcohol modulates BLA network states in a sex-dependent manner

Because of the well documented sex differences in alcohol related behaviors (Melón et al., 2013; Barkley-Levenson and Crabbe, 2015; Becker and Koob, 2016; Sneddon et al., 2019), we treated female C57BL/6J to the same acute alcohol paradigm as described in males (Figure 1B). Similar to the males, we did not find any significant differences between the two vehicle injections in the vehicle/vehicle control experiments in females (Figure 1-1B). We did find that vehicle significantly decreased high θ power as compared to baseline (p = 0.0273, 95% C.I = [0.2699, 0.4296]; Figure 1H), similar to what we observed in the males. Additionally, there was no significant difference between the male and female C57BL/6J BLA LFP response to the vehicle injection (figure not shown).

In response to acute alcohol exposure, we found that alcohol significantly decreased the γ band power in female C57BL/6J mice as compared to vehicle (p = 0.0014, 95% C.I = [0.2955, 0.9441]; Figure 1D, F, H; Figure 1-1D), a unique signature from the males. Interestingly, this reduction in γ power represents a blunting of the increase in power exhibited by the vehicle injection (Figure 1F). Although alcohol decreased BLA power in different frequency bands in males and females, there were no direct significant differences between groups (figure not

198 shown). Collectively, these data suggest that acute ethanol modulates the BLA network
199 differently in male and female mice.

200 *Alcohol modulation of BLA network states involves δ subunit-containing $GABA_AR$s*

201 Previous literature has supported the role of δ-$GABA_AR$s in mediating the effects of alcohol on
202 tonic inhibition, drinking and withdrawal behaviors (Wallner et al., 2003; Santhakumar et al.,
203 2007; Melón et al., 2018; Darnieder et al., 2019). Therefore, to test whether alcohol is mediating
204 its effects on BLA network states through δ-$GABA_AR$s, we repeated the same procedure in male
205 and female *Gabrd*$^{-/-}$ mice. We found that vehicle injections significantly increased BLA power at
206 low γ frequencies only in the vehicle/alcohol condition in male *Gabrd*$^{-/-}$ mice as compared to
207 baseline (p = 0.0121, 95% C.I = [-1.062, -0.1299]; Figure 2A-B). In both *Gabrd*$^{-/-}$ males and
208 females, we did not find any significant difference between vehicle injections (Figure 2-1).

209 Unlike C57BL/6J males, acute alcohol significantly decreased the low γ band of *Gabrd*$^{-/-}$ males
210 (p = 0.020, 95% C.I = [0.07, 0.78]; Figure 2A-B) and *Gabrd*$^{-/-}$ females (p = 0.0012, 95% C.I =
211 [0.1731, 0.5352]; Figure 2C-D) as compared to vehicle. This effect was similar to, but not as
212 robust an effect, as in C57BL/6J females. However, direct comparisons between male C57BL/6J
213 and male *Gabrd*$^{-/-}$ mice or between C57BL/6J females and *Gabrd*$^{-/-}$ females did not detect
214 significant differences in the ability of alcohol to modulate oscillatory states (figure not shown).
215 Collectively, these data suggest that the loss of the $GABA_AR$ δ subunit impacts the network
216 effect of alcohol more profoundly in males and induces a similar network effect as observed in
217 C57BL/6J females.

218 *Ability of repeated alcohol exposure to modulate BLA network states is dependent on δ subunit-*
219 *containing $GABA_AR$s*

Since we established that acute alcohol could modulate specific oscillatory frequencies in the BLA, we were interested in how BLA LFPs changed over time in response to repeated doses of alcohol. Male C57BL/6J and *Gabrd*$^{-/-}$ mice received vehicle (0.9% saline) or low dose (1 g/kg i.p.) alcohol for five consecutive days (Figure 3A). We did not find significant effects of repeated vehicle injections across days in either the male C57BL/6J or male *Gabrd*$^{-/-}$ mice (Figure 3-1A, C).

Interestingly, in response to repeated alcohol treatment, we found a change in the baseline low γ power from the first to last day (BASE2-BASE1) in male C57BL/6J mice ($p = 0.0252$, 95% C.I = [0.03785, 0.6384]; Figure 3-2A), which may be an anticipatory change associated with repeated alcohol administration. In response to alcohol treatment, we observed a significant increase in low γ power from the first to last day of exposure (EtOH-BASE2) ($p = 0.0305$, 95% C.I = [-0.7087, -0.03272]; Figure 3C) along with an increase in BEC (first: 161.4 mg/dl, last: 189.8 mg/dl; $p < 0.0001$, 95% C.I = [-160.4, -95.81]; Figure 3A). In contrast, we did not observe significant effects of repeated alcohol on baseline or treatment in male *Gabrd*$^{-/-}$ mice (Figure 3D; Figure 3-2C).

Direct comparison between male C57BL/6J and *Gabrd*$^{-/-}$ mice on the first day of alcohol exposure does not reveal any significant changes within baseline (Figure 4-1A), but did find that male C57BL/6J mice had significantly decreased high θ ($p = 0.0138$, 95% C.I = [-1.061, -0.1088]) and β ($p = 0.0022$, 95% C.I = [-0.7193, -0.1659]) BLA power as compared to male *Gabrd*$^{-/-}$ mice in response to alcohol exposure (Figure 4A). By the last day, there were significant decreases within the baseline period specifically in the low ($p = 0.0343$, 95% C.I = [-0.9892, -0.03576]) and high γ ($p = 0.0062$, 95% C.I = [-0.5908, -0.09553]) BLA power in male C57BL/6J mice as compared to male *Gabrd*$^{-/-}$ mice (Figure 4-1B), again likely attributed to the role of the

243 $GABA_AR$ δ subunit in the anticipatory effects of repeated alcohol exposure. In response to
244 repeated alcohol administration, we observed a significant increase in the high γ frequency range
245 in male C57BL/6J mice compared to male *Gabrd*$^{-/-}$ mice (p = 0.0203, 95% C.I = [0.02211,
246 0.2899]; Figure 4B). Overall, these results suggest a blunted impact of acute and repeated
247 alcohol exposure on BLA oscillatory states in mice lacking the $GABA_AR$ δ subunit. Further,
248 these data indicate a role for δ-$GABA_A$Rs in adapting to alcohol exposure over time, as well as
249 anticipating alcohol treatment as shown by the changes in baseline in male C57BL/6J mice, but
250 not *Gabrd*$^{-/-}$ mice.

