A SINGLE ALCOHOL PRE-EXPOSURE ALTERS DORSOLATERAL STRIATAL AMPA RECEPTOR DEPENDENT BINGE AND COMPULSIVE-LIKE DRINKING

by

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Dedicated to my wonderful husband, Devon Bauer.

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ABSTRACT

Background

Compulsive alcohol drinking is a defining characteristic of alcohol use disorder and the dorsolateral striatum (DLS) is implicated in regulating this inflexible behavior. AMPA receptors have been implicated in both goal-directed (dorsomedial striatal dependent) and DLS dependent inflexible behaviors with antagonism in the DLS and general DLS inhibition altering inflexible behavior including habit and compulsion. Discrepancies exist in the preclinical models used to investigate compulsive-like alcohol. The purpose of these experiments was to establish a robust model of compulsive-like quinine adulterated alcohol (QuA) drinking in C57BL/6J male and female mice, assess associated AMPA receptor protein expression in the dorsal striatum, and to antagonize DLS AMPA receptors during compulsive-like QuA drinking using a model of binge-like alcohol drinking, Drinking-in-the-Dark (DID).

Methods

In aim 1, C57BL/6J mice were allowed free access to 20% (v/v) alcohol (alcohol history), or water (water history) for two hours each day beginning three hours into the dark cycle for 23 days. On days 15 and 22 mice were given QuA to test for compulsive-like QuA drinking. 24-hours following the last DID session brain slices were taking for DLS and DMS AMPA receptor western blot. In aim 2, C57BL/6J mice were given a total of 21 days alcohol history, to establish a compulsive-like phenotype, or water history, prior to infusion. On days 22 and 24 mice were given a bilateral infusion of one of three concentrations of NBQX, an AMPA receptor antagonist, into the DLS, immediately prior to DID where the DID solution was either alcohol or QuA.

Results

We found that three weeks, not two, is sufficient to produce robust compulsive-like QuA drinking in C57BL/6J mice. We failed to replicate our compulsive-like DID model in aim 2 and found that infusion of NBQX reduced 2-hour alcohol drinking and reduced 2-hour QuA drinking when QuA was the second solution presented on infusion days in male water history mice only.

We also found that NBQX reduced 20-minute front-loading in female alcohol history mice on alcohol intake and trended toward QuA intake. Overall locomotor activity was affected by drug infusions.

Conclusions

Together, these data suggest that compulsive-like alcohol drinking can be achieved following three-weeks DID and DLS infusion of NBQX reduces both alcohol and QuA drinking in a sex and drinking history dependent way, and these effects may be reliant on an initial single QuA or alcohol exposure.

INTRODUCTION

Alcohol Use Disorder

Alcohol use disorder (AUD) is a chronic disease that often results in compulsive drinking or drinking despite clear evidence of harmful consequences (American Psychiatric Association, 2013). The dorsolateral striatum (DLS) is a brain region that has been implicated in mediating inflexible behaviors such as habit and compulsion (Belin, Belin-Rauscent, Murray, & Everitt, 2013; Everitt & Robbins, 2016; Guiliano, Belin, & Everitt; 2019). A leading hypothesis in alcohol addiction research is that the shift from goal-directed, dorsomedial striatum (DMS) dependent, to compulsive behaviorally inflexible, dorsolateral striatum (DLS) dependent, alcohol drinking may be result of a shift in synaptic inputs from the prefrontal cortex into the dorsal striatum such that long-term potentiation differentially modulates the behavioral output (Everitt, Belin, Exonomidou, Pelloux, Dalley, & Robbins, 2008; Everitt & Robbins, 2013; Lüscher, Robbins, & Everitt, 2020). Additionally, recent data suggests that this shift to compulsive-like alcohol drinking may be due to the inability to weaken the cortical inputs into the DLS (Guilianao, Belin, & Everitt, 2019). However, the exact mechanisms underlying compulsive-like alcohol drinking remain unknown.

AMPA Receptors and Striatal MSNs

Postsynaptic AMPA gated medium spiny neurons (MSNs) in the dorsal striatum receive excitatory cortical input which then cause inhibition of the D1 or D2 dopamine pathways that project to a variety of reward related brain regions, as part of the corticostriatal circuit (Shiflett & Balleine, 2011; Bamford, Wightman, & Sulzer, 2019). AMPA receptors are ionotropic glutamate receptors that are involved in fast-acting neurotransmission in the CNS and are vital for long term potentiation (LTP). LTP is dependent on the membrane insertion of post-synaptic AMPA receptors, where glutamate binding to AMPA receptors is necessary to cause post-synaptic depolarization. AMPA receptors are dimers of dimers, composed of four different subunits (GluA1-4), with the most common subunit composition being GluA1 and GluA2. Additionally, AMPA receptors are permeable to sodium only, unless they lack the GluA2 subunit, in which GluA2 lacking AMPA receptors are permeable to both sodium and calcium (Henley &

Wilkinson 2016; Malenka & Bear, 2004). The development of compulsive-like alcohol drinking is likely mediated by the region specific glutamatergic input into the AMPA gated MSNs in the dorsal striatum resulting in the shift from goal-directed to compulsive alcohol drinking behavior (Yin & Knowlton, 2006; Ballenie, Delgado, & Hikosaka, 2007; Koob & Volkow, 2010; Everitt & Robbins, 2016). In further support of this, Wang et al. (2012) found that repeated ethanol exposure increases synaptic AMPA receptors in the DMS in rats demonstrating goal-directed operant responding and Ma et al. (2018) demonstrated that ethanol seeking behavior can be mediated by the optogenetic induction of LTP and long-term depression (LTD), both AMPA receptor dependent phenomena, pre and postsynaptically on DMS MSNs. Additionally, Corbit, Nie, and Janak, 2014 found that antagonism of DLS AMPA receptors returned habitual alcohol responding rats to goal directed. Thus, investigation of glutamate and AMPA receptors in the behavioral shift from goal-directed to compulsive-like alcohol drinking within the dorsal striatum is advantageous to further understand AUD.

Compulsive-like Alcohol Drinking in Humans and Rodents

Compulsive alcohol drinking in the human population is exemplified by making decisions to consume alcohol with clear knowledge of a negative consequence. For instance, a compulsive alcohol drinker may choose to drink alcohol during work hours being fully aware that this behavior may result in the loss of income or a person may compulsively drink a non-beverage alcohol solution like methanol being fully aware that this may result in an aversive taste and poisoning (Lachenmeier, Rehm, & Gmel, 2007; Green, Neff, Giuliano, Lee, Turchin, & Kunkel, 2018). Compulsive-like alcohol drinking has been modeled in mice by adulterating the alcohol solution, in which mice freely consume, with the bitter compound, quinine (Hopf & Lesscher, 2014). Adulterating the alcohol solution with quinine results in a highly aversive flavor with no other known negative consequence. While previous research has aimed to model compulsive-like alcohol drinking in mice, discrepancies in compulsive-like quinine drinking models and definitions exist. Generally, the agreed upon definition is that compulsive mice will consume the same amount of quinine-adulterated alcohol (QuA) as regular alcohol whereas non-compulsive mice would consume significantly less QuA than regular alcohol and that the development of compulsive alcohol drinking can be established through having a sufficient

alcohol drinking history (Lesscher, Van Kerkhof, & Vanderschuren, 2010; Sneddon, White, & Radke, 2019).

Compulsive-like Alcohol Drinking Models

Alcohol histories and consumption models sufficient to produce compulsive-like alcohol drinking vary, with compulsive-like alcohol drinking concluded in as little as 24-hours limited-access to as much as six weeks two-bottle choice access, although the exact timeframe to establish compulsive-like alcohol drinking is unknown (Lei, Wegner, Yu, Simms, & Hopf, 2016; Fulenwider, Nennig, Price, Hafeez, & Schank, 2019). Further, concentration of quinine used varies widely, ranging from 30 to 1000 μM concentrations (Lei, Wegner, Yu, Simms, & Hopf, 2016; Blegen, da Silvia E Silva, Bock, Morisot, Ron, & Alvarez, 2018; Bocarsly et al., 2019; Fulenwider, Nennig, Price, Hafeez, & Schank, 2019; Siciliano et al., 2019). Additionally, most of the compulsive-like alcohol drinking papers have not investigated this behavior in female mice. Thus, there is a critical need to further establish a robust model of compulsive-like alcohol drinking and to investigate whether compulsive-like alcohol drinking is a sexually dimorphic behavior.

Previous Research

Previously, researchers have used the competitive AMPA/Kainate receptor antagonist, NBQX, to investigate the role of AMPA receptors in alcohol drinking. NBQX has as relatively high binding affinity for AMPA receptors (K_D = 47 nM; Dev, Petersen, Honoré, & Henley, 1996) and is more selective for AMPA receptors by 30 fold than it is for kianate receptors, making it a useful pharmacological agent to assess AMPA receptors (Sheardown, Nielsen, Handsen, Jacobsen, & Honoré, 1990). Previous work investigating the role of AMPA receptors and ethanol consumption have demonstrated efficacy of NBQX, both systemically to reduce ethanol consumption (Stephens & Brown, 1999; Ruda-Kucerova et al., 2018) and site specifically in the DMS (Wang et al., 2012) and DLS (Corbit, Nie, & Janak, 2014) to reduce operant responding for ethanol but not sucrose. Additionally, work from our laboratory has shown that systemic injection of NBQX reduces binge-like alcohol consumption in male, but not female, C57BL/6J during Drinking-in-the-Dark (DID; Bauer, Garcy, & Boehm, 2020). While the previously

mentioned research has demonstrated the role of AMPA receptors in alcohol consumption, no work to our knowledge has demonstrated the role of DLS AMPA receptors in a limited-daily access alcohol consumption model of compulsive binge-like drinking. To expand on this literature, we sought to develop a robust model of compulsive-like alcohol drinking using male and female mice and to test compulsive like alcohol drinking at two and three weeks alcohol history, assess associated AMPA receptor protein levels in the DMS and DLS, and investigate the effect of direct manipulation of DLS AMPA receptors in compulsive and non-compulsive mice on regular alcohol and QuA drinking.

AIM 1 SPECIFIC AIMS

Goals

The goals of this aim are to 1) validate that robust quinine resistant alcohol drinking can be achieved at 0.5 mM quinine following 14-days (21-days if not achieved) of limited-access alcohol consumption, 2) provide molecular evidence for the hypothesized DLS AMPA receptor changes associated with compulsive-like QuA drinking in hypothesis 2 and 3, and 3) investigate any sex specific effects of quinine resistant alcohol drinking and sex specific differences in AMPA receptor expression.

Hypotheses

Hypothesis 1

Male and female mice with an alcohol history will show robust quinine resistant alcohol drinking as defined by 1) consuming significantly more QuA than water history controls, 2) consuming significantly more regular alcohol on the first day of alcohol exposure than (i.e. day 1) than water history mice consume of QuA to ensure any QuA intake is not due to novelty of fluid, and 3) consuming the same amount of alcohol on the day prior to QuA testing (i.e. baseline) as QuA.

Hypothesis 2

There will be an increase in AMPA receptor expression in both males and females following alcohol exposure.

Hypothesis 3

Females will consume more alcohol than males, quinine resistance and molecular changes following alcohol consumption will not be sexually dimorphic.

Hypothesis 4

Locomotor activity will not be affected by sex, drinking history, or test day during consumption on baseline or QuA test days.

AIM 1 METHODS

Animals

Naïve adult male and adult female C57BL/6J mice (PND 63-68 at test start date, n = 7-9/group) were acquired from Jackson Laboratories (Bar Harbor, ME). Animals were individually housed in a vivarium with 12h:12h reverse light-dark cycle for one week prior to the start of experiments. Throughout the experiment, animals received food and water ad libitum with the exception of water bottle removal during the two-hour DID sessions. Procedures were approved by the IUPUI School of Science Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals (The National Academic Press, 2003).

Drinking-in-the-Dark (DID)

DID is a limited-access model of binge-like alcohol consumption. Animals received one 10 mL ball-bearing sipper tube of 20% v/v alcohol in tap water into their home cages in place of the regular water bottle. This occurred three hours into the dark cycle for two-hours for a total of 23 days. C57BL/6J mice demonstrate binge-like alcohol consumption during DID, achieving two-hour intakes approaching 3.0 g/kg and blood alcohol levels surpassing 0.08 g/dL (Kasten & Boehm, 2014). Consumption was measured by reading the sipper tubes to the nearest 0.025 mL, and volumes were adjusted for leak based on the volume leaked from a tube in an empty cage on the same rack.

Solutions

190 proof alcohol was purchased from Pharmco, Inc (Brookfield, CT) and was added to tap water to create a 20% v/v alcohol solution for use in DID. On test days (day 15 and 22) the 20% ethanol solution was adulterated with quinine-hemisulfate for a quinine-adulterated alcohol concentration of 0.5 mM (0.1957 g/L) for experiment animals and tap water was adulterated with the same concentration for quinine-adulterated water (QuW) for western-blot control animals.

Locomotor Monitoring

Home cage locomotor activity was monitored during baseline and test day drinking sessions. Data were collected using the Opto M-3 system (Columbus Instruments) which collects locomotor activity by summating beam breaks over a set time-interval from infrared beams that surround the perimeter of the home cage. Locomotor data are shown total ambient counts representing total beam breaks across the DID session.

Western Blot

Western-blot analyses were performed on DMS and DLS brain punches. 24-hours after the last DID session ended brains were extracted, rinsed in PBS, sliced in 2 mm coronal sections to expose the striatum, and DMS and DLS punches were taken. Brain punches were homogenized in RIPA buffer (RIPA buffer with protease inhibitor (1ml of RIPA buffer containing 100ul of 10X PI and 10ul of 10X PMSF). Each well was loaded with 20µg protein in 20 µl. Primary antibodies used were Anti-Glutamate Receptor 1 Rabbit polyclonal Antibody purchased from Millipore and Anti Glur2 polyclonal antibody purchased from Thermo Fischer Scientific. Samples were counterbalanced by sex and drinking history across gels, normalized to beta-actin, and shown as percent control where the average of the male-water history control mice samples are the control for their respective gel. Male, water-history mice were chosen as a control because they are water history mice, although female, water-history mice would have also sufficed as the control group chosen is relatively arbitrary.

Statistical Analyses

Statistical testing using ANOVA, correlation, and regression were performed in R and GraphPad Prism. All statistical assumptions were either passed or corrected for. Baseline alcohol drinking was analyzed with repeated-measures ANOVA in alcohol history mice only (sex X day). Compulsive-like QuA drinking was analyzed with ANOVA (drinking history X sex; drinking history/day (water history QuA intake vs alcohol history day 1 intake) X sex) and repeated-measures ANOVA (day X sex). Comparison of QuA test 1 and QuA test 2 were done using repeated-measures ANOVA (drinking history X sex X QuA test X drinking bin; drinking history X sex X QuA test; and QuA test X sex). Locomotor data were analyzed on baseline days

and QuA test days with repeated-measures ANOVA (drinking history X sex X drinking bin). Western-blot were analyzed for each protein and brain region with ANOVA (drinking history X sex). Differences were considered significant at $p < 0.05$.

AIM 1 RESULTS

Compulsive-like Alcohol Drinking

Baseline Drinking

Baseline alcohol drinking (days 1-14) for male and female C57BL/6J mice are shown in figure 2A. Alcohol consumption is displayed in grams consumed per kilogram of body weight per two hours. Water consumption is displayed in milliliters consumed per kilogram of body weight per two hours. Repeated-measures ANOVA with Greenhouse-Geisser corrections of baseline drinking in the alcohol history mice by sex revealed a significant main effect of day [$F(13,195) = 4.78$, $p < 0.0001$, $\eta^2_G = 0.20$] and sex [$F(1,15) = 8.49$, $p = 0.01$, $\eta^2_G = 0.10$] and no interactions.

Effect of 14-Days DID on QuA Drinking

The effect of a 14-day drinking history on QuA consumption is shown in figure 2. Several analyses were run to test our hypothesis that quinine-resistant alcohol drinking can be achieved following 14 days DID. First, mice underwent DID for 14 days (n=7-9) and received either water (water history) or alcohol (alcohol history) and were tested for quinine-resistant alcohol drinking on day 15. Two-way ANOVA of drinking history and sex revealed a main effect of sex [$F(1,28) = 11.28$, $p = 0.002$, $\eta^2_G = 0.29$], and a trend toward a main effect of drinking history, [$F(1,28) = 4.181$, $p = 0.0504$, $\eta^2_G = 0.13$], and no other interactions, demonstrating that alcohol history mice do not differ in QuA intakes as compared to water history mice; figure 2B. Next, we tested whether water history mice drank significantly less quinine-adulterated alcohol than alcohol history mice drank on their first day of alcohol consumption. Two-way ANOVA of sex and drinking history/day (water history QuA intake vs alcohol history alcohol day 1 intake) revealed a trend towards a main effect of sex, [$F(1,28) = 3.503$, $p = 0.0718$, $\eta^2_G = 0.11$], no significant effect of drinking history, or interactions, demonstrating that water history mice did not differ in intake of QuA as compared to alcohol history mice's consumption of unadulterated alcohol on day 1; figure 2C. Finally, we tested whether alcohol history mice drank the same amount of QuA on day 15 as regular alcohol on day

14. Repeated-measures ANOVA of sex and day revealed a main effect of sex, [$F(1,15) = 21.96$, $p < 0.001$, $\eta^2_G = 0.41$], a main effect of day, [$F(1,15) = 8.88$, $p = 0.009$, $\eta^2_G = 0.24$], and no interactions, demonstrating that alcohol history mice drank less QuA on day 15 than unadulterated alcohol on day 14; figure 2D. Together, these data demonstrate that 14-days alcohol history is not sufficient to produce robust quinine-resistant alcohol drinking and suggests that female alcohol history mice drink more than male alcohol history mice during a DID session, regardless of alcohol or quinine-adulterated alcohol solution.

Effect of 21-Days DID on QuA Drinking

The effect of a 21-day drinking history on quinine-adulterated alcohol consumption is shown in figure 3. The following analyses were run to test our hypothesis that quinine-resistant alcohol drinking can be achieved following 21 days DID. Mice underwent 6 more days of DID, a total of 21-days DID (n = 7-9) and received either water (water history) or alcohol (alcohol history) and were tested for quinine-resistant alcohol drinking on day 22. Baseline alcohol drinking (days 1-21) for male and female C57BL/6J mice are shown in figure 3A. Alcohol consumption is displayed in grams consumed per kilogram of body weight per two hours. Water consumption is displayed in milliliters consumed per kilogram of body weight per two hours. Repeated-measures ANOVA of baseline drinking by sex in the alcohol history mice revealed a significant main effect of day [$F(19, 285) = 4.4$, $p < 0.0001$, $\eta^2_G = 0.20$],sex [$F(1,15) = 12.21$, $p = 0.003$, $\eta^2_G = 0.14$], and no interactions. Two-way ANOVA of sex and drinking history on consumption revealed a main effect of drinking history, [$F(1, 28) = 12.27$, $p = 0.002$, $\eta^2_G = 0.30$], no effect of sex, and no interaction, demonstrating that the alcohol history mice drank significantly more QuA than water mice on QuA test day (day 22), regardless of sex; figure 3B. Next, we tested whether water history mice drank significantly less quinine-adulterated alcohol than alcohol history mice drank on their first day of alcohol consumption. Two-way ANOVA of sex and day/drinking history on consumption revealed a main effect of day/drinking history, [$F(1, 30) = 4.661$, $p = 0.039$, $\eta^2_G = 0.13$], and no interaction, demonstrating that water history mice drank significantly less QuA on day 22 than alcohol history mice drank on day 1, regardless of sex; figure 3C. Finally, we tested whether alcohol history mice drank the same amount of QuA on day 22 as they drank on the day prior to QuA testing. Two-way repeated-measures ANOVA of QuA and baseline intake in alcohol history mice by sex revealed a main

effect of sex, [F(1,15) = 4.572, p = 0.0494, $\eta^2_G = 0.15$], no effect of day, and interaction, demonstrating that alcohol history mice drank the same amount of QuA as unadulterated alcohol and that females drink more than males of either solution; figure 3D. Together, these data demonstrate that compulsive-like quinine-resistant alcohol drinking can be sufficiently achieved in both males and females following three weeks of alcohol history and that, on average, female alcohol history mice drink more than male alcohol history mice during a DID session, regardless of alcohol or QuA solution.

Exploratory Analyses on QuA Test Days for Compulsive-like QuA Drinking

Relationship Between History, Sex, and QuA Test Day on QuA Intake

Given the robust quinine resistant alcohol drinking seen after 21 days alcohol history, and not 14 days alcohol history, we sought to further investigate the relationship between drinking history, sex, and QuA test day on QuA intake through exploratory post-hoc analyses; figure 4. Repeated-measures three-way ANOVA of drinking history, sex, and QuA test day on QuA intake revealed a significant main effect sex, [F(1,28) = 6.2, p = 0.018, $\eta^2_G = 0.144$], drinking history, [F(1,28) = 10.38, p = 0.003, $\eta^2_G = 0.219$], an interaction of sex and QuA test day, [F(1,28) = 4.32, p = 0.046, $\eta^2_G = 0.036$], and no other interactions; figure 4A. Additionally, this ANOVA yielded a trend toward a main effect of QuA test day [(1,28) = 4.18, p = 0.0502, $\eta^2_G = 0.034$], and trend toward an interaction of drinking history and QuA test day, [F(1,28) = 3.29, p = 0.08, $\eta^2_G = 0.027$]. To further investigate the interaction of sex and QuA test day on QuA drinking, we ran a post-hoc repeated-measures ANOVA on QuA drinking, revealing a main effect of sex, [F(1,30) = 5.66, p = 0.023, $\eta^2_G = 0.13$], a trend toward an effect of QuA test day, [F(1,30) = 4.01, p = 0.054, $\eta^2_G = 0.026$], and a trend toward an interaction of QuA test day and sex, [F(1,30) = 3.73, p = 0.063, $\eta^2_G = 0.025$], and no other interactions; figure 4A. Due to the non-significant p-values and small effect sizes of the trends, these data will not be interpreted as a significant effect.

Further, to confirm that differences between quinine-resistant alcohol drinking in the alcohol mice between QuA test day 1 and QuA test day 2 were not due, in part, to differences in baseline drinking (i.e. day prior to QuA test day) we assessed differences in percent baseline QuA intake between QuA test day and sex. Repeated-measures two-way ANOVA of sex and

QuA test day in alcohol history mice on QuA drinking did not reveal a significant effect of sex, or QuA test day on percent baseline QuA intake for alcohol history mice, or any interactions; figure 4B. These data support the fact that female mice drank significantly more QuA than male mice and that alcohol history mice drank significantly more QuA that water history mice, regardless of test day. Interestingly, neither alcohol history or water history mice significantly increased or decreased their QuA intake from QuA test 1 to QuA test 2, nor did intake of QuA differ as a function of baseline drinking between sex or QuA test day.

Exploratory Analysis of Escalation of Compulsive-like Alcohol Drinking

Temporal Dynamics of Compulsive-like QuA Drinking

To further illuminate the underlying mechanism driving the behavioral differences between QuA test 1 and QuA test 2 in producing compulsive-like quinine-resistant alcohol drinking, we investigated the temporal dynamics of compulsive-like drinking escalation behavior across the QuA DID sessions through exploratory post-hoc analyses; figure 5. First, we assessed intake across DID sessions in 30-minute drinking bins by sex, drinking history, and QuA test day. Repeated-measures ANOVA with Greenhouse-Geisser correction revealed a significant main effect of sex, [$F(1,28) = 6.37$, $p = 0.01$, $\eta^2_G = 0.71$], drinking history, [$F(1,28) = 1.0.68$. $p = 0.002$, $\eta^2_G = 0.05$], drinking bin, [$F(3,84) = 27.66$, $p < 0.0001$, $\eta^2_G = 0.33$), an interaction between sex and drinking bin, [$F(3,84) = 5.20$, $p = 0.002$, $\eta^2_G = 0.085$], an interaction of drinking bin and QuA test day [$F(3,84) = 6.27$, $p < 0.001$, $\eta^2_G = 0.062$] and no other interactions; figure 5A. To probe the two-way interaction between sex and drinking bin, we ran post-hoc separate one-way ANOVAs on alcohol consumption. Simple effects one-way ANOVA of sex on QuA consumption revealed a significant main effect of sex, [$F(1,30) = 5.76$, $p = 0.02$, $\eta^2_G = 0.16$]. Post-hoc one-way ANOVA with Greenhouse-Geisser correction of drinking bin on QuA consumption revealed a significant main effect of drinking bin, [$F(3,93) = 22.62$, $p > 0.0001$, $\eta^2_G = 0.35$]. Bonferroni corrected paired t-test revealed that males did not differ in the amount of QuA consumed across drinking bin (all p's > 0.05). Bonferroni corrected paired t-tests revealed that females drank significantly more QuA at 0-30 minutes than at 60-90 minutes ($p < 0.0001$), more at 0-30 minutes than at 90-120 ($p < 0.0001$), more at 30-60 than at 90-120 minutes ($p < 0.04$), and more at 60-90 minutes than at 90-120 minutes ($p < 0.001$), regardless of QuA test day

or drinking history; figure 5A and 5B. These data demonstrate that female, but not male mice, consume greater amounts of QuA in the first 30-minutes of DID than at almost any other timepoint during the DID session and that male, but not female, mice escalate in their consumption (but not front-load) throughout the DID session, regardless of drinking history or QuA test day. Next, we probed the interaction between drinking bin and QuA test day. To probe this interaction, we used separate one-way ANOVAs to determine if there is a main effect of drinking bin and QuA test day on alcohol consumption. One-way ANOVA of QuA test day did not reveal a significant effect of QuA test day, though there was a trend, [$F(1,31) = 3.43$, $p = 0.07$, $\eta^2_G = 0.02$]. Because we did not find a significant simple effect for QuA test day on drinking bin, we will not further probe this interaction. These data demonstrate interesting differences is the in the pattern of intake across DID session between sex and drinking bin. Importantly, when assessing the effect of QuA intake across drinking history, sex, and drinking bin, female mice consume significantly more QuA than male mice and alcohol history mice consistently consume significantly more QuA than water history mice.

Exploratory Analysis of Front-Loading Behavior During Compulsive-like Alcohol Drinking

Temporal Dynamics of Compulsive-like QuA Drinking

In continuing to investigate of the temporal dynamics between QuA test day, drinking history, and sex on QuA intake, we investigated differences in 30-minute front-loading behavior between the groups in exploratory post-hoc analyses; figure 5. Because the alcohol history mice consistently consume more QuA than the water history, we chose to assess front-loading behavior by looking at first 30-minutes as percent of the entire 2-hour DID session, to account for total intake differences. Repeated-measures ANOVA on QuA intake in the first 30 minutes as percent 2-hr intake of QuA test day, drinking history, and sex on 30-minute QuA intake as percent total session, revealed a main effect of QuA test day, [$F(1,28) = 12.04$, $p = 0.0017$, $\eta^2_G = 0.103$], an interaction of QuA test day and drinking history, [$F(1,28) = 6.036$, $p = 0.02$, $\eta^2_G = 0.055$], a trend toward a main effect of sex, [$F(1,28) = 3.228$, $p = 0.083$, $\eta^2_G = 0.078$], and no other interactions. Follow-up t-test of drinking history on QuA intake on QuA test 1 revealed a significant main effect of drinking history, such that alcohol history mice ($M = 44.36$, $SD =$

18.98) consumed significantly more of their total percent intake in the first thirty minutes of the DID session compared to water history mice (M = 25.19, SD = 16.98) on QuA test 1, [t(2.99) = 30, p = 0.006]; figure 5C. Follow-up t-test of drinking history on QuA intake on QuA test 2 revealed that alcohol history mice (M = 48.79, SD = 20.45) do not differ in their total percent intake in the first thirty minutes of the DID session compared to water history mice (M = 49.35, SD = 29.68) on QuA test 2; figure 5C. Additionally, paired t-test of water history mice across QuA test day revealed that water history mice consume significantly more of their total percent intake in the first thirty minutes of the DID session on QuA test 2 (M = 49.35, SD = 29.68) than on QuA test 1 (M = 25.19, SD = 16.98), [t(3.73) = 14, p = 0.002]; figure 5C. Finally, we tested whether front-loading behavior was altered in alcohol history mice on QuA test day compared to the day prior (baseline). We ran a repeated-measures ANOVA of sex and QuA test day as percent baseline (i.e. day before QuA testing) on QuA intake in the first 30-minutes of the DID session. Repeated-measures ANOVA of sex and QuA test day on 30-min QuA intake as percent baseline revealed a main effect of QuA test day, [F(1,30) = 11.34, p = 0.002, η^2_G = 0.041], andan interaction of QuA test day and sex, [F(1,30) = 4.34, p = 0.046, η^2_G = 0.016]; figure 5D. Post-hoc t-test of sex on QuA test 1 revealed that female mice (M = 60.20, SD = 11.64) front-load significantly more QuA than male mice (M = 25.04, SD = 26.34), [t(3.64) = 15, p = 0.002]; figure 5D. Post-hoc t-test of sex on QuA test 2 revealed no effect of sex on QuA intake.