251 *Sex differences in BLA network states in response to repeated alcohol exposure*

252 Repeated alcohol exposure in female C57BL/6J and *Gabrd*$^{-/-}$ mice involved acute alcohol or
253 vehicle exposure on day one and the repeated alcohol exposure days two to five (Figure 3B). We
254 will be using their second day of exposure in our repeated alcohol comparisons, which were not
255 significantly different in female C57BL/6J mice (Figure 3-3A). Neither day one nor day two
256 were significantly different from day five in female *Gabrd*$^{-/-}$ mice (Figure 3-3B-C). Therefore,
257 we continued to use day two as the first day of repeated exposure in our analysis.

258 We did not observe significant effects of vehicle exposure across days in female C57BL/6J
259 (Figure 3-1B) or female *Gabrd*$^{-/-}$ mice (Figure 3-1D). Interestingly, unlike the males, we did not
260 observe any significant effect of repeated alcohol in C57BL/6J female and *Gabrd*$^{-/-}$ females
261 across days (Figure 3D, F; Figure 3-2B, D) despite an increase in BEC in female C57BL/6J mice
262 from the first to last day of exposure (first: 151.1 mg/dl, last: 257.3 mg/dl; p = 0.0072, 95% C.I
263 = [-166.0, -46.32; Figure 3B).

Direct comparison between C57BL/6J males and females did not reveal significant differences at any frequency range within the baseline period (Figure 4-1C) or in the effect of alcohol from baseline (Figure 4C) on the first day of exposure. However, by the last day, we found an increase in high θ (p = 0.0435, 95% C.I = [0.01858, 1.216]) and a decrease in high γ power within the baseline of males, but not in the females (p = 0.0122, 95% C.I = [-0.5198, -0.06395]; Figure 4-1D). In response to repeated alcohol exposure, males exhibited a significantly reduced power in the high θ frequency range in C57BL/6J males with no effect in females (p = 0.0433, 95% C.I = [-1.085, -0.01596]; Figure 4D). There was no significant difference between male and female BECs on day one or day five suggesting the same level of alcohol intoxication modulates BLA LFPs differentially in the two sexes. Collectively, these data suggest that male C57BL/6J mice are becoming more sensitive to the sedative effects of alcohol as demonstrated by the significant effects of repeated alcohol administration on γ frequency ranges, an effect that involves the $GABA_AR$ δ subunit (Pian et al, 2008).

Repeated alcohol exposure alters δ expression on PV interneurons in the BLA

Alcohol exposure can change the expression of $GABA_AR$ subunits (Liang et al., 2004; Olsen et al., 2012; Lindemeyer et al., 2014; Follesa et al., 2015) and sex differences in $GABA_AR$ δ subunit expression has been reported (Maguire et al., 2005). Changes in the expression of the $GABA_AR$ δ subunit, whether through genetic deletions or hormone fluctuations during pregnancy, can alter specific oscillation frequencies in the hippocampus (Ferando and Mody, 2013, 2015). Therefore, we hypothesized that altered expression of the $GABA_AR$ δ subunit on PV interneurons in the BLA may contribute to our observed sex differences in BLA network states. Thus, we examined whether there were any potential sex differences in $GABA_AR$ δ subunit expression in the BLA or in δ expression associated with alcohol exposure. We observed

a higher δ expression on PV interneurons in naive female C57BL/6J mice (*M* = 1002542, *SEM* = 45011) as compared to naive male C57BL/6J mice (*M* = 538252, *SEM* = 12440; t [424] = 10.39, p < 0.0001; Figure 5B) with no change to PV immunoreactivity (female: *M* = 1464808, *SEM* = 51507; male: *M* = 1448517, *SEM* = 97320; Figure 5A). Interestingly, vehicle treatment alone reduced PV immunoreactivity in females compared to males ($p < 0.0001$, 95% C.I = [267746, 621366]; Figure 5D) and also reduced δ expression on PV neurons in females as compared to males ($p < 0.0001$, 95% C.I = [35568, 126727]; Figure 5E). These data demonstrate baseline sex differences in the expression and lability of $GABA_AR$ δ expression on PV interneurons in the BLA.

Repeated alcohol treatment reduced PV immunoreactivity in males as compared to vehicle ($p < 0.0001$, 95% C.I = [147380, 518402]; Figure 5D). In contrast, repeated alcohol exposure in females did not alter PV immunoreactivity but did significantly reduce $GABA_AR$ δ expression on PV interneurons compared to vehicle ($p = 0.0018$, 95% C.I = [15583, 98517]; Figure 5E), an effect that was not observed in males.

Comparing males and females exposed to repeated alcohol, PV immunoreactivity ($p = 0.0195$, 95% C.I = [22163, 362911]; Figure 5D) and δ expression on PV interneurons are reduced in females as compared to males ($p < 0.0001$, 95% C.I = [54848, 142689; Figure 5E). These data implicate that changes in $GABA_AR$ δ expression on PV interneurons may mediate sex differences and the response to repeated alcohol exposure.

Discussion

Network states have been shown to correlate with behavioral states and accumulating evidence demonstrates that signature oscillatory states in the BLA are associated with fear and anxiety

states (Likhtik et al., 2013; Stujenske et al., 2014; Davis et al., 2017; Antonoudiou et al., 2021). In fact, optogenetically driving specific oscillatory states influences the behavioral expression of fear (Ozawa et al., 2020) and learned helplessness (Antonoudiou et al., 2021). However, limited studies have examined the physiological, pathological, or pharmacological mechanisms mediating transitions between network and behavioral states. Recent work has demonstrated that chronic stress can perturb oscillations in the BLA and a clinically effective antidepressant treatment can restore the "healthy" network state (Antonoudiou et al., 2021). Here, we examine the impact of alcohol on BLA network states. Given the anxiolytic effects of alcohol, we posited that alcohol may be capable of shifting the network state towards the anxiolytic state. We demonstrate that acute alcohol exposure is capable of altering BLA network states and that there are sex differences in the effect of alcohol on BLA network states, affecting different frequencies in males and females. These data are the first to demonstrate that alcohol is capable of modulating network states associated with affective states.