Further, we also assessed whether 30-minute raw front-loading intakes differed by sex, drinking history, or QuA test day. Three-way repeated-measures ANOVA of sex, drinking history, and QuA test day revealed a significant main effect of sex [F(1,28) = 6.37, p = 0.01, η^2_G = 0.14], drinking history [F(1,28) = 10.67, p = 0.002, η^2_G = 0.22], a trend toward a main effect of QuA test day [F(1,28) = 3.84, p = 0.06, η^2_G = 0.03), a trend toward an interaction of sex and QuA test day [F(1,28) = 3.99, p = 0.055, η^2_G = 0.034); data are shown in figure 5A and 5B's first 30-minute bin, and no other interactions. Together these data highlight important differences in front-loading between QuA test days and drinking history where female and alcohol history mice front-load alcohol significantly more than male or water history mice, Regardless of sex, a single previous QuA exposure caused water history mice to consume the same proportion of QuA as the alcohol history mice when assessed as 30-minute intakes per entire DID session on QuA2.

Overall, these data suggest that quinine-resistant compulsive like alcohol drinking may be achieved after three weeks, but not two weeks alcohol history. These differences may be due to

the development and alteration in 'wanting' behavior and represent motivational changes from QuA test 1 and QuA test 2 on front-loading behavior of the QuA solution, such that water history mice do not 'want' QuA to the same extent as alcohol history mice (see discussion for more on 'wanting' behavior). Importantly, the effect of a single QuA session altered later front-loading behavior in water history mice, demonstrating that a single DID session can alter alcohol related drinking behaviors. Additionally, the effect of single QuA expsoure altering later consumption during QuA could be due to repeated expsoure to the discusting properties of QuA, where an initial QuA exposure is required for mice to get over the intial aversiveness.

Locomotor Activity

Baseline Locomotor Activity

Locomotor data were collected during the first and second baseline and QuA sessions; figure 6. We assessed the locomotor activity across 30-min activity bins during the two-hour baseline alcohol drinking sessions across sex, drinking history, activity bin, and baseline day. Data are displayed as ambulatory counts per 30-minute time bin, figure 6A and 6C.

Repeated-measures ANOVA of drinking history, activity bin, baseline day, and sex on baseline locomotor activity revealed significant main effects of drinking history [$F(1,28) = 9.15$, $p = 0.005$, $\eta^2_G = 0.13$], activity bin [$F(3,84) = 79.94$, $p < 0.0002$, $\eta^2_G = 0.028$], and baseline day [$F(1,28) = 20.92$, $p < 0.0001$ $\eta^2_G = 0.13$], and interactions between drinking history and activity bin [$F(3,84) = 5.27$, $p = 0.002$, $\eta^2_G = 0.03$], activity bin and baseline day [$F(3,84) = 112,12$, $p < 0.0001$, $\eta^2_G = 0.38$], and drinking history, activity bin, and baseline day [$F(3, 84) = 7.12$, $p = 0.002$, $\eta^2_G = 0.037$], as well as a trend toward an interaction between drinking history and baseline day [$(1,28) = 4.14$, $p = 0.051$, $\eta^2_G = 0.029$], and no other interactions. To probe these interactions, we first looked for simple main effects. First, we tested the interaction of drinking history and activity bin by assessing the effect of activity bin in the alcohol history mice on locomotor activity. Repeated-measures ANOVA in the alcohol history mice across activity bin revealed a significant main effect of activity bin, [$F(3,93) = 9.16$, $p > 0.001$, $\eta^2_G = 0.05$]. Bonferroni-corrected paired t-tests in alcohol history mice revealed that alcohol history mice had significantly more ambulatory activity at 30 minutes compared to 90 minutes ($p < 0.001$), at 30 compared to 120 minutes ($p = 0.04$), and at 60 minutes compared to 90 minutes ($p < 0.001$). We

next tested the effect of activity bin in the water history mice on locomotor activity. Repeated-measures ANOVA in the water history mice across activity bin revealed a significant main effect of activity bin, [$F(3,93) = 5.23$, $p = 0.002$, $\eta^2_G = 0.031$]. We found through Bonferroni-corrected paired t-tests that water history mice had significantly more ambulatory activity 30 minutes compared to 60 minutes ($p < 0.001$), at 30 compared to 60 minutes ($p = 0.017$), and at 30 minutes compared to 120 minutes ($p = 0.01$). Next, we probed the interaction of baseline day and activity bin. Repeated-measured ANOVA across activity bin for baseline day 1 on locomotor activity revealed a significant main effect of activity bin, [$F(3,93) = 3.78$, $p = 0.013$, $\eta^2_G = 0.013$]. Bonferroni-corrected paired t-tests on baseline day 1 across activity bin revealed that mice had significantly more ambulatory activity at 30 minutes compared to 60 minutes ($p = 0.027$). Repeated-measures ANOVA across activity bin for baseline day 2 on locomotor activity revealed a significant main effect of activity bin, [$F(3,93) = 8.19$, $p < 0.0001$, $\eta^2_G = 0.06$]. Bonferroni-corrected paired t-tests on baseline day 2 across activity bin revealed that mice had significantly more ambulatory activity at 30 minutes compared to 60 minutes ($p = 0.002$), at 30 minutes compared to 90 ($p < 0.0001$), and at 30 minutes compared to 120 ($p = 0.003$). We then assessed the trending interaction between drinking history and baseline test day. Repeated-measures ANOVA across baseline days in the alcohol history mice did not reveal any significant effects ($p > 0.05$). Repeated-measures ANOVA across baseline days in the water history mice did not reveal any significant effects ($p > 0.05$). Because these data were collected during baseline drinking days, water history mice were only consuming water and alcohol history mice were consuming alcohol. Therefore, the differences observed between drinking history could be due to the acute effect of alcohol on locomotor activity. These data demonstrate that regardless of sex or drinking history, activity was greatest in the first 30-minutes of baseline drinking.

QuA Test Day Locomotor Activity

Next, we looked at the locomotor activity across 30-min activity bins during the two-hour QuA DID sessions across sex, drinking history, activity bin, and QuA test day, figure 6B and 6D. Repeated-measures ANOVA of sex, drinking history, activity bin, and QuA test day with Greenhouse-Geisser corrections during the two-hour QuA Did session revealed a significant main effect of drinking history, [$F(1,28) = 8.28$, $p = 0.00755$, $\eta^2_G = 0.14$], activity bin [$F(3,84) = 5.25$, $p = 0.002$, $\eta^2_G = 0.028$], and no other interactions. These data demonstrate that water

history mice are more active than alcohol history mice during the QuA DID sessions regardless of QuA test day or sex. Locomotor activity varied by activity bin, but this did not interact with sex, drinking history, or QuA test day.

AMPA Receptor Protein Expression

To test our AMPA receptor protein expression hypothesis, we assessed whether compulsive-like alcohol drinking via 3-weeks alcohol history would alter DMS and DLS GluA1 and GluA2 AMPA receptor protein expression. Following the final QuA test day, mice were returned to whatever solution they previously consumed (i.e. water or alcohol) on day 23, and 24-hours later brains were extracted and DMS and DLS regions were extracted. Two-way ANOVA of sex and drinking history did not alter GluA1 or GluA2 protein expression in any brain region, (all p's > 0.05; F's < 1.57, p's > 0.22); figure 7.

AIM 2 SPECIFIC AIMS

Goals

Aim 2A

The goals of this aim are to investigate the effects of AMPA receptor antagonism on quinine resistant drinking within the DLS in mice with and without an alcohol history sufficient to produce compulsive-like QuA drinking, at three different drug concentrations.

Aim 2B

The goals of this aim are to investigate sex differences of AMPA receptor antagonism on quinine resistant drinking within the DLS in animals with and without an alcohol history sufficient to produce compulsive-like QuA drinking at three different drug concentrations.

Hypotheses

Aim 2A

Hypothesis 1

DLS AMPA receptor antagonism via NBQX will decrease quinine resistant drinking, but not regular alcohol drinking in animals with an alcohol history. This effect will be concentration dependent.

Hypothesis 2

DLS AMPA receptor antagonism via NBQX will not affect QuA drinking or regular alcohol drinking in animals without a sufficient alcohol history.

Hypothesis 3

Locomotor activity will not be affected by infusion of NBQX at any dose, sex, or alcohol history.

Aim 2B

Hypothesis 1

DLS AMPA receptor antagonisms via NBQX will decrease quinine resistant drinking, but not regular alcohol drinking in animals with an alcohol history in both males and females.

Hypothesis 2

Females will drink significantly more than males regardless of condition.

Hypothesis 3

Males will be affected to a greater extent than females by AMPA receptor antagonism via NBQX.

Hypothesis 4

Locomotor activity will not be affected by infusion of NBQX at any dose, sex, or alcohol history.

AIM 2 METHODS

Animals

Naïve adult male and adult female C57BL/6J mice (PND 62-90 at test start date; n = 7-11 per group, N = 120 mice total) were acquired from Jackson Laboratories (Bar Harbor, ME). Animals were individually housed in a vivarium with 12h:12h reverse light-dark cycle for at least one week prior to the start of experiments. Throughout the experiment, animals received food and water ad libitum with the exception of water bottle removal during the two-hour DID sessions. Procedures were approved by the IUPUI School of Science Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals (The National Academic Press, 2003). A total of 159 mice underwent bilateral cannulation surgery. Following, a total of 39 mice were excluded from this experiment. 22 mice were removed from the experiment because their headcaps came off prior to infusion and 17 mice were excluded due to missed placements. Missed placements were identified by the experimenter, blinded to alcohol drinking history, sex, and dose, were defined by anything outside of the DLS according to Paxinos and Franklin's Mouse Brain Atlas.

Drinking-in-the-Dark (DID)

DID is a limited-access model of binge-like alcohol consumption. Animals received one 10 mL ball-bearing sipper tube of 20% v/v alcohol in tap water into their home cages in place of the regular water bottle. This occurred 2.5-3.5 hours into the dark cycle for two-hours for a total of 23 days. Animals are still very active during this time period and this adjustment to DID should not affect the results of the experiment (Rhodes, Best, Belknap, Finn, & Crabbe, 2005).. Consumption was measured by reading the sipper tubes to the nearest 0.025 mL, and volumes were adjusted for leak based on the volume leaked from a tube in an empty cage on the same rack

Solutions

190 proof alcohol was purchased from Pharmco, Inc (Brookfield, CT) and was added to tap water to create a 20% v/v alcohol solution for use in DID. On test days (either day 22 or 24)

the 20% ethanol solution was adulterated with quinine-hemisulfate for a quinine-adulterated alcohol concentration of 0.5 mM (0.1957 g/L).

Locomotor Monitoring

Home cage locomotor activity was monitored during mock injections and infusion days during the drinking sessions. Data were collected using the Opto M-3 system (Columbus Instruments) which collects locomotor activity by summating beam breaks over a set time-interval from infrared beams that surround the perimeter of the home cage. Locomotor data are shown as total beam breaks across the DID session.

Bilateral Cannulation

The DLS was targeted for bilateral cannulations using a Kopf stereotaxic alignment system at predetermined coordinates, (M/L: +/- 2.5 mm, A/P: + 0.38 mm, and D/V: - 3.0 mm). Briefly, mice were anesthetized with isoflurane, heads were shaved, and eyes were moistened with sterile lubricant eye ointment. The dorsal scalp was sterilized with povidone-iodine solution (10%) and sterile alcohol prep pads (isopropyl alcohol 70% v/v antiseptic) (repeated 3 times). Following, a midline incision was made extending from bregma to lambda (about 4.21 mm wide). Two holes were drilled through the skull for simultaneous placement of guide cannula (10-mm long, 25-gauge) and a third and fourth hole were drilled 2mm anterior of the guide cannula holes for placement of anchor screws. Cement was applied to the skull surrounding the guide cannula and on the screws. The mice received a single subcutaneous injection of 5 mg/mL carprofen (a post-operative pain treatment) at 10 mL/kg and were placed a heating pad until recovery from anesthesia (approximately 30-mintues). Following, mice were given ad-libitum food and water and at least 24-hours of recovery prior to DID.

Stylet Changing and Habituation

Stylets were changed daily for all of the mice. Mice were restrained in the five days leading up to microinjections in increasing time periods to mitigate the effect of restraint stress during microinjections, although it is possible that this caused a stress sensitization. The first

restraint length was 30 seconds, and the length increased by 30 seconds each day until the restraint time reached that of the length of an infusion for the mock injection (2 minutes and 30 seconds).

Microinjections

Mock injections were given on day 20 immediately prior to DID by using microinjectors that extended 0.5 mm past the cannula and restraining the mouse for the duration of a microinjection Mice were microinjected on day 22 and 24 with one of three drug concentrations (saline (control), 0.6 µg/µl, or 2.0 µg/µl NBQX) into the DLS. On day 23 mice were returned to their original drinking solution without infusion. Mice were injected with the same drug concentration on both infusion days. The drug concentration groups were assigned based on 2-hour consumption levels on the mock injection day. Two segments of PE-20 tubing were attached to the end two 10 µl Hamilton syringes, the syringes were locked into a Cole-Parmer dual infusion pump, and tips of the microinjectors extended 0.5 mm below the end of the guide cannula to reach the DLS. Saline or NBQX (250 nl per side) was be microinjected at a rate of 125 nl/min. Injectors stayed in place for at least 30 seconds following infusion to allow drug diffusion away from the injectors.