It has been demonstrated that PV interneurons are critical in orchestrating oscillatory states in the BLA (Antonoudiou et al., 2021). PV interneurons in the BLA express a high density of δ-$GABA_A$Rs, which have been suggested to be a target for low dose alcohol (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003; Hanchar et al., 2006; Santhakumar et al., 2007). However, the actions of alcohol directly on these receptors remains somewhat controversial (Borghese et al., 2006; Korpi et al., 2007). It is important to note that the majority of these studies focus solely on principal neurons; GABAergic interneurons, on the other hand, have a unique receptor subunit composition in which the δ subunit has been shown to partner with the α1 subunit, and have been demonstrated to generate tonic GABAergic currents which are highly sensitive to low concentrations of ethanol (Glykys et al., 2007). Thus, we proposed that the high expression of δ

subunit-containing $GABA_ARs$ on PV interneurons in the BLA may confer unique sensitivity to the effects of alcohol and, given the role of these interneurons in coordinating oscillations, may mediate the effects of alcohol on BLA network states. Here we demonstrate that δ-$GABA_ARs$ influence the ability of alcohol to alter specific oscillatory states in the BLA, blunting the ability to shift network states. Specifically, we observed a reduction of β power from acute ethanol in male wild-type mice that was blunted in mice lacking δ-$GABA_ARs$ (Figure 1, 2). Others have found reductions of β power in the nucleus accumbens shell during alcohol relapse related to reduced synchrony of the local network, suggesting this reduction we find in response to alcohol may also be from reduced synchrony (Hadar et al., 2016). This power detected in the β frequency may arise from the neighboring high θ oscillator, given the lack of a clear β peak (Figure 1-1C). Regardless, these data suggest that δ subunit-containing $GABA_ARs$ are important players in mediating the effects of alcohol on oscillatory states related to mood/anxiety; although, it is also possible that other $GABA_AR$ subtypes are involved. Previous studies demonstrated that δ has a specific role in lower frequencies as compared to higher frequencies (Antonoudiou et al., 2021), which may be true for the effects of alcohol as well. Further studies are required to investigate the impact of other $GABA_AR$ subtypes in mediating the ability of alcohol to modulate BLA network states given that previous studies have implicated other $GABA_AR$ subtypes, such as the γ2 subunit, in anxiety-like behavior (Chandra et al., 2005) and alcohol withdrawal severity (Buck and Hood, 1998). It is also possible that alcohol's indirect effects on receptor expression, neurotransmitter availability, and other neuromodulators could account for the changes in BLA oscillations observed here (Morrow et al., 2001; Fleming et al., 2009; Olsen and Liang, 2017). For example, the effects of alcohol have been suggested to be mediated through the action of neuroactive steroids (Morrow et al., 2001; Finn et al., 2009; Finn and Jimenez, 2018) and given

355 recent evidence that allopregnanolone can alter BLA network states (Antonoudiou et al., 2021),
356 this may be an indirect mechanism whereby alcohol could modulate BLA network states.
357 Arguing against this indirect mechanism is the evidence that alcohol exerts unique effects on
358 BLA network states compared to allopregnanolone (Antonoudiou et al., 2021).

359 Since expression of δ subunit-containing GABAARs have been shown to be sensitive to ovarian
360 steroid hormone modulation and are implicated in sex differences in alcohol intake (Darnieder et
361 al, 2019), we hypothesized that there may be sex differences in the ability of alcohol to modulate
362 BLA network states through actions on these receptors. In fact, we do observe sex differences in
363 the modulation of BLA network states by alcohol even though both male and female C57BL/6J
364 mice reach similar BEC levels after alcohol exposure (Figure 3A, B). Interestingly, the loss of
365 the $GABA_AR$ δ subunit in males shifts the alcohol modulation of the BLA network state towards
366 the signature that we observe for female C57BL/6J mice (Figure 1, 3) and we believe that the
367 observed sex differences in the expression of δ subunit-containing $GABA_ARs$ in the BLA
368 (Figure 5) may underlie these differences. Further, there are well-documented sex differences in
369 responses to alcohol, alcohol related anxiety-like behavior, and estrous-cycle dependent δ
370 expression (Maguire et al., 2005; Rhodes et al., 2005; Barkley-Levenson and Crabbe, 2015),
371 consistent with our observations of sex differences in the alcohol-induced modulation of network
372 states. Additionally, sex differences have been reported in neural oscillations in major depressive
373 disorder with oscillatory signatures of susceptibility (Thériault et al., 2021). Future studies are
374 required to evaluate the relationship between the capacity of alcohol to modulate network states
375 and voluntary alcohol consumption, the anxiolytic effects of alcohol, and the anxiogenic effects
376 of alcohol withdrawal.

To investigate whether the effect of alcohol on network states may be altered after repeated exposure, we treated mice with low dose alcohol for up to five days. Interestingly, we found that BLA network states changed before alcohol exposure. Given the evidence that the amygdala is involved in valence encoding and assignment, it is possible that network state changes before the alcohol exposure is reflective of the anticipation or expectation of the event, which has been demonstrated in a Pavlovian conditioning paradigm (Pignatelli and Beyeler, 2019; Tallot et al., 2020). We found robust effects of repeated alcohol exposure on the BLA LFP response of male C57BL/6J mice which was significantly different from the male *Gabrd*$^{-/-}$ and female C57BL/6J mice. In fact, we found no differences in the extent of the effect of repeated alcohol on network states in female C57BL/6J or *Gabrd*$^{-/-}$ mice despite similar BEC levels between male and female C57BL/6J mice. It is possible that the change in BLA power in male C57BL/6J mice across repeated alcohol exposure is due to the increase in BEC rather than an adaptation to the injections. Indeed, our dose response data shows that higher doses of alcohol do have larger effects on BLA network states. Alcohol administration prominently affected γ band oscillations in the BLA, a network activity that has been associated with local network synchrony, affective learning and memory consolidation, (Bocchio et al., 2017; Antonodiou et al., 2021; Headley et al., 2021). Given the critical role of PV interneurons in the generation of BLA γ oscillations (Antonodiou et al., 2021; Headley et al., 2021), alcohol may directly modulate PV interneuron signaling. It's possible that reduction in tonic inhibition of PV interneurons in female and *Gabrd*$^{-/-}$ mice makes PV interneurons more susceptible to the effects of acute ethanol leading to disruption in the generation of γ network oscillations in BLA.

Since the downregulation of the δ subunit has been thought to confer tolerance to alcohol (Olsen and Liang, 2017), the reduction of δ subunit in female, but not male, C57BL/6J mice could

explain the lack of effects on LFPs after repeated alcohol exposure. Further, GABAAR agonists and positive allosteric modulators, like neurosteroids which exert effects through the δ subunit, can block tolerance to the sedative effects of alcohol (Debatin and Barbosa, 2006; Barbosa and Morato, 2007). This could explain why alcohol does not change the BLA network in female C57BL/6J and *Gabrd*$^{-/-}$ mice, who have reductions in δ-$GABA_AR$ expression, after repeated administration like it does in male C57BL/6J mice. However, this study did not directly measure sensitization or tolerance to the effects of alcohol and future studies could link network and behavioral changes. Lastly, we found that δ expression on PV interneurons is increased in naive female C57BL/6J mice compared to males. Because we did not see any baseline differences in BLA network states between male and female C57BL/6J mice, this difference in expression may not impact BLA oscillations, but expression of δ in females does influence the response to alcohol exposure.

The literature and recent findings demonstrate a strong role for PV interneurons in oscillation generation (Antonoudiou et al., 2021) giving support to the likely fact that alcohol's effects on PV interneurons are influencing the oscillations. However, due to the heterogeneity of the interneuron population in the BLA, it is possible other interneuron types, like somatostatin, cholecystokinin, or PKC-δ expressing cells may be involved in effecting oscillations (Klausberger et al., 2005). Furthermore, another major influence on BLA oscillations are other brain areas with strong network connections to the BLA, such as the medial prefrontal cortex (mPFC), which is heavily implicated in addiction (Goldstein and Volkow, 2011; Davis et al., 2017; Ozawa et al., 2020).

Forced alcohol injections or alcohol induced aversion can cause stress to mice which may contribute to the observed effects (Eckardt et al., 1974). However, our experimental plan was

designed to dissociate any stressful or unpleasant effects of the infusion from the effects of alcohol. Although we observed significant effects of vehicle injections on BLA LFP responses compared to baseline (Figure 1-1), we did not find any sensitization or tolerance to the injections in the acute alcohol experiment or across days in the repeated alcohol experiment (Figure 1-1, 2-1, 3-1), similar to what has been reported previously (Antonoudiou et al., 2021). Thus, we are confident that the observed effects of alcohol on BLA oscillatory states is due to the effects of alcohol rather than an aversive experience related to the route of administration. Further, our data suggest that the effects of alcohol may mitigate the stress-induced effects on the BLA network state.

To our knowledge, this is the first demonstration that alcohol can modulate oscillations in the BLA, which have been implicated in governing behavioral states. Numerous studies have investigated the relationship of BLA network states to behavioral states; however, few studies have investigated mechanisms mediating transitions between BLA network states. The current study demonstrates that alcohol can induce a transition between network states associated with fear and anxiety, which may mediate the impact of alcohol on anxiety states. Future work is required to investigate how changes in the BLA relate to other connected areas implicated in alcohol use and anxiety, such as the central amygdala, mPFC, nucleus accumbens, BNST, and ventral striatum (Janak and Tye, 2015). Recordings of oscillations are stable over long periods of time and thus can be examined throughout the addiction cycle from intoxication to withdrawal to preoccupation in specific brain areas to understand how alcohol changes communication between these areas. Thus, this novel approach may demonstrate utility in understanding the trajectory from first exposure to alcohol dependence and the contribution of both the positive and negative reinforcing effects of alcohol.

446 **References**

447 Agoglia A, Herman M (2018) The Center of the Emotional Universe: alcohol, stress, and CRF1
448 amygdala circuitry. Alcohol 72:61–73.

449 Antonoudiou P, Colmers PLW, Walton NL, Weiss GL, Smith AC, Nguyen DP, Lewis M, Quirk
450 MC, Barros L, Melón LC, Maguire JL (2021) Allopregnanolone Mediates Affective
451 Switching Through Modulation of Oscillatory States in the Basolateral Amygdala.
452 Biological Psychiatry.

453 Barbosa ADE, Morato GS (2007) Influence of epipregnanolone on the modulation of rapid
454 tolerance to ethanol by neurosteroids. Revista Brasileira de Psiquiatria, 29(4), 337–345.

455 Barkley-Levenson AM, Crabbe JC (2015) Genotypic and sex differences in anxiety-like
456 behavior and alcohol-induced anxiolysis in High Drinking in the Dark selected mice.
457 Alcohol 49:29–36.

458 Bartos M, Vida I, Jonas P (2007) Synaptic mechanisms of synchronized gamma oscillations in
459 inhibitory interneuron networks. Nature Reviews Neuroscience 8:45–56.

460 Becker JB, Koob GF (2016) Sex Differences in Animal Models: Focus on Addiction.
461 Pharmacological Reviews 68:242–263.

462 Bocchio M, Capogna M (2014) Oscillatory Substrates of Fear and Safety. Neuron 83:753–755.

463 Bocchio M, Nabavi S, Capogna M (2017) Synaptic Plasticity, Engrams, and Network
464 Oscillations in Amygdala Circuits for Storage and Retrieval of Emotional Memories.
465 Neuron 94(4) 731–743.

466 Borghese CM, Stórustovu SÍ, Ebert B, Herd MB, Belelli D, Lambert JJ, Marshall G, Wafford
467 KA, Harris RA (2006) The δ subunit of γ-aminobutyric acid type A receptors does not
468 confer sensitivity to low concentrations of ethanol. Journal of Pharmacology and
469 Experimental Therapeutics 316:1360–1368.