Blood Ethanol Concentrations (BECs)

On day 24, immediately following DID mice had retro-orbital sinus bloods taken. Blood plasma was spun down on the centrifuge at 14000 RPMs for 5-minutes, plasma was pipetted out, and bloods were stored at -20 degrees F. BECs were determined using an Analox EtOH Analyzer (Analox Instruments, Lunenburg, MA). The Analox was calibrated with a 5 µl injection of 100 mg/dl EtOH standard. Following calibration, blood plasma was then briefly vortexed and approximately 5 µls were pipetted into the analyzer. BECs were immediately displayed and cataloged. The Analox was recalibrated every 5-10 samples to ensure accurate readouts.

Brain Extraction

Following the completion of the behavioral data collection, brains were taken for verification of cannula placement. Mice were cervically dislocated, brains were extracted, and flash-frozen in 2-methylbutane at -20 to -40 degrees C. Brains were stored in the -80 degree F freezer.

Verification of Cannula Placement

Brains were taken from the freezer and sliced on the cryostat at 40 microns thick and placed onto microscope slides. Brain slices were stained with cresyl violet and placements were determined by looking at the slides under a microscope. Verified correct cannula placement are displayed in figure 10.

Statistical Analyses

Statistical testing using ANOVA and correlation were performed in R and GraphPad Prism. All statistical assumptions were either passed or corrected for. The following analyses were required for hypothesis testing. Baseline alcohol drinking for alcohol history mice at each drug concentration was analyzed with repeated-measures ANOVA (sex X day) to determine if alcohol consumption differed by day or sex for each dose. Effect of order of solution presented on infusion days were analyzed for alcohol and QuA intakes with ANOVA (drug concentration X sex X drinking history X order of solution) to determine if order of solution presented (QuA or alcohol) caused differential effects on alcohol consumption. Specific hypothesis testing for the effect of NBQX on alcohol and QuA drinking was analyzed with ANOVA *(*drug concentration X sex X drinking history). Sex specific hypothesis testing in water history and alcohol history mice for the effect of NBQX on alcohol and QuA drinking was analyzed with ANOVA (drug concentration X sex). Locomotor data were analyzed in the same manner as the consumption data but with repeated-measures ANOVA across 30-minute activity bin. All additional analysis was to probe interactions or for exploratory analysis. Blood ethanol concentrations were correlated with alcohol and QuA consumption using Pearson's correlation. Differences were considered significant at $p < 0.05$.

AIM 2 RESULTS

DLS AMPA Receptor Antagonism

Baseline Alcohol Drinking

Baseline alcohol drinking (days 1-21) for male and female C57BL/6J mice are shown in figure 9. Based on the results of Aim 1, we utilized three weeks DID alcohol history to establish compulsive-like alcohol drinking. Alcohol consumption is displayed in grams consumed per kilogram of body weight per two hours. Water consumption is displayed in milliliters consumed per kilogram of body weight per two hours. Repeated-measures ANOVA with Greenhouse-Geisser corrections of baseline drinking in the alcohol history mice across sex and drug concentration revealed a significant main effect of day [$F(20, 900) = 3.2$, $p < 0.0001$, $\eta^2_G = 0.05$], and no interactions. The observed change in alcohol intake across days is consistent with the drinking pattern in Aim 1. All mice included in the infusion data had cannula placements histologically verified for correct placement into the DLS. Figure 10 shows our placements for each cannula.

The Effect of Infusion Order on DLS NBQX 2-Hour Alcohol and QuA Drinking

The effect of DLS infusion of NBQX on 2-hour alcohol and QuA consumption is shown in figure 10. Mice underwent DID for 21 days (n = 7-11) and received either water (water history) or alcohol (alcohol history); figure 9. On days 22 and 24 mice were microinjected with one of three concentrations of NBQX immediately prior DID and were given either alcohol or QuA, instead of their regular DID solution. The drug concentration groups were assigned based on 2-hour consumption levels on the mock injection day. Importantly, ANOVA on mock injection day revealed no significant differences between drug concentration or sex in the alcohol history or water history mice on consumption during DID, (p's > 0.05); data not shown, demonstrating that drug groups did not differ on alcohol intake on the mock injection day. Next, we began hypothesis testing. In order to do this we needed to test whether order of solution presented (either alcohol first and QuA second or vice versa) had an effect on consumption across drug concentration, sex, and drinking history on infusion days to ensure we could collapse

data across infusion day. We looked at these factors separately by solution (alcohol or QuA) because we had separate hypotheses for each solution on how NBQX differed for each solution, not how NBQX's effect interacts between each solution. ANOVA of drug concentration, sex, drinking history, and order of solution on alcohol intake on infusion days revealed a trend toward a main effect of drinking history, [$F(1, 82) = 3.95$, $p = 0.05$, $\eta^2_G = 0.046$], such that alcohol history mice drank significantly more alcohol than water history mice on infusion days regardless of sex, dose, or solution order, and no other effects or interactions; data not shown. ANOVA of drug concentration, sex, drinking history, and order of solution on QuA intake on infusion days revealed a significant main effect of solution order on QuA consumption, [$F(1,82) = 10.41$, $p = 0.002$, $\eta^2_G = 0.11$], a significant interaction of sex, drinking history, drug concentration, and solution order, [$F(2,82) = 3.97$, $p = 0.02$, $\eta^2_G = 0.09$] on QuA consumption, and no other effects or interactions. Because of this, all alcohol infusion data are analyzed and displayed collapsed across infusion day, while QuA infusion data are analyzed and displayed separately by infusion day and have group sizes ranging from n = 3-6.

QuA 2-Hour Drinking in the Saline Control Group

Further, we tested whether QuA intakes differed in the saline drug concentration based on QuA solution order, sex, and drinking history to determine if alcohol history mice displayed compulsive-like QuA intakes. ANOVA of QuA solution order, sex, and drinking history in the saline infused mice revealed a main effect of QuA solution order, such that regardless of sex or drinking history, saline mice drank significantly more QuA when it was presented second as compared to when QuA was presented first, [$F(1, 30) = 7.83$, $p = 0.008$, $\eta^2_G = 0.21$], and no other main effects or interactions. Importantly, QuA intake did not differ based on drinking history in the saline infused animals ($p > 0.05$) meaning that the alcohol history mice did not demonstrate compulsive-like alcohol drinking.

The Effect of DLS NBQX on 2-Hour Alcohol and QuA Drinking

Next, we tested our specific hypotheses. First, we tested the effect of NBQX in the DLS on regular alcohol drinking and QuA drinking in mice with an alcohol history. ANOVA of sex and drug concentration in the alcohol history mice on alcohol drinking revealed no significant

effects or interactions, (p's > 0.05); figure 11A. ANOVA of sex and drug concentration in the alcohol history mice on QuA drinking revealed no significant effect of NBQX for either sex or drug concentration when QuA was given first, (p's > 0.05), or when QuA was given second, (p's > 0.05), though a trend toward an interaction of sex and dose emerged, [$F(2,22) = 2.41$, 0.11, $\eta^2_G = 0.18$]; figures 11B and 11C. Next, we tested the effect of NBQX in the DLS on regular alcohol drinking and QuA drinking in mice without an alcohol history. ANOVA of sex and drug concentration in the water history mice on alcohol drinking revealed a significant main effect of drug concentration, [$F(2, 49) = 3.74$, $p = 0.03$, $\eta^2_G = 0.13$], and no interactions; figure 11D. ANOVA of sex and drug concentration in the water history mice on QuA drinking revealed no significant effect of NBQX for either sex or drug concentration when QuA was given first, (p's > 0.05), but when QuA was given second, drug concentration significantly reduced QuA drinking, [$F(2,21) = 4.05$, $p = 0.03$, $\eta^2_G = 0.27$), and a sex and drug concentration interaction trended toward significance, [$F(2,21) = 2.34$, $p = 0.12$, $\eta^2_G = 0.18$], an no interactions were detected; figures 11E and 11F. Further hypothesis testing using one-way simple ANOVAs investigating whether males were affected to a greater extent than females in water history and alcohol history groups for both alcohol and QuA intake revealed that male, but not female, water history mice significantly reduced their alcohol intake by drug concentration, [$F(2,27) = 6.9$, $p = 0.004$, $\eta^2_G = 0.34$); figure 11D, and that male, but not female, water history mice significantly reduced their QuA intake when QuA was given second by drug concentration, [$F(2,11) = 4.40$, $p = 0.039$, $\eta^2_G = 0.45$]; figure 11F.

These data demonstrate that antagonism of AMPA receptors in the DLS reduced both alcohol and QuA drinking in water history mice only and that this effect is specific to male mice. Further, order of QuA presentation mattered, where QuA presented second caused an increase in intake across sex and drinking history compared to when QuA was presented first. This could be due to a single alcohol pre-exposure altering the susceptibility to DLS AMPA receptor antagonism in male water history mice or due to the overall disgust of QuA. It could be that QuA is so aversive that the mice require the bitter alcohol exposure prior to QuA exposures to get past the initial aversiveness.

Exploratory Analysis of DLS AMPA Receptor Antagonism on Front-Loading Behavior

The Effect of Infusion Order on DLS NBQX 20-Minute Alcohol and QuA Drinking

We then looked at the effect of DLS infusion of NBQX in the same mice on 20-minute front-loading of alcohol and QuA consumption through exploratory post-hoc analyses; figure 12. While drug concentration groups were assigned based on 2-hour mock injection intakes, ANOVA of 20-minute intake on mock injection day revealed no significant differences or interactions between drug concentration or sex in the alcohol history or water history group on consumption during DID, (p's > 0.05); data not shown. Next, we tested whether order of solution presented (either alcohol first and QuA second or vice versa) had an effect on consumption across drug concentration, sex, and drinking history on infusion days. ANOVA of drug concentration, sex, drinking history, and order of solution on 20-minute alcohol intake revealed a main effect of sex, [$F(1,82) = 8.02$, $p = 0.006$, $\eta^2_G = 0.08$], an interaction of order of solution presented and sex, [$F(1,82) = 5.46$, $p = 0.02$, $\eta^2_G = 0.06$], a trend toward and interaction of drug concentration and drinking history, [$F(2,82) = 2.52$, $p = 0.09$, $\eta^2_G = 0.06$], and no other interactions. Simple-effects analysis to follow-up the interaction of sex and order of solution presented, using one-way ANOVA of the effect of sex on 20-minute alcohol intake revealed a main effect of sex, [$F(1,49) = 4.25$, $p = 0.04$, $\eta^2_G = 0.08$]. Simple-effects analysis of the interaction of sex and order of solution presented using one-way aNOVA of the effect of order on 20-minute alcohol intake did not reveal a main effect ($p < 0.05$). Because no significant differences emerged, data will be analyzed and displayed collapsed across solution order for 20-minute alcohol drinking. ANOVA of drug concentration, sex, drinking history, and order of solution on 20-minute QuA intake revealed a main effect of drinking history, [$F(1,82) = 8.64$, $p = 0.004$, $\eta^2_G = 0.095$] such that alcohol history mice consumed significantly more QuA in the first 20-minutes than water history mice, a trend toward an effect of drug concentration, [$F(2,82) = 2.72$, $p = 0.07$, $\eta^2_G = 0.06$], a trend toward an interaction of sex and drug concentration, [$F(2, 82) = 2.48$, $p = 0.09$, $\eta^2_G = 0.057$], and no other interactions.

QuA 20-Minute Drinking in the Saline Control Group

Further, we tested whether QuA intake differed in the saline group based on QuA solution order, sex, and history to determine if the alcohol history mice displayed compulsive-

like drinking in the first 20-minutes of drinking. ANOVA of QuA solution order, sex, and drinking history in the saline infused mice revealed no effect or interactions on 20-minute QuA intake (p's > 0.05). Importantly, QuA intake did not differ based on drinking history history in the saline infused animals (p > 0.05), meaning that the alcohol history mice did not demonstrate compulsive-like alcohol drinking in their front-loading behavior relative to water history mice.

The Effect of DLS NBQX on 20-Minute Alcohol and QuA Drinking

Next, we applied the same analyses to the 20-minute front-loading intakes as we did to test our specific hypothesis for the 2-hour drinking data. First, we tested the effect on NBQX in the DLS on 20-minute alcohol drinking and 20-minute QuA drinking in mice with an alcohol history. ANOVA of sex and drug concentration in the alcohol history mice on 20-minute alcohol drinking violated Levene's test for homogeneity of variance, as result, we ran separate one-way ANOVAs. One-way ANOVA of sex on 20-minute alcohol drinking in the alcohol history group revealed a main effect of sex, [(1,49) = 4.25, p = 0.04, η^2_G = 0.08], such that male mice drank significantly more alcohol than female mice; figure 12A. Kruskal-Wallis rank sum test of 20-minute alcohol intake in the alcohol history mice revealed a trend toward a main effect of dose, [$\chi^2(2)$ = 5.25, p = 0.07, η^2_G = 0.07], given the moderate effect size, it is likely that NBQX reduced alcohol drinking in the first 20-minutes for the alcohol history mice; figure 12A. ANOVA of sex and drug concentration in the alcohol history mice on 20-minute QuA drinking revealed a trend toward an effect of drug concentration, [F(2,45) = 2.30, p = 0.11, η^2_G = 0.09], and a trend toward an interaction of sex and drug concentration, [F(2,45) = 2.30, p = 0.11, η^2_G = 0.09], suggesting that NBQX affected QuA intake; figure 12B. Next, we tested the effect of NBQX in the DLS on QuA drinking and on regular alcohol drinking in mice without an alcohol history. ANOVA of sex and drug concentration in the water history mice on 20-minute alcohol drinking revealed a trend toward a main effect of sex, [F(1,49) =4.01, p = 0.051, η^2_G = 0.08], but no other effects or interactions, such that male mice drank significantly more alcohol than female mice; figure 12C. ANOVA of sex and drug concentration in the water history mice on 20-minute QuA drinking revealed a trend toward a main effect of dose, [F(2,49) = 2.82, p = 0.07, η^2_G = 0.10], but no other effects or interactions, suggesting that NBQX reduced QuA consumption in the first 20-minutes of DID; figure 12D. Further, one-way simple ANOVAs investigating whether males were affected to a greater extent than females in water history and alcohol history

mice for both alcohol and QuA intake revealed that female, but not male, alcohol history mice significantly reduced their 20-minute alcohol intake by drug concentration, [$F(2,24) = 5.94$, $p = 0.008$, $\eta^2_G = 0.33$]; figure 12A. The same trend appeared for female, but not male, alcohol history mice in that they trended toward a significant reduction in 20-minute QuA intake by drug concentration, [$F(2,24) = 2.67$, $p = 0.09$, $\eta^2_G = 0.19$]; figure 12B. These data show that antagonism of AMPA receptors in the DLS reduced 20-minute front-loading alcohol and QuA drinking in female alcohol history mice only, although drug concentration trended toward an effect on QuA drinking for water history mice. Interestingly, male mice consumed significantly more alcohol than female mice overall.