470 Buck KJ, Hood HM (1998) Genetic association of a GABAA receptor y2 subunit variant with
471 severity of acute physiological dependence on alcohol. Mammalian Genome 9:975–978.

472 Chandra D, Korpi ER, Miralles CP, de Blas AL, Homanics GE (2005) GABAA receptor γ2
473 subunit knockdown mice have enhanced anxiety-like behavior but unaltered hypnotic
474 response to benzodiazepines.

475 Darnieder LM, Melón LC, Do T, Walton NL, Miczek KA, Maguire JL (2019) Female-specific
476 decreases in alcohol binge-like drinking resulting from GABAA receptor delta-subunit
477 knockdown in the VTA. Scientific Reports 9:1–11.

478 Davis P, Zaki Y, Maguire J, Reijmers LG (2017) Cellular and oscillatory substrates of fear
479 extinction learning. Nature Neuroscience 20:1624–1633.

480 Debatin T, Barbosa ADE (2006) Effect of isopregnanolone on rapid tolerance to the anxiolytic
481 effect of ethanol. Brazilian Journal of Psychiatry 28(1), 18–23.

482 Eckardt MJ, Skurdal AJ, Brown JS (1974) Conditioned taste aversion produced by low doses of
483 alcohol, Physiological Psychology.

484 Ferando I, Mody I (2015) In vitro gamma oscillations following partial and complete ablation of
485 δ subunit-containing GABAA receptors from parvalbumin interneurons.
486 Neuropharmacology 88:91–98.

487 Ferando I, Mody I (2014) Interneuronal GABAA receptors inside and outside of synapses.
488 Current Opinion in Neurobiology 26:57–63.

489 Ferando I, Mody I (2013) Altered gamma oscillations during pregnancy through loss of δ
490 subunit-containing GABAA receptors on parvalbumin interneurons. Frontiers in Neural
491 Circuits 7:1–14.

492 Finn DA, Beckley EH, Kaufman KR, Ford MM (2009) Manipulation of GABAergic steroids:
493 Sex differences in the effects on alcohol drinking- and withdrawal-related behaviors.
494 Hormones and Behavior 57:12–22.

495 Finn DA, Jimenez VA (2018) Dynamic Adaptation in Neurosteroid Networks in Response to
496 Alcohol In: Handbook of Experimental Pharmacology (Grant KA, Lovinger DM eds), pp1–
497 24. Springer, Berlin, Heidelberg.

498 Fleming RL, Manis PB, Morrow AL (2009) The effects of acute and chronic ethanol exposure
499 on presynaptic and postsynaptic γ-aminobutyric acid (GABA) neurotransmission in cultured
500 cortical and hippocampal neurons. Alcohol 43.

501 Follesa P, Floris G, Asuni GP, Ibba A, Tocco MG, Zicca L, Mercante B, Deriu F, Gorini G
502 (2015) Chronic Intermittent Ethanol Regulates Hippocampal GABAA Receptor Delta
503 Subunit Gene Expression. Frontiers in Cellular Neuroscience 9:445.

504 Freeman WJ, Rogers LJ, Holmes MD, Silbergeld DL (2000) Spatial spectral analysis of human
505 electrocorticograms including the alpha and gamma bands, Journal of Neuroscience
506 Methods.

507 Frigo M, Johnson SG (1998) FFTW: An adaptive software architecture for the FFT In:
508 Proceedings of the International Conference on Acoustics, Speech, and Signal Processing,
509 pp1381–1384.

510 Fuchs T, Jefferson SJ, Hooper A, Yee P-H, Maguire J, Luscher B (2017) Disinhibition of
511 somatostatin-positive GABAergic interneurons results in an anxiolytic and antidepressant-
512 like brain state. Molecular Psychiatry 22:920–930.

513 Goldstein, RZ, & Volkow, ND (2011) Dysfunction of the prefrontal cortex in addiction:
514 neuroimaging findings and clinical implications. Nature reviews. Neuroscience, 12(11),
515 652–669.

516 Gilpin NW, Koob GF (2008) Neurobiology of Alcohol Dependence Focus on Motivational
517 Mechanisms. Alcohol Research & Health: the journal of the National Institute on Alcohol
518 Abuse and Alcoholism 31:185–195.

519 Glykys J, Peng Z, Chandra D, Homanics GE, Houser CR, Mody I (2007) A new naturally
520 occurring GABAA receptor subunit partnership with high sensitivity to ethanol. Nature
521 Neuroscience 10:40–48.

522 Hadar R, Voget M, Vengeliene V, Haumesser J K, van Riesen C, Avchalumov Y, … Winter C
523 (2017) Altered neural oscillations and elevated dopamine levels in the reward pathway
524 during alcohol relapse. Behavioural Brain Research, 316, 131–135.

525 Hanchar HJ, Chutsrinopkun P, Meera P, Supavilai P, Sieghart W, Wallner M, Olsen RW (2006)
526 Ethanol potently and competitively inhibits binding of the alcohol antagonist Ro15-4513 to
527 4/6 3 GABAA receptors. Proceedings of the National Academy of Sciences 103:8546–
528 8551.

529 Janak PH, Tye KM (2015) From circuits to behaviour in the amygdala. Nature 517:284–292.

530 Jurado-Barba R, Sion A, Martínez-Maldonado A, Domínguez-Centeno I, Prieto-Montalvo J,
531 Navarrete F, Salud García-Gutierrez M, Manzanares J, Rubio G (2020)
532 Neuropsychophysiological Measures of Alcohol Dependence: Can We Use EEG in the
533 Clinical Assessment? Frontiers in Psychiatry 11.

534 Klausberger T, Marton LF, O'Neill J, Huck JHJ, Dalezios Y, Fuentealba P, Suen WY, Papp E,
535 Kaneko T, Watanabe M, Csicsvari J, Somogyi P (2005) Complementary Roles of
536 Cholecystokinin- and Parvalbumin-Expressing GABAergic Neurons in Hippocampal
537 Network Oscillations. Journal of Neuroscience 25:9782–9793.

538 Korpi ER, Debus F, Linden AM, Malécot C, Leppä E, Vekovischeva O, Rabe H, Böhme I, Aller
539 MI, Wisden W, Lüddens H (2007) Does ethanol act preferentially via selected brain
540 GABAA receptor subtypes? the current evidence is ambiguous. Alcohol 41:163–176.

541 Kruse W, Eckhorn R (1996) Inhibition of sustained gamma oscillations (35-80 Hz) by fast
542 transient responses in cat visual cortex. Proceedings of the National Academy of Sciences
543 93:6112–6117.

544 Kushner MG, Maurer E, Menary K, Thuras P (2011) Vulnerability to the Rapid ("Telescoped")
545 Development of Alcohol Dependence in Individuals with Anxiety Disorder*. Journal of
546 Studies on Alcohol and Drugs 72:1019–1027.

547 Liang J, Cagetti E, Olsen RW, & Spigelman I (2004) Altered pharmacology of synaptic and
548 extrasynaptic GABAA receptors on CA1 hippocampal neurons is consistent with subunit
549 changes in a model of alcohol withdrawal and dependence. The Journal of pharmacology
550 and experimental therapeutics, 310(3), 1234–1245.

551 Likhtik E, Stujenske JM, Topiwala MA, Harris AZ, Gordon JA (2013) Prefrontal entrainment of
552 amygdala activity signals safety in learned fear and innate anxiety. Nature Publishing Group
553 17:106–113.

554 Lindemeyer AK, Liang J, Marty VN, Meyer EM, Suryanarayanan A, Olsen RW, Spigelman I
555 (2014) Ethanol-Induced Plasticity of GABAA Receptors in the Basolateral Amygdala.
556 Neurochemical Research 39.

557 Maguire JL, Stell BM, Rafizadeh M, Mody I (2005) Ovarian cycle-linked changes in GABAA
558 receptors mediating tonic inhibition alter seizure susceptibility and anxiety. Nature
559 Neuroscience 8:797–804.

560 Mann EO, Mody I (2010) Control of hippocampal gamma oscillation frequency by tonic
561 inhibition and excitation of interneurons. Nature Neuroscience 13.

562 Mason JE, LeBouthillier DM, Asmundson GJG (2018) Relationships between health behaviors,
563 posttraumatic stress disorder, and comorbid general anxiety and depression. Cognitive
564 Behaviour Therapy 48:184–199.

565 Mcdonald AJ (1992) Projection Neurons of the Basolateral Amygdala: A Correlative Golgi and
566 Retrograde Tract Tracing Study, Brain Research Bulletin.

567 Melón LC, Nasman JT, John AS, Mbonu K, Maguire JL (2018) Interneuronal δ-GABAA
568 receptors regulate binge drinking and are necessary for the behavioral effects of early
569 withdrawal. Neuropsychopharmacology.

570 Melón LC, Wray KN, Moore EM, Boehm SL (2013) Sex and age differences in heavy binge
571 drinking and its effects on alcohol responsivity following abstinence. Pharmacology
572 Biochemistry and Behavior 104:177–187.

573 Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi Z-P, Lagenaur C, Tretter V,
574 Sieghart W, Anagnostaras SG, Sage JR, Fanselow MS, Guidotti A, Spigelman I, Li Z,
575 Delorey TM, Olsen RW, Homanics GE (1999) Attenuated sensitivity to neuroactive
576 steroids in-aminobutyrate type A receptor delta subunit knockout mice. Proceedings of the
577 National Academy of Sciences 96:12905–12910.

578 Mihalek RM, Bowers BJ, Wehner JM, Kralic JE, VanDoren MJ, Morrow AL, Homanics GE
579 (2001) GABAA-Receptor delta Subunit Knockout Mice Have Multiple Defects in
580 Behavioral Responses to Ethanol. Alcoholism: Clinical and Experimental Research
581 25:1708–1718.

582 Morrow AL, VanDoren MJ, Penland SN, Matthews DB (2001) The role of GABAergic
583 neuroactive steroids in ethanol action, tolerance and dependence, Brain Research Reviews.
584 Elsevier.

585 Olsen RW, & Spigelman I (2012) GABAA Receptor Plasticity in Alcohol Withdrawal. In J. L.
586 Noebels (Eds.) et. al., Jasper's Basic Mechanisms of the Epilepsies. (4th ed.). National
587 Center for Biotechnology Information (US).

588 Olsen RW, Liang J (2017) Role of GABAA receptors in alcohol use disorders suggested by
589 chronic intermittent ethanol (CIE) rodent model. Molecular Brain 10.

590 Oppenheim A v., Schafer RW, Buck JR (1999) Discrete-Time Signal Processing, 2nd ed. Upper
591 Saddle River, NJ.

592 Ozawa M, Davis P, Ni J, Maguire J, Papouin T, Reijmers L (2020) Experience-dependent
593 resonance in amygdalo-cortical circuits supports fear memory retrieval following extinction.
594 Nature Communications 11:1–16.

595 Pape HC, Pare D, Driesang RB, Paré D, Driesang RB (1998) Two types of intrinsic oscillations
596 in neurons of the lateral and basolateral nuclei of the amygdala. Journal of Neurophysiology
597 79:205–216.

598 Pavlov I, Savtchenko LP, Song I, Koo J, Pimashkin A, Rusakov DA, Semyanov A (2014) Tonic
599 GABAA conductance bidirectionally controls interneuron firing pattern and
600 synchronization in the CA3 hippocampal network. Proceedings of the National Academy of
601 Sciences 111:504–509.

602 Pian JP, Criado JR, Walker BM, & Ehlers CL (2008) Differential effects of acute alcohol on
603 EEG and sedative responses in adolescent and adult Wistar rats. Brain research, 1194, 28–
604 36.

605 Pignatelli M, Beyeler A. (2019) Valence coding in amygdala circuits. Current Opinion in
606 Behavioral Science 26 97–106.

607 Rhodes JS, Best K, Belknap JK, Finn DA, Crabbe JC (2005) Evaluation of a simple model of
608 ethanol drinking to intoxication in C57BL/6J mice. Physiology & Behavior 84:53–63.