Together, these data demonstrate interesting sex differences in front-loading behavior and 2-hour alcohol intakes. While males were generally unaffected by DLS AMPA receptor antagonism at 20-minutes, female alcohol history mice reduced alcohol and QuA intakes, but when bottles were read again at 2-hours male water history mice had reduced alcohol intakes and QuA intakes when given QuA second, but female mice were unaffected. Interestingly, neither male or female water history mice had altered QuA intakes across test day, meaning that whatever effect a single alcohol pre-exposure had at 2-hour intakes did not affect 20-minute intakes. Further, because of the trend of NBQX in 20-minute water QuA intakes, front-loading could have been suppressed at 20-minute QuA intakes regardless of order but only had a persistent effect at QuA test 2 in male mice. These data overarchingly highlight important pharmacological differences of the AMPA receptor between sex and drinking history in alcohol and QuA drinking.

Locomotor Activity

Two-hour locomotor activity taken during the 2-hour DID session on infusion days is displayed in figure 13 and 20-minute locomotor activity taken during the 2-hour DID on infusion days is displayed in figure 14, n = 6-10 per group. Locomotor data were collected on both infusion days in 5-minute data bins. Data are shown as total ambulatory counts in either 20-minute summated locomotor activity from the 5-minute bins or in 5-minute bins.

Effect of NBQX on Locomotor Activity During the 2-Hour DID Session

First, we tested whether NBQX had an effect on locomotion across groups in the 2-hour DID session. Repeated-measures ANOVA with Greenhouse-Geisser correction of sex, drug concentration, and locomotor bin in the alcohol history mice revealed a main effect of locomotor bin during QuA intake, [$F(5,195) = 3.68$, $p = 0.01$, $\eta^2_G = 0.03$]; figure 13B, but not during alcohol intake, (p's > 0.05), and no other effects or interactions; figure 13A. Repeated-measures ANOVA of sex, drug concentration, and locomotor bin in the water history mice on alcohol intake during infusion revealed a main effect of locomotor bin on alcohol intake, [$F(5,205) = 8.27$, $p < 0.001$, $\eta^2_G = 0.09$]; figure 13C, and on QuA intake, [$F(5,205) = 5.90$, $p < 0.001$, $\eta^2_G = 0.06$], and no other effects or interactions; figure 13D. Further, we tested whether locomotor activity was affected by dose or drinking history in our sex-specific hypothesis. Repeated-measures ANOVA of locomotor bin and drug concentration in male alcohol history mice revealed no effect of drug concentration of locomotor bin during alcohol or QuA intake, (p's > 0.05), and no other effects or interactions. Repeated-measures ANOVA with Greenhouse-Geisser corrections of locomotor bin and drug concentration in female alcohol history mice revealed a main effect of locomotor bin during QuA intake, [$F(5,105) = 3.02$, $p = 0.03$, $\eta^2_G = 0.05$], but not during alcohol intake, ($p > 0.05$), and no other effects or interactions. Repeated-measures ANOVA with Greenhouse-Geisser corrections of locomotor bin and drug concentration in male water history mice revealed a main effect of locomotor bin during alcohol intake, [$F(5,115) = 4.46$, $p = 0.02$, $\eta^2_G = 0.09$] and during QuA intake [$F(5,115) = 3.63$, $p = 0.02$, $\eta^2_G = 0.06$], and no other effects or interactions. Repeated-measures ANOVA with Greenhouse-Geisser corrections of locomotor bin and drug concentration in female water history mice revealed a main effect of locomotor bin during alcohol intake, [$F(5,90) = 3.37$, $p = 0.001$, $\eta^2_G = 0.20$] and during QuA intake, [$F(5,90) = 2.83$, $p = 0.04$, $\eta^2_G = 0.09$], and no other effects or interactions. Together, these data demonstrate that infusion of NBQX into the DLS can reduce alcohol drinking without altering general locomotor activity.

Effect of NBQX on Locomotor Activity During the 2-Hour DID Session Compared to Mock Injection Day

Because locomotor activity was so low across groups (compared with the locomotor activity in Aim 1), we also assessed locomotor activity during the mock infusion day across drug

concentration and sex in water history and in alcohol history mice across 20-minute activity bins for comparison purposes (n=4-7 per group). Three-way RM ANOVA of sex, drug concentration, and activity bin in alcohol history mice did not real any significant effects or interactions (all p's > 0.05). On average, locomotor activity in the alcohol history mice during mock injection did not reach over 1,300 total ambulatory counts at any time bin, meaning the lower activity observed in our infusions is not specific to the infusion day. Three-way RM ANOVA of sex, drug concentration, and activity bin in the water history mice revealed a main effect of activity bin [$F(5,140) = 9.61$, $p > 0.0001$, $\eta^2_G = 0.087$], but no other significant effects or interactions (all other p's > 0.05). On average, locomotor activity in the water history mice during mock injection did not reach over 1,500 total ambulatory counts at any time bin, meaning the lower activity observed in our infusions is not specific to the infusion day. These data provide evidence that locomotor data was lower in all mice in Aim 2, as compared to Aim 1, and that the reduced intakes are not due to the effect of infusion or stress of infusion on the mouse.

Effect of NBQX on Locomotor Activity During the 20-Minute DID Session

Next, we tested whether NBQX had an effect of locomotion across groups in 5-minute bins across the first 20-minutes of DID. Repeated-measures ANOVA of sex, drug concentration, and locomotor bin in the alcohol history mice revealed a main effect of locomotor bin on alcohol intake, [$F(3,120) = 8.30$, $p < 0.001$, $\eta^2_G = 0.09$]; figure 14A, on QuA intake, [$F(3,120) = 1.12$, $p < 0.001$, $\eta^2_G = 0.10$], and no other effects or interactions; figure 14B. Repeated-measures ANOVA of sex, drug concentration, and locomotor bin in the water history mice on alcohol intake during infusion revealed a main effect of locomotor bin on alcohol intake, [$F(3,117) = 10.86$, $p < 0.001$, $\eta^2_G = 0.09$]; figure 14C, and on QuA intake, [$F(3,117) = 3.82$, $p = 0.03$, $\eta^2_G = 0.05$], and no other effects or interactions; figure 14D. Further, we tested whether locomotor activity was affected by drug concentration or drinking history in our sex-specific hypothesis. Repeated-measures ANOVA with Greenhouse-Geisser corrections of locomotor bin and drug concentration in male alcohol history mice revealed a main effect of locomotor bin on alcohol intake, [$F(3,57) = 4.70$, $p = 0.007$, $\eta^2_G = 0.08$] and on QuA intake [$F(3,57) = 7.81$, $p < 0.001$, $\eta^2_G = 0.15$], and no other effects or interactions. Repeated-measures ANOVA with Greenhouse-Geisser corrections of locomotor bin and drug concentration in female alcohol history mice revealed a main effect of locomotor bin on alcohol intake, [$F(3,63) = 4.47$, $p = 0.02$, $\eta^2_G =$

0.10],on QuA intake [$F(3,63) = 11.35$, $p < 0.001$, $\eta^2_G = 0.09$], and no other effects or interactions. Repeated-measures ANOVA with Greenhouse-Geisser corrections of locomotor bin and drug concentration in female water history mice revealed a main effect of locomotor bin on alcohol intake, [$F(3,48) = 5.56$, $p = 0.01$, $\eta^2_G = 0.16$], a trend toward an interaction of drug concentration and locomotor bin during alcohol intake, [$F(6,48) = 2.16$, $p = 0.06$, $\eta^2_G = 0.13$*],* and no other effects or interactions. Repeated-measures ANOVA with Greenhouse-Geisser corrections of locomotor bin and drug concentration in male water history mice revealed a main effect of locomotor bin on alcohol intake, [$F(3,69) = 5.44$, $p = 0.005$, $\eta^2_G = 0.08$], a trend toward an effect of locomotor bin on QuA intake [$F(3,69) = 2.93$, $p = 0.08$, $\eta^2_G = 0.07$*],* and no other effects or interactions. Together, these data demonstrate that antagonisms of AMPA receptors in the DLS does not affect overall locomotion behavior in the first 20-minutes or across the entire two-hour DID session and that the locomotion behavior in the mice varies across DID session.

Blood Ethanol Concentrations (BECs)

BECs correlated with QuA or alcohol intake are shown in figure 15. BECs were taking immediately following DID on the last day of infusions. Because of this, we only have BECs from one of the infusion datapoints from each mouse. Alcohol and QuA consumption are displayed in grams consumed per kilogram of body weight per two hours. BECs are displayed as mg/dL. Results from a Pearson correlation indicate that both alcohol ($n = 31$; $r = 0.69$, $p < 0.000$) and QuA ($n = 28$; $r = 0.81$, $p < 0.000$) 2-hour intakes significantly and positively predicted BECs; figure 15. These results indicate that bottle readings were likely accurate for both alcohol and QuA intakes because the amount of fluid consumed positively correlates with BECs.

DISCUSSON

Summary of Findings

The goal of the present experiment was to develop a model of compulsive-like alcohol drinking, begin to understand the role of dorsal striatal AMPA receptors in control of alcohol and compulsive-like QuA drinking, and to identify any sex-specific differences in compulsive-like alcohol drinking in C57BL/6J mice. These data demonstrate a robust three-criteria model for compulsive-like QuA drinking, the importance of an alcohol history in compulsive-like binge drinking and motivation for alcohol reward, and identify that a single alcohol exposure can alter DLS response to AMPA receptor antagonism on alcohol and QuA drinking in C57BL/6J mice.

Compulsive-like QuA Drinking Model

We first aimed to define and model compulsive-like QuA drinking in C57BL/6J mice after having identified significant gaps in the literature pertaining to alcohol drinking history, concentration of quinine used, and inclusion of female mice. Compulsive-like alcohol drinking is usually defined by alcohol history mice consuming the same amount of QuA as alcohol. While this model ensures that mice that are deemed compulsive maintain alcohol intakes and thus, BECs, at the same level regardless of whether the alcohol solution is adulterated with quinine or not, it fails to account for maintained levels of drinking due to solution novelty or due to the inability of the solution to be adequately aversive. To address these issues, we concluded that mice demonstrating robust compulsive-like quinine resistant alcohol drinking must have an alcohol history long enough to produce QuA consumption significantly greater than mice without an alcohol history, must consume more alcohol on the first day of alcohol exposure than water history mice would consume of QuA, and must consume the same amount of QuA as alcohol on the day prior to QuA testing. We found that three weeks, but not two weeks, alcohol history is sufficient to produce compulsive-like QuA drinking using a quinine concentration of 0.5 mM. This concentration of quinine has been used before to test compulsive like QuA drinking and is highly concentrated relative to other more commonly used quinine concentrations in the literature, which we had hoped would increase the validity of our model (Blegen, da Silva E Silva, Bock, Morisot, Ron, D, & Alvarez, 2018; Bocarsly et al., 2019; Siciliano et al., 2019).

Counter to our findings, the two week alcohol history has been determined to suffice for producing compulsive-like alcohol drinking under the definition that compulsive-mice drink the same amount of QuA and regular alcohol at baseline, though this was tested across lower concentrations of QuA and in male mice only (Lesscher, Van Kerkhof, & Vanderschuren, 2010). Our findings begin to fill the gaps in the literature for compulsive-like QuA drinking, addressing discrepancies between alcohol drinking history, sex differences, aversiveness of the QuA, and novelty of solution. These data are the first of their kind to provide a robust three criteria model for compulsive-like alcohol drinking using a highly concentrated QuA solution using male and female C57BL/6J mice.

Escalation and Front-Loading

In attempt to illuminate the underlying behavioral processes causing differences in compulsive-like alcohol drinking between QuA test 1 and QuA test 2 in the mice, we examined the pattern of drinking across QuA test session, sex, and drinking history throughout the two-hour DID session. When we compared intakes of QuA on test day 1 versus test day 2 across drinking history and sex we found that QuA test day did not affect overall intakes in alcohol history mice, meaning that the mice with an alcohol history consumed statistically the same amount of QuA after a two-week alcohol history as after a three-week alcohol history at the 2-hour measurement. Further, we found that on QuA test 1 alcohol history and water history mice increased their drinking throughout the DID session across 30-minute bins, effectively escalating their QuA drinking across the DID session. In addition, at QuA test 1 alcohol history mice front-loaded, as determined as 30-minute intake shown as percent 2-hour intake, significantly more that water history mice. While neither 2-hour QuA intakes at test 1 nor escalation of QuA drinking differ between alcohol history or water history mice, alcohol history mice front-loaded significantly more that water history mice. During DID with either C57BL/6J mice or high alcohol preferring mice front-loading alcohol drinking behavior can be established, such that mice increase their alcohol intakes across repeated alcohol exposures within a short time interval (e.g. 15 minutes; Wilcox et al., 2014; Linsenbardt & Boehm, 2014; 2015). This front-loading behavior is thought to represent an increase in motivation to the alcohol reward either through mechanisms of learned behavioral associations, pharmacological drug effects, or a combination

of both. Overall, these data demonstrate distinct patterns of QuA intake for water and alcohol history mice.

We interpret our findings relating to front-loading and escalation in QuA drinking to be related to the motivating properties of alcohol. We hypothesize that alcohol history mice are highly motivated for alcohol even when the alcohol is adulterated with quinine such that they will front-load and continue to escalate in their intakes throughout the DID session. This may be related to incentive-sensitization theory which would suggest that the compulsive nature of addiction is result of neural adaptations causing a hyper-sensitivity to the incentive motivational properties of drug cues via the repeated use of a drug (Robinson & Berridge, 1993; 2008, 2016). Incentive-sensitization theory also suggests that 'wanting' and 'liking' are two facets of addiction, where 'wanting' or incentive-sensitization is dependent on mesolimbic dopamine and manifests the motivational aspect of addiction, and 'liking' manifests as the actual pleasure of the reward. Our front-loading finding may support the 'wanting' aspect of incentive-sensitization as the alcohol history mice appeared to be highly motivated for the alcohol drinking. Whereas the escalation in intakes across the DID session could relate to the 'liking' aspect of incentive-sensitization where the pleasurable aspect is the acute experience of alcohol itself. Further, these findings would imply that compulsive-like alcohol drinking is heavily dependent on neural mechanisms of motivation, as incentive sensitization requires 'wanting' but not 'liking'. A major limitation to our interpretation as it relates to Incentive-sensitization is that Incentive-sensitization theory would require the mice to develop sensitization to the locomotor effects of alcohol. Although we report that the locomotor activity significantly increased at baseline 2 compared to baseline 1, which could be sensitization to the locomotor effects of alcohol, we did not directly test this. Future research should directly test incentive-sensitization theory and compulsive-like QuA drinking by utilizing reward cues to test the motivating aspects of compulsive-like alcohol drinking. These data demonstrate explicit patterns of consumption indicating a specific behavioral phenotype associated with compulsive-like QuA drinking.

A Single-Alcohol Exposure Alters Compulsive-like QuA Drinking

Incentive-sensitization theory states that the motivational properties of 'wanting' rely on repeated drug exposure to develop. We found that when water history mice were faced with QuA for the second time, they significantly increased their front-loading behavior and it did not differ

from alcohol history mice. This would suggest that a single exposure to the rewarding properties of alcohol; albeit adulterated with quinine, cause an increase in motivated response for the effects of alcohol. Interestingly, previous reports investigating QuA drinking in C57BL/6J mice have found that just one alcohol drinking session can cause aversion resistant QuA drinking in a limited daily access consumption model, although the quinine-concentration used was relatively mild (0.1 mM), providing further support that one alcohol drinking session can increase the motivation for the rewarding properties of alcohol (Lei, Wegner, Yu, Simms, & Hopf, 2016). Recently, Darevsky, Gill, Vitale, Hu, Wegner, & Hopf, (2019) found that rats given over 3 months of alcohol access demonstrate quinine resistant alcohol drinking in a 20-minute drinking session, while still consuming the same volume of QuA as regular alcohol. These conflict-resistant rats demonstrated less variable response patterns in their intakes which may be reflective of a greater motivational drive to consume the alcohol, which the authors refer to as a head down and push strategy. The authors gave the rats multiple QuA sessions and data were analyzed by combining QuA drinking sessions and in alcohol history animals only, making interpretation of a single QuA session's effect on motivation to drink difficult to parse out in either animals with or without an alcohol history. Importantly, Giuliano et al., (2018) found that compulsive alcohol drinking rats demonstrate an increase in motivation for alcohol responding relative to non-compulsive rats. Our data could suggest that an alcohol drinking history is necessary to produce robust compulsive-like QuA drinking, and that a single-alcohol exposure (via QuA) can alter the motivational response to alcohol consumption which may be related to the development of compulsive-like QuA drinking. Together, our findings in combination with previous research highlight a critical need to address the relationship between the development of compulsive-like QuA drinking and motivation for the rewarding effects of alcohol.

Sex Differences in Compulsive-like QuA Drinking

To control for potential differences between baseline and QuA test days on front-loading behavior, we further investigated front-loading drinking in alcohol history mice as compared to their front-loading during baseline, i.e. front-loading as percent baseline. We found that male alcohol history mice front-loaded only to approximately 25% of their baseline intakes, whereas female alcohol history mice front-loaded to approximately 65% of their baseline intakes on QuA test 1. On QuA test 2, both male and female alcohol history mice front-loaded to approximately

65% of their baseline intakes. This finding suggests that QuA reduces overall front-loading compared to baseline, and that front-loading for female mice is more resistant to the aversiveness of QuA compared to male. This is consistent with a recent paper finding that female mice require a higher concentration of QuA to reduce drinking in a model of aversion-resistant alcohol drinking (Fulenwider, Nennig, Price, Hafeex, & Schank; 2019). It could be that the neural mechanisms underlying motivation for reward are different for male and female mice, resulting in altered motivation for reward (Becker, Berkley, Geary, Hampson, Herman, & Young, 2007; Becker, 2009). Recently, Kawa & Robinson (2019) identified that female mice showed greater motivation for cocaine, developing incentive-sensitization faster than male mice. A similar relationship may be what we are seeing in our compulsive-like QuA drinking model, where males need an additional week or so to develop the same level of motivation, as compared to baseline front-loading, for alcohol as female mice. Further research involving both males and females on compulsion and motivation neural circuitry in regard to alcohol is necessary to further understand this sex-specific relationship.

Compulsive-like QuA Drinking and Associated Dorsal Striatal AMPA Receptor Protein Levels

Because of the well-established role of the DMS and DLS in goal-directed and inflexible behaviors, respectively, and the importance of AMPA receptors in gating the MSN activity within the striatum, we sought to identify whether AMPA receptor protein levels differed between the alcohol and water history mice. We found that GluA1 and GluA2 AMPA receptor protein levels were unaffected in either the DMS or DLS brain regions by drinking history or sex when we assessed total protein levels. This report is consistent with Wang et al. 2012 who did not find differences in AMPA receptor protein levels in rats with an alcohol history responding in a goal-directed manner, although the authors did find significant differences when they assessed changes in synaptic AMPA receptor proteins in the DMS. Future experiments should take a similar approach and assess both homogenate and synaptosomal AMPA receptor protein levels in association with compulsive-like QuA drinking. It is likely that dorsal striatal AMPA receptor changes occurred following ethanol exposure given the nature of AMPA receptors and their role in mechanisms of potentiation (Henley & Wilkinson; 2013). Multiple previous reports found changes in AMPA receptors in the DMS and DLS following repeated alcohol exposure

though one experiment found that 24-hours abstinence was sufficient to return DLS AMPA receptors to baseline (Ma, Barbee, Wang, & Wang, 2017; Lagström, Danielsson, Söderpalm, Ericson, & Adermark; 2019). Given we did not take brains until 24-hour after the final DID session it is possible that we missed a critical window. Future research should center on the causal relationship between this striatal AMPA receptor subunit specific relationship and compulsive-like alcohol drinking by directly agonizing or antagonizing specific AMPA receptor subunits and measuring 'wanting' behavior.

Failure to Replicate Compulsive-like QuA Drinking

In Aim 1 we demonstrated what we consider to be a robust criterion for compulsive-like QuA drinking in male and female C57BL/6J mice. We then extended this model into Aim 2 to manipulate compulsive-like drinking through infusion of NBQX into the DLS. When we did this, we were not able to replicate our compulsive-like alcohol drinking. That is, saline infused mice consumed significantly less QuA than alcohol, and water and alcohol history mice did not differ in their consumption of QuA. We also found floor effects for the QuA drinking on infusion day. These differences could be due to a number of things. For example, it is possible that the QuA is too aversive and a prior QuA exposure is required for animals to push through and consume the solution. In Aim 1, though a week apart, the mice did receive two QuA exposures and the second exposure resulted in increased consumption. Another primary difference between Aim 1 and Aim 2 is that the mice all underwent stereotaxic surgery and were restrained for increasing periods of time prior to infusion. The stress of a surgery or of the restraint could have been a contributing reason that the mice did not demonstrate compulsive-like QuA drinking. We included the restraining as a way to habituate the animals to the stress of being restrained during the infusion. It is possible that we caused a stress sensitization to occur, though we have no evidence to support this. Regardless, the failure to replicate our compulsive-like QuA drinking greatly limits our ability to manipulate compulsive-like alcohol drinking.

Order of Solution Presented Affects DLS AMPA Receptor Antagonism Alcohol and QuA Consumption

We sought to directly assess the role of DLS AMPA receptors in controlling compulsive-like alcohol drinking by applying our model of compulsive-like QuA drinking and antagonized

DLS AMPA receptors. When we employed our QuA drinking model from aim 1, we were unable to replicate our robust definition of compulsive-like QuA drinking. Other than the bilateral cannulation surgeries, the only element to differ in our infusion experiment compared to our aim 1 drinking experiment is the additional exposure to QuA. To account for these differences, we hypothesize that two separate phenomena may be occurring in water history and alcohol history mice on compulsive-like QuA drinking. First, in the water history mice, given that a single QuA exposure in aim 1 resulted in increased motivation to consume alcohol as measured during front-loading behavior when given QuA for a second time, the single QuA pre-exposure may be key to achieving motivational aspects of addiction which could be related to the development of compulsion. This development of increased motivated behavior may be related to the phenotype of compulsive-like QuA drinking, but this single-exposure was not enough to establish robust compulsive-like 2-hour QuA drinking as seen in alcohol history mice. Second, alcohol history mice that were given a pre-exposure to QuA in aim 1 may have had any neophobia to the QuA mitigated at QuA test 2 by this first QuA exposure. Any reductionist or negative valence effect that could possibly have occurred in the alcohol history mice at QuA test 1 would have theoretically been deterred by the prior QuA exposure. Indeed, others who use QuA to study compulsion give multiple QuA exposures and report results as the average of the QuA test sessions, often following a QuA habituation day, obscuring the potential to investigate the initial effects of a first QuA exposure in alcohol history animals (Seif, et al., 2013; 2015; Wegner, Hu, De Oliveira Sergio, Darevsky, Kwok, Lei, & Hopf, 2019). Although we do not know exactly why we were unable to replicate our compulsive-like QuA model, it is clear that a single-QuA pre-exposure alters consummatory behaviors on later QuA consumption. Future research should investigate such effects accounting for both novelty/aversiveness of solution and relevance of a single QuA exposure by giving multiple QuA exposures and reporting any differences across QuA tests in both water history and alcohol history mice.

In support of the idea that an initial QuA exposure affects future reward behaviors, we found that the order of solution presented first (i.e. QuA or alcohol) affected AMPA receptor antagonism. Specifically, water history mice were unaffected by NBQX infusions when QuA was the first solution presented, but if QuA was the second solution presented NBQX significantly reduced QuA intake in male mice at the 2-hour timepoint. NBQX also reduced alcohol drinking in male water history mice at the two-hour timepoint, but order of solution

presented did not matter for alcohol drinking. These data suggest that the aversiveness of QuA may inhibit acute DLS AMPA receptor dependent pharmacological adaptations to produce motivational salience to the rewarding properties of alcohol that a single experience with unadulterated alcohol produced during the DID session in male water history mice. This finding is supported by recent data from Lerner et al., 2015, who demonstrated the role of DLS DA neurons for salience signaling through optogenetic manipulation, finding DA neurons to respond for both appetitive and aversive stimuli. The DLS DA response for salience my differ for alcohol and QuA causing our varying effect of NBQX infusions on QuA consumption, where QuA consumption may not be affected by NBQX in the DLS. Finally, these DLS depended mechanisms may also be due to DA and glutamate modulation of cholinergic interneurons, as tonically active cholinergic interneurons can be modulated by DA and glutamate in response to motivationally salient stimuli (Doig, Magill, Apicella, Bolam, & Sharott, 2014; Cai & Ford, 2018; Cox & Witten; 2019). The effect of order on consumption could also be due to a floor effect which is reduced at the second QuA exposure. It is possible the aversiveness of QuA was reduced in water history mice following a prior alcohol exposure and the order effect observed is simply a disgust effect, rather than a physiological mechanism of alcohol. Further, the ability to detect an effect of NBQX on drinking could have been hampered by these floor effects. Future research is needed to disentangle this effect. Importantly, overall locomotor activity in the mice was not affected by drug infusion at any timepoint and BECs predicted both alcohol and QuA consumption, meaning the observed effect on consumption is not due to antagonism of striatal motor networks and our bottle readings were accurate.

Sex Specific Effects of DLS AMPA Receptor Antagonism on 20-minute Alcohol and QuA Consumption

Female mice were unaffected by NBQX at the 2-hour timepoint regardless of drinking history, but 20-minute front-loading of alcohol was significantly reduced and trended toward significant for QuA intakes in female alcohol history mice. This finding would suggest that the DLS mechanisms for reward differ by sex and drinking history and are transient in alcohol history female mice. Additionally, the fact that front-loading but not overall 2-hour drinking was reduced in female alcohol history mice, but not water history or male mice, suggests that DLS AMPA receptors may modulate the mechanisms underlying motivation and overall ‘wanting’

behavior for reward in females with an alcohol drinking history. NBQX also trended toward significantly reducing QuA intake in the first 20-minutes in male and female water history mice but this effect was likely undetectable because of how low overall drinking was for water history mice, likely producing a floor effect. The sex specific effects observed could potentially be due to differing drug concentrations needed to produce an effect in each sex as seen in our previous work where I.P. injections of NBQX in male, but not female mice reduced alcohol drinking (Bauer, Garcy, & Boehm, under revision). The sex effect could also be due to an AMPA specific alteration in response to binge-like alcohol drinking. Recently, Finn et al. (2018) demonstrated that sex influences response to binge-like alcohol drinking such that nucleus accumbens transcriptional properties between male and female mice differ in response to alcohol and Cannady et al. (2017) demonstrated that alcohol self-administration in rats cause an increase in GluA1 AMPA receptor subunit phosphorylation in the central amygdala, basolateral amygdala, and the nucleus accumbens core compared to sucrose drinking rats. Further, the sex specific effects of NBQX on front-loading in female alcohol history mice may be due to differences in pharmacodynamics as infusion of NBQX in the DLS, in males only, has demonstrated to cause antagonistic effects up to 45-minutes in previous studies (Wang et al., 2012; Corbit, Nie, & Janak, 2014), and to as much as 2 hours here in this experiment. Because AMPA receptors are essential for mechanisms of long-term potentiation and acute ethanol decreases while chronic ethanol increases extracellular glutamate concentrations, the efficacy of NBQX in the DLS may differ drastically by alcohol history and sex. We speculate that female mice may have been able to clear NBQX faster than males due to neuroadaptations as result of chronic ethanol (Roberto et al., 2004; Ding et al., 2013; Tiwari et al., 2014; for further review of alcohols actions on glutamate please see Banerjee, 2014 & Goodwani et al., 2017).