609 Santhakumar V, Wallner M, Otis TS (2007) Ethanol acts directly on extrasynaptic subtypes of
610 GABAA receptors to increase tonic inhibition. Alcohol 41:211–221.

611 Silberman Y, Bajo M, Chappell AM, Christian DT, Cruz M, Diaz MR, Kash T, Lack AK,
612 Messing RO, Siggins GR, Winder D, Roberto M, Mccool BA, Weiner JL (2009)
613 Neurobiological mechanisms contributing to alcohol-stress-anxiety interactions. Alcohol
614 43:509–519.

615 Smith JP, Randall CL (2012) Anxiety and Alcohol Use Disorders: Comorbidity and Treatment
616 Considerations. Alcohol Research: Current Reviews 23:414–431.

617 Sneddon EA, White RD, Radke AK (2019) Sex Differences in Binge-Like and Aversion-
618 Resistant Alcohol Drinking in C57BL/6J Mice. Alcoholism: Clinical and Experimental
619 Research 43:243–249.

620 Sohal VS, Zhang F, Yizhar O, Deisseroth K (2009) Parvalbumin neurons and gamma rhythms
621 enhance cortical circuit performance. Nature 459:698–702.

622 Stujenske JM, Likhtik E, Topiwala MA, Gordon JA (2014) Fear and Safety Engage Competing
623 Patterns of Theta-Gamma Coupling in the Basolateral Amygdala. Neuron 83:919–933.

624 Substance Abuse and Mental Health Services Administration (2020) Key Substance Use and
625 Mental Health Indicators in the United States: Results from the 2019 National Survey on
626 Drug Use and Health. Rockville, MD.

627 Sundstrom-Poromaa I, Smith DH, Gong QH, Sabado TN, Li X, Light A, Wiedmann M,
628 Williams K, Smith SS (2002) Hormonally regulated α4 β2 δ GABAA receptors are a target
629 for alcohol. Nature Neuroscience 5:721–722.

630 Tallot L, Graupner M, Diaz-Mataix L, & Doyère V (2020) Beyond Freezing: Temporal
631 Expectancy of an Aversive Event Engages the Amygdalo-Prefronto-Dorsostriatal Network.
632 Cerebral cortex (New York, N.Y. : 1991), 30(10), 5257–5269.

633 Thériault RK, Manduca JD, Perreault ML (2021) Sex differences in innate and adaptive neural
634 oscillatory patterns link resilience and susceptibility to chronic stress in rats. Journal of
635 Psychiatry and Neuroscience 46:E258–E270.

636 Thomas SE, Randall CL, Carrigan MH (2003) Drinking to Cope in Socially Anxious
637 Individuals: A Controlled Study. Alcoholism: Clinical and Experimental Research 27:1937–
638 1943.

639 Tye KM, Prakash R, Kim S-Y, Fenno LE, Grosenick L, Zarabi H, Thompson KR, Gradinaru V,
640 Ramakrishnan C, Deisseroth K (2011) Amygdala circuitry mediating reversible and
641 bidirectional control of anxiety. Nature 471.

642 Wallner M, Hanchar HJ, Olsen RW (2003) Ethanol enhances a4b3d and a6b3d GABAA
643 receptors at low concentrations known to affect humans. Proceedings of the National
644 Academy of Sciences 100:15218–15223.

Figure Legends

Figure 1. Acute alcohol alters BLA network activity differently in male and female C57BL/6J mice. **A** Representative targeting of BLA LFP recordings. **B,** Acute alcohol exposure paradigm consisted of LFP recordings during baseline (60 minutes), vehicle injection (0.9% saline i.p.; 60 minutes), and a treatment injection (0.9% saline or 1 g/kg alcohol i.p.; 60 minutes). This dose was determined through a dose response (Figure 1-2). **C,** Representative male spectrogram of normalized β power (15-30 Hz) and **D,** representative female spectrogram of normalized γ power (40-70 Hz) from acute alcohol exposure. **E,** Average normalized β power in males and **F,** average normalized γ power in females during acute injections of vehicle and alcohol (1 g/kg i.p.; dose response shown in Figure 1-2). Dots represent the mean and the shaded region represents SEM. **G,** Normalized power area for vehicle/alcohol acute exposure in males (n = 11) and **H,** females (n = 8). #$p < 0.05$ vs. baseline, *$p < 0.05$, **$p < 0.01$ vs. vehicle. Acute vehicle exposure does not alter BLA network activity in C57BL/6J mice (Extended Data Figure 1-1). Summaries of ANOVA and multiple comparison tests can be found in Table 1-1 and 1-2.

Figure 1-1. Acute vehicle exposure does not alter BLA network activity in C57BL/6J mice. **A,** Normalized power area for vehicle/vehicle acute exposure in male (n = 13) and **B,** female (n = 6) mice. **C,** Power spectral density of baseline, vehicle, and 1 g/kg alcohol injection over 0-80 Hz in male and **D,** female mice. #$p < 0.05$, ##$p < 0.01$ vs. baseline.

Figure 1-2. Effects of an alcohol dose response on BLA network states. **A,** Power area difference of 2-5, 6-12, and 15-30 Hz frequency ranges across alcohol doses in male C57BL/6J mice (0.5 n = 8; 1.0 n = 8; 1.5 n = 4; 2.0 n = 7). Shaded region indicates doses that caused high

immobility in mice. Normalized power area for vehicle/alcohol exposure across doses for **B,** 2-5 Hz**, C,** 6-12 Hz**, D,** 15-30 Hz**, E,** 40-70 Hz**, F** 80-120 Hz**.** *$p < 0.05$, **$p < 0.01$ vs. vehicle.

Figure 2. Acute alcohol produces similar effects in *Gabrd*$^{-/-}$ mice as female C57BL/6J mice. **A,** Representative male and **C,** female spectrogram of normalized γ power from acute alcohol exposure. **B,** Normalized power area for vehicle/alcohol acute exposure in male ($n = 9$) and **D,** female *Gabrd*$^{-/-}$ mice ($n = 8$). #$p < 0.05$ vs. baseline, *$p < 0.05$, **$p < 0.01$ vs. vehicle. Acute vehicle exposure does not alter BLA network activity in *Gabrd*$^{-/-}$ mice (Extended Data Figure 2-1). Summaries of ANOVA and multiple comparison tests can be found in Table 2-1 and 2-2.

Figure 2-1. Acute vehicle exposure does not alter BLA network activity in *Gabrd*$^{-/-}$ mice. **A,** Normalized power area for vehicle/vehicle exposure in male ($n = 8$) and **B,** female mice ($n = 7$).

Figure 3. Repeated alcohol exposure exaggerates BLA network modulation in male C57BL/6J mice. **A,** Experimental paradigm (left) of the repeated alcohol exposure procedure in male C57BL/6J and *Gabrd*$^{-/-}$ mice of BLA LFP recordings during baseline (60 minutes) and vehicle or alcohol injections (0.9% saline or 1 g/kg alcohol i.p.; 60 minutes) over five days. BEC measurements (right) of male C57BL/6J mice ($n = 6$) taken 15 minutes after alcohol exposure (1 g/kg i.p.) on day one, two, and five of exposure. ***$p < 0.0001$. **B,** Repeated alcohol paradigm (left) for female C57BL/6J and *Gabrd*$^{-/-}$ mice which includes the acute alcohol exposure and the repeated alcohol exposure as day two (first day) to five (last day). Justification for using day two instead of day one is in Extended Data Figure 3-3. BEC measurements (right) of female C57BL/6J mice ($n = 5$) taken 15 minutes after alcohol exposure (1 g/kg i.p.) on day one, two, and five of exposure. **C,** Change in effect of alcohol on the first and last day of exposure in male (first day $n = 8$; last day $n = 6$) and **D,** female (first day $n = 10$; last day $n = 8$) C57BL/6J mice

and **E,** male (first day $n = 8$; last day $n = 8$) and **F,** female (first day $n = 8$; last day $n = 7$) *Gabrd*$^{-/-}$ mice. *$p < 0.05$ vs first exposure. Repeated vehicle exposure does not change BLA network activity (Extended Data Figure 3-1). Baseline network activity is modulated by repeated alcohol in male C57BL/6J mice (Extended Data Figure 3-2). Summaries of ANOVA and multiple comparison tests can be found in Table 3-1 and 3-2.

Figure 3-1. Repeated vehicle exposure does not change BLA network activity in C57BL6/J and *Gabrd*$^{-/-}$ mice. **A,** Power area difference between vehicle and baseline for the first and last day of exposure in male (first day $n = 4$; last day $n = 3$) and **B,** female (first day $n = 5$; last day $n = 6$) C57BL/6J mice and **C,** male (first day $n = 4$; last day $n = 4$) and **D,** female (first day $n = 7$; last day $n = 7$) *Gabrd*$^{-/-}$ mice.

Figure 3-2. Repeated alcohol modulates baseline network activity in male C57BL/6J mice. **A,** Change in baseline on the first and last day of alcohol exposure in male and **B,** female C57BL/6J mice and **C,** male and **D,** female *Gabrd*$^{-/-}$ mice. *$p < 0.05$.

Figure 3-3. Effects of acute alcohol on day one (acute) and day five do not differ in female *Gabrd*$^{-/-}$ mice. **A,** Normalized power area of alcohol injections during the acute alcohol injection and day two in female C57BL/6J mice ($n = 10$). **B,** Normalized power area of alcohol injections during the acute alcohol injection and day two in female *Gabrd*$^{-/-}$ mice. **B,** Normalized power area of alcohol injections during acute alcohol injection and day five in female *Gabrd*$^{-/-}$ mice. **$p < 0.01$.

Figure 4. δ-$GABA_AR$ and sex dependent effects of repeated alcohol on BLA network activity. **A,** Comparison between male C57BL/6J and *Gabrd*$^{-/-}$ mice in the response to alcohol on the first and **B,** last day of exposure. **C,** Comparison between male and female C57BL/6J mice in the

response to alcohol on the first and **D,** the last day of exposure. *$p < 0.05$, **$p < 0.01$. Repeated alcohol has δ-$GABA_AR$ and sex dependent effects on baseline network activity (Extended Data Figure 4-1).

Figure 4-1. δ-$GABA_ARs$ and sex dependent effects of baseline network modulation after repeated alcohol. **A,** Comparison between male C57BL/6J and *Gabrd*$^{-/-}$ mice in the change in baseline on the first and **B,** last day of exposure. **C,** Comparison between male and female C57BL/6J mice in the change in baseline on the first and **D,** last day of exposure. *$p < 0.05$.

Figure 5. Repeated alcohol reduces δ-$GABA_AR$ expression on PV interneurons in female C57BL/6J mice. Integrated density of **A,** PV immunoreactivity and **B,** δ expression on PV interneurons in naive male (cell $n = 224$, animal $n = 4$) and female (cell $n = 202$, animal $n = 4$) C57BL/6J mice. **C,** Representative images from male and female C57BL/6J mice who received repeated vehicle or alcohol. Integrated density of **D,** PV immunoreactivity and **E,** δ expression on PV interneurons in the BLA of male (vehicle: cell $n = 112$, animal $n = 3$; alcohol: cell $n = 97$, animal $n = 3$) and female (vehicle: cell $n = 117$, animal $n = 3$; alcohol: cell $n = 169$, animal $n = 3$) C57BL/6J mice who received repeated vehicle or alcohol. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.

Table 1-1. Summary of ANOVA tests for acute alcohol experiments in C57BL/6J mice.

Table 1-2. Summary of multiple comparisons tests for acute alcohol experiments in C57BL/6J mice.

Table 2-1. Summary of ANOVA tests for acute alcohol experiments in *Gabrd*$^{-/-}$ mice.

730 **Table 2-2.** Summary of multiple comparisons tests for acute alcohol experiments in *Gabrd*$^{-/-}$
731 mice.

732 **Table 3-1.** Summary of ANOVA tests for repeated alcohol experiments.

733 **Table 3-2.** Summary of multiple comparisons tests for repeated alcohol experiments.

734