Although our model of compulsive-like QuA drinking did not replicate as we had hoped, the fact that female alcohol history mice have attenuated front-loading ‘wanting’ behavior but persist in their intakes throughout the DID session such that NBQX did not alter 2-hour drinking compared to saline mice could mean that female alcohol history mice indeed do demonstrate components of compulsive-like alcohol drinking. This is supported in aim 1, when female mice, but not male mice, did not differ from QuA test 1 or 2 in their front-loading as compared to their baseline front-loading. Additionally, our finding that DLS AMPA antagonism reduces motivated front-loading behavior in female alcohol history mice could be due to the indirect effect of

AMPA receptor antagonism on either D1 and D2 dopamine (DA) pathways and cholinergic interneurons that inhabit the dorsal striatum. Though there is a well-established role for DLS DA in motivation and reward signaling (Palmiter, 2008; Gardoni & Bellone, 2015), the exact mechanism in relation to compulsive-like behavior is not defined. Our results could be interpreted as to suggest that DLS dependent motivation for alcohol is related to compulsive-like QuA drinking, and this effect may differ between sexes. DiFeliceantonio & Berridge (2016) found that, in female rats, stimulation of dopamine or opioid receptors in the DLS enhances incentive motivation triggered by Pavlovian reward cues. In agreeance, our finding that 20-minute front-loading is reduced by DLS AMPA receptor antagonism in female mice suggests the importance of sex specific differences in incentive motivation and the DLS. Importantly, Giuliano, Belin, & Everitt, 2019 found that anterior DLS DA dependence predicts compulsive-like alcohol seeking behavior. Together, these data highlight the importance of DLS dependent mechanisms of reward and motivation for the development of compulsive-like QuA drinking.

Concluding Remarks and Future Directions

Ultimately, while we were able to develop a robust model of compulsive-like alcohol drinking in aim 1, our major finding centers on how a single alcohol session can alter subsequent DLS AMPA receptor dependent compulsive-like QuA drinking and non-compulsive QuA drinking. Importantly, we have found sex differences in both susceptibility to DLS AMPA receptor antagonism in water and alcohol history mice. These differences may be mediated by motivationally relevant ‘wanting’ phenotypes related to the development of compulsive-like alcohol drinking as demonstrated in front-loading behavior, though this interpretation is relatively speculative. Future research should directly measure how neurons in the DLS respond to a single alcohol or QuA session, measure this in relation to the development of compulsive-like QuA drinking and investigate the role of individual differences in compulsion.

FIGURES

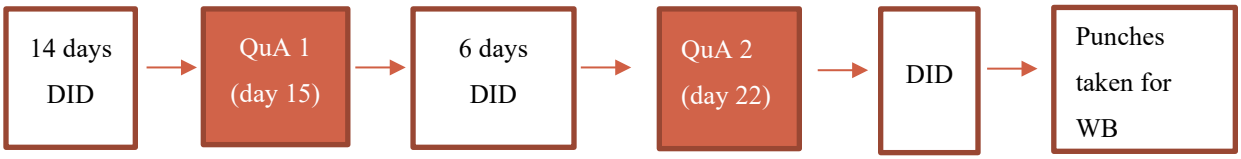

Figure 2. Aim 1 Experimental Timeline.

Mice first underwent 14 days of either water or alcohol DID. Then on day 15 mice were given QuA during DID and compulsive-like QuA drinking was assessed. Mice were then given an additional 6 days of alcohol or water exposure during DID. On day 22 mice were again tested for compulsive-like QuA drinking. On day 23 mice underwent DID of either water or alcohol as a washout drinking day before western-blots which were taken on day 24.

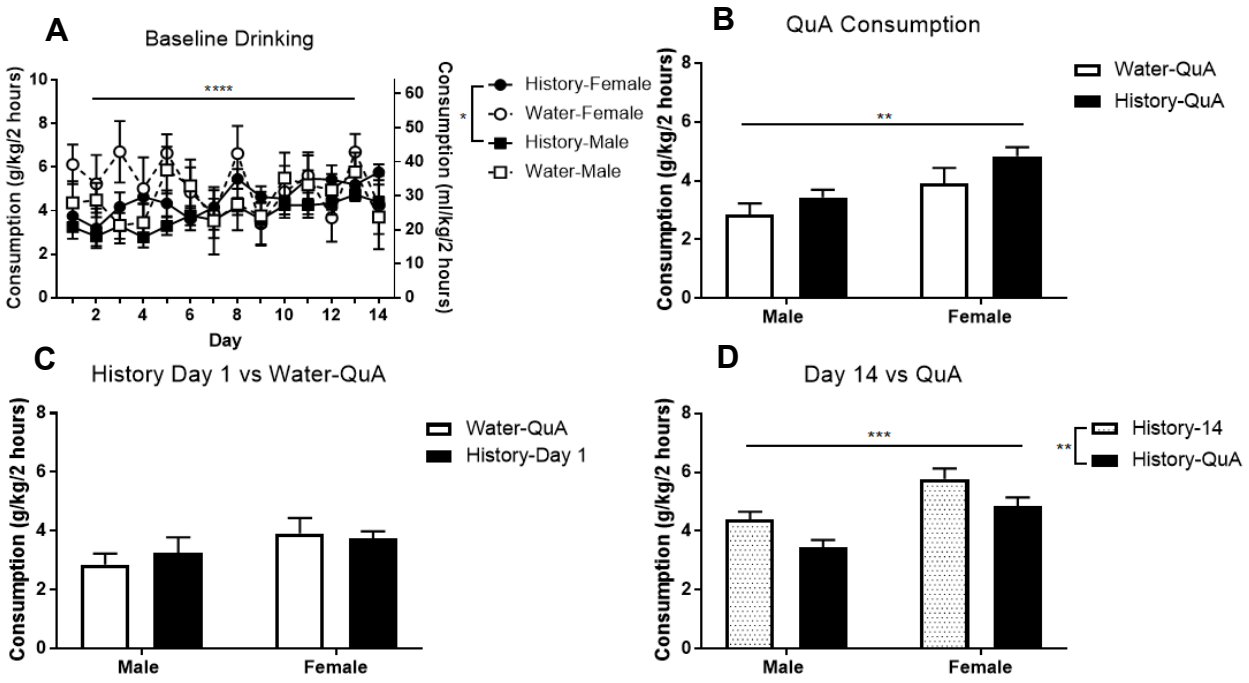

Figure 2. The Effect of 14-Days Drinking History on Quinine-Adulterated Alcohol Consumption.

A. Average daily DID alcohol or water consumption across day for each drinking history and sex. RM ANOVA of sex and day in the alcohol history mice revealed a main effect of day (****$p < 0.0001$) and sex, (*$p < 0.05$). B. Alcohol history and water history mice do not differ in QuA consumption. ANOVA of sex and drinking history on QuA drinking revealed a main effect of sex, (**$p < 0.01$). C. Alcohol history mice do not differ on alcohol consumption on their first day of alcohol drinking as compared to water history consumption of QuA. ANOVA of sex and day/history (history mice day 1 vs water mice on QuA test day) revealed no effects, (p's > 0.05). D. Alcohol history mice drank significantly more alcohol on the day prior to QuA test day than QuA on test day. RM ANOVA of sex and day in the alcohol history mice revealed a main effect of sex (***$p < 0.001$) and day ($p < 0.01$). Data are shown as mean +/- standard error of the mean, n = 7-9 per group.

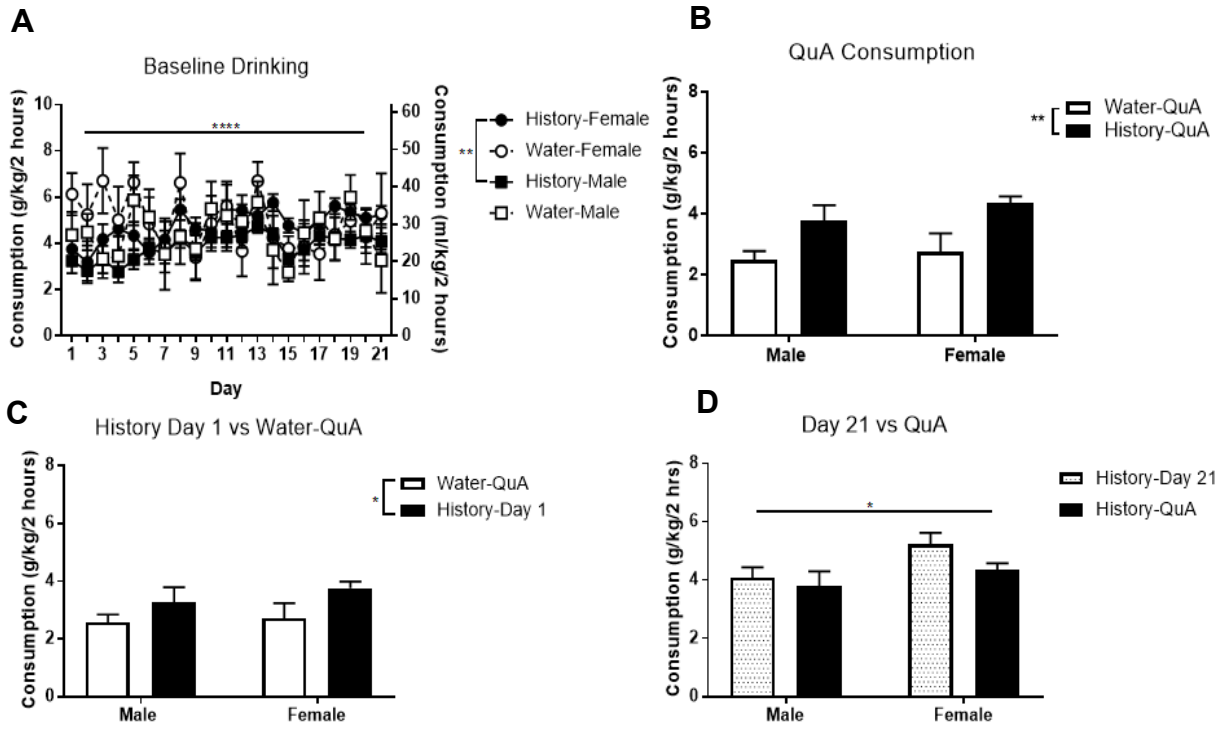

Figure 3. The Effect of 21-Days Alcohol History on Quinine-Adulterated Alcohol Consumption.

A. Average daily DID alcohol or water consumption across day for each drinking history and sex. RM ANOVA of sex and day in the alcohol history animals revealed a main effect of day (****$p < 0.0001$) and sex, (**$p < 0.01$). B. Two-way ANOVA of sex and history on QuA drinking revealed a main effect of alcohol history, (**$p < 0.01$). C. Two-way ANOVA of sex and day/drinking history (history mice day 1 vs water mice on QuA test day) revealed a main effect of day/drinking history, (*$p < 0.05$). D. RM two-way ANOVA of sex and day in the alcohol history mice revealed a main effect of sex (*$p < 0.05$). Data are shown as mean +/- standard error of the mean, n = 7-9 per group.

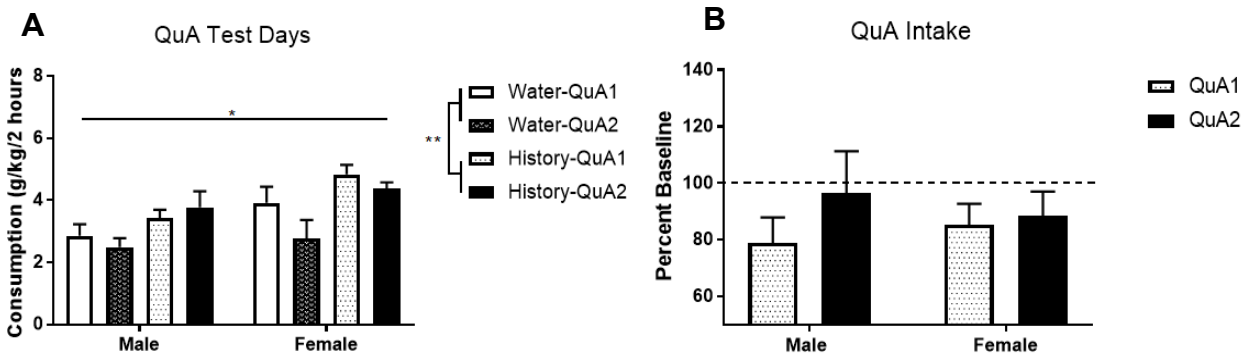

Figure 4. The Effect of QuA Test Day on QuA Consumption.

A. Average daily DID alcohol or water consumption across day for each drinking history and sex. RM ANOVA of sex and day in the alcohol history animals revealed a main effect of day (****$p < 0.0001$) and sex, (**$p < 0.01$). B. Two-way ANOVA of sex and history on QuA drinking revealed a main effect of alcohol history, (**$p < 0.01$). C. Two-way ANOVA of sex and day/drinking history (history mice day 1 vs water mice on QuA test day) revealed a main effect of day/drinking history, (*$p < 0.05$). D. RM two-way ANOVA of sex and day in the alcohol history mice revealed a main effect of sex (*$p < 0.05$). Data are shown as mean +/- standard error of the mean, n = 7-9 per group.

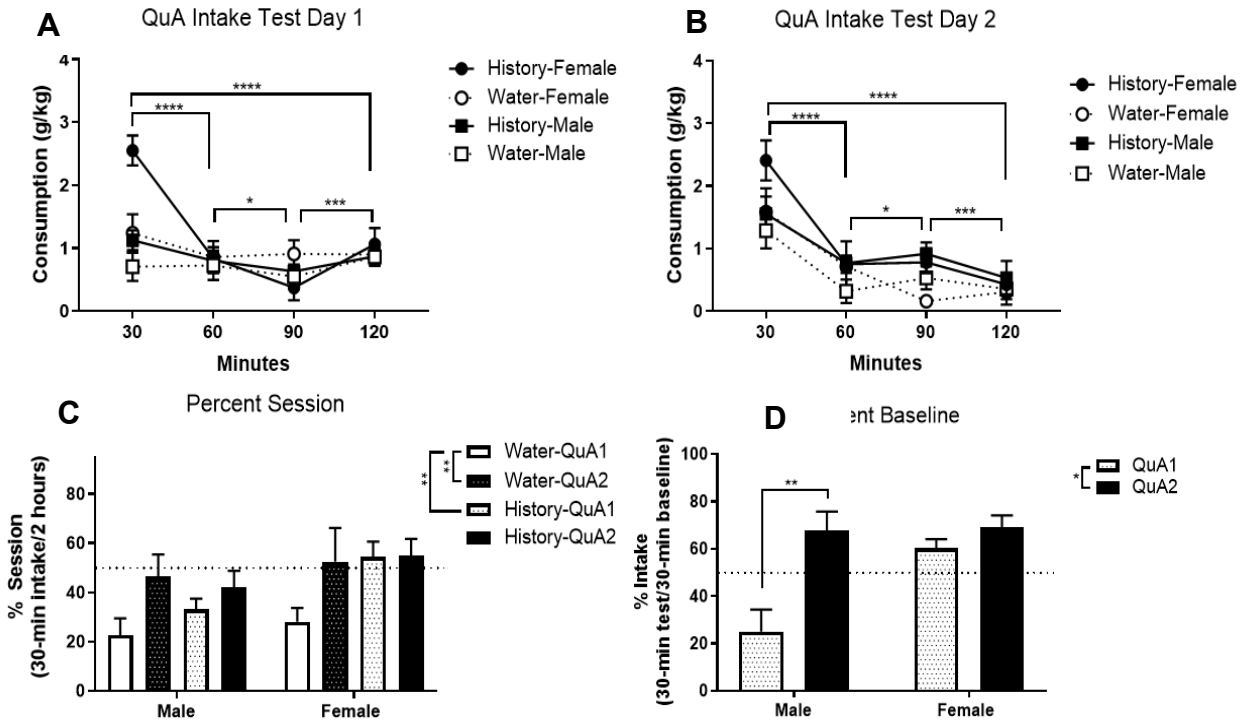

Figure 5. Escalation and Front-loading Behavior of QuA Intake on QuA Test Days.

A and B. Average QuA consumption across 30-minute drinking bins by sex, drinking history, and QuA test day. ANOVA revealed a main effect of sex, drinking history, and drinking bin (p's < 0.05). Bonferroni corrected paired t-tests show that female mice drank significantly more QuA at 0-30 minutes than at 30-60 minutes (****$p > 0.0001$) or at 90-120 minutes (****$p < 0.0001$). Female mice drank significantly more QuA at 30-60 minutes than at 60-90 (*$p < 0.05$), and they drank more from 90-120 than from 60-90 minutes (***$p < 0.001$). C. Percent intake in the first thirty minutes of the entire DID session across drinking history, sex, and QuA test day. Paired t-test of drinking history on QuA test 1 revealed an effect of drinking history (**$p < 0.01$). Paired t-test of water history mice on QuA test day revealed an effect of QuA test day (**$p < 0.01$). D. Alcohol history mice 30-minute percent baseline intake by QuA test day. RM ANOVA of sex and QuA test day revealed a main effect of QuA test day (*$p < 0.05$) and an interaction of sex and test day ($p < 0.05$). Paired t-test of QuA test day in males revealed an effect of QuA test day (*$p < 0.05$). Data are shown as mean +/- standard error of the mean, n = 7-9 per group.

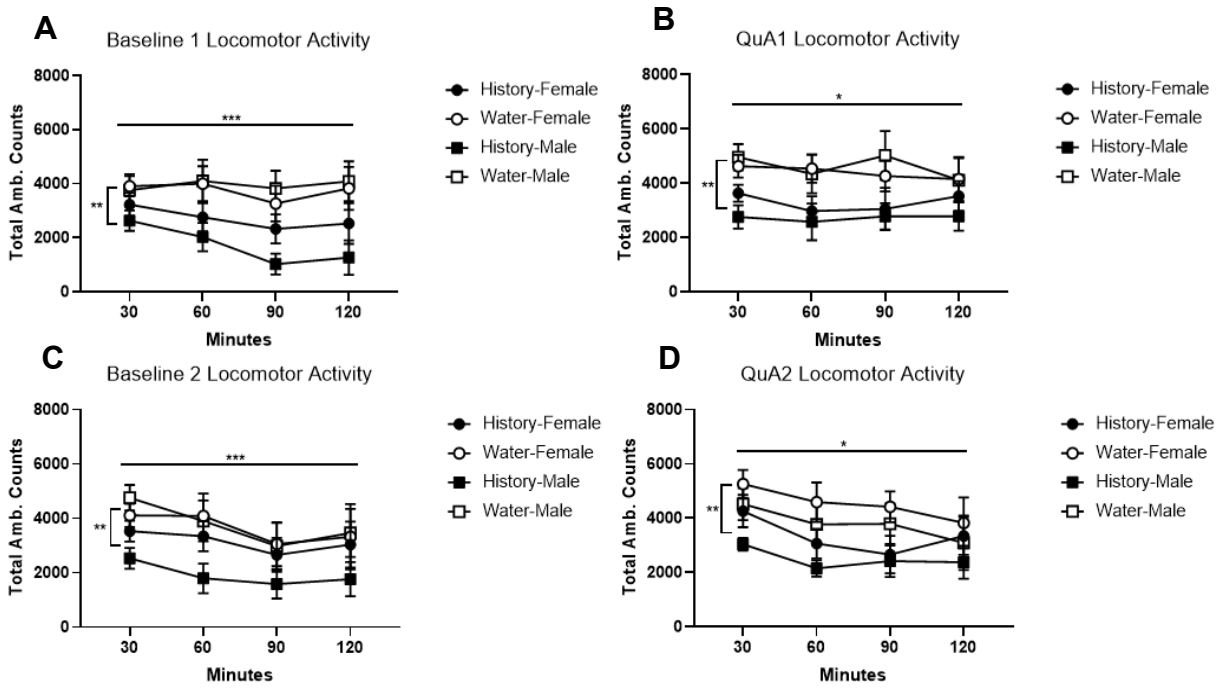

Figure 6. Locomotor Activity During Aim 1 Consumption.

A and C. Average baseline locomotor activity across 30-minute drinking bins by sex, drinking history, and baseline day. RM ANOVA of drinking history, activity bin, baseline day, and sex on baseline locomotor activity revealed a main effect of drinking history, (**$p = 0.005$), activity bin (***$p < 0.0002$), baseline day ($p < 0.0001$), an interaction of drinking history and activity bin ($p = 0.002$), an interaction of activity bin and baseline day ($p < 0.0001$), an interaction of drinking history, activity bin, and baseline day ($p = 0.002$), and a trend toward an interaction of drinking history and baseline day ($p = 0.051$). Bonferroni-corrected paired t-tests in alcohol history mice revealed that alcohol history mice had significantly more ambulatory activity at 30 minutes compared to 90 minutes ($p < 0.001$), at 30 compared to 120 minutes ($p = 0.04$), and at 60 minutes compared to 90 minutes ($p < 0.001$). We found through Bonferroni-corrected paired t-tests that water history mice had significantly more ambulatory activity 30 minutes compared to 60 minutes ($p < 0.001$), at 30 compared to 60 minutes ($p = 0.017$), and at 30 minutes compared to 120 minutes ($p = 0.01$). Bonferroni-corrected paired t-tests on baseline day 1 across activity bin revealed that mice had significantly more ambulatory activity at 30 minutes compared to 60 minutes ($p = 0.027$). Bonferroni-corrected paired t-tests on baseline day 2 across activity bin revealed that mice had significantly more ambulatory activity at 30 minutes compared to 60 minutes ($p = 0.002$), at 30 minutes compared to 90 ($p < 0.0001$), and at 30 minutes compared to 120 ($p = 0.003$). B and D. Average locomotor activity on QuA test days across 30-minute drinking bins by sex, drinking history, and test day. RM ANOVA of sex, drinking history, activity bin, and QuA test day during the two-hour QuA Did session revealed a main effect of drinking history, (**$p = 0.00755$) and activity bin (*$p = 0.002$). Data are shown as mean +/- standard error of the mean, n = 7-9 per group.

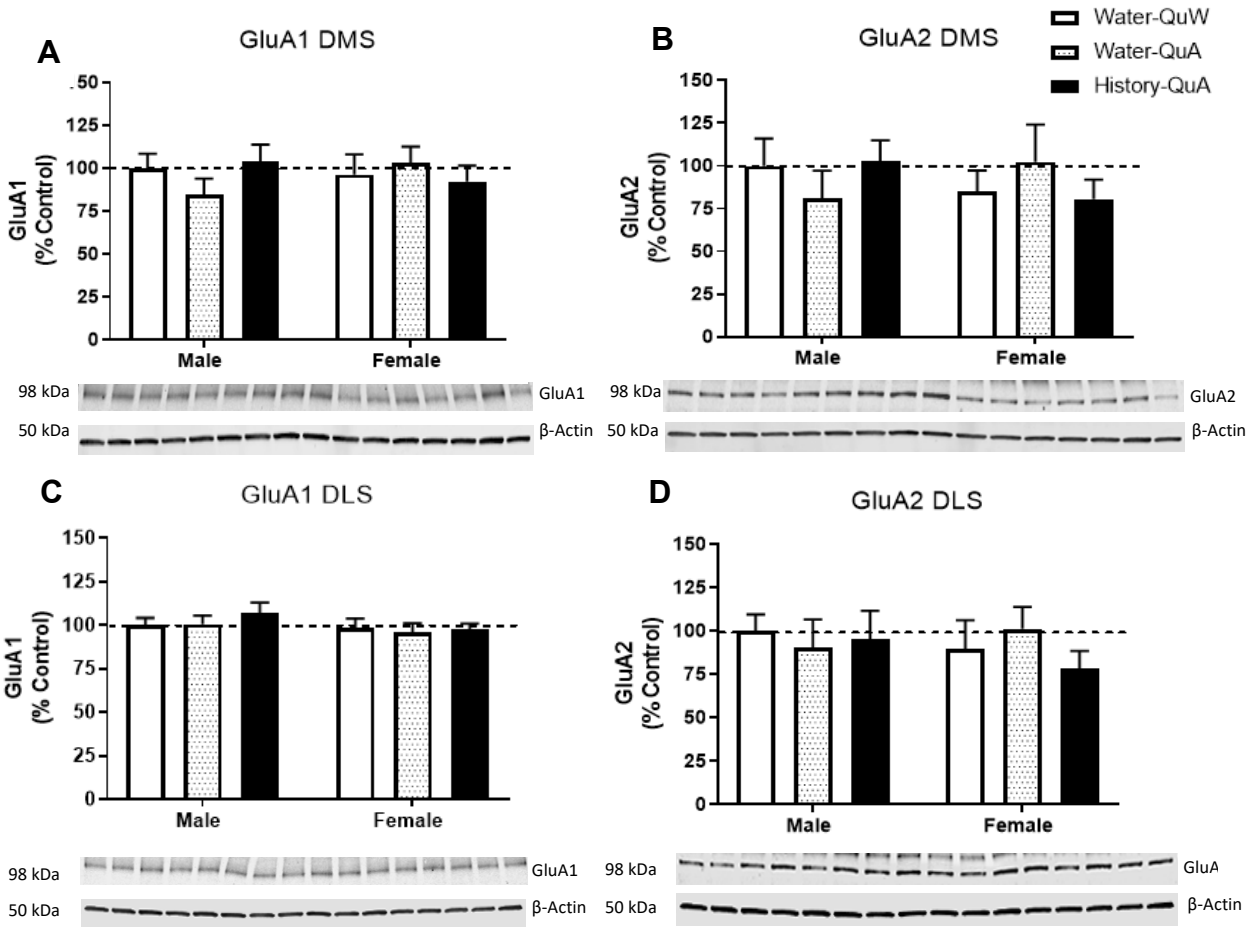

Figure 7. AMPA Receptor Protein Levels.

A. GluA1 protein levels in the DMS, B. GluA2 protein levels in the DMS, C. GluA1 protein levels in the DLS, D. and GluA2 protein levels in the DLS for mice who received only water and QuW on test days, water and QuA on test days, or alcohol and QuA on test days. ANOVA revealed no effect of sex or alcohol history on AMPA receptor protein levels, (all p's < 0.05). Order of condition in the representative samples are as follows, left to right; Male Alcohol History, Female Water History, Male Water History QuW, Female Alcohol History, Male Water History, Female Water history QuW, repeating this pattern across gels. Data are shown as mean +/- standard error of the mean, n = 7-9 per group.

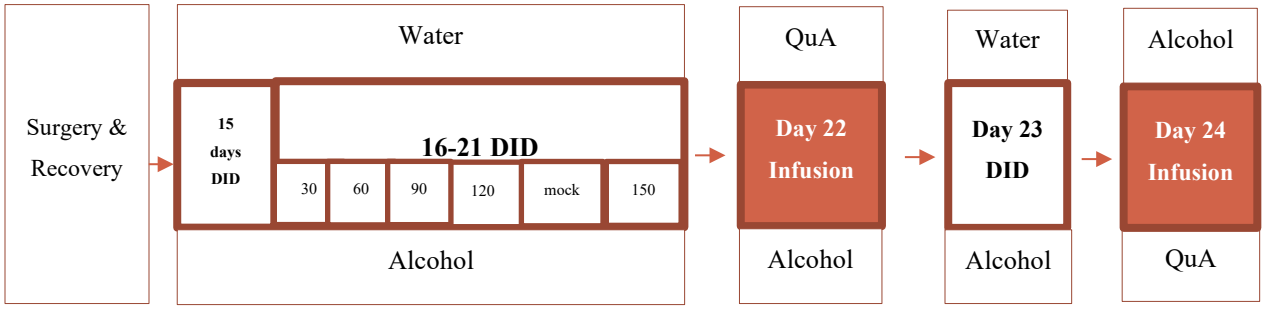

Figure 8. Aim 2 Experimental Timeline.

Mice first underwent bilateral canulation surgery and recovery. Mice were given a total of 21 days of DID of either alcohol or water. On days 22 and 24 mice were microinjected with NBQX or saline during alcohol or QuA drinking sessions.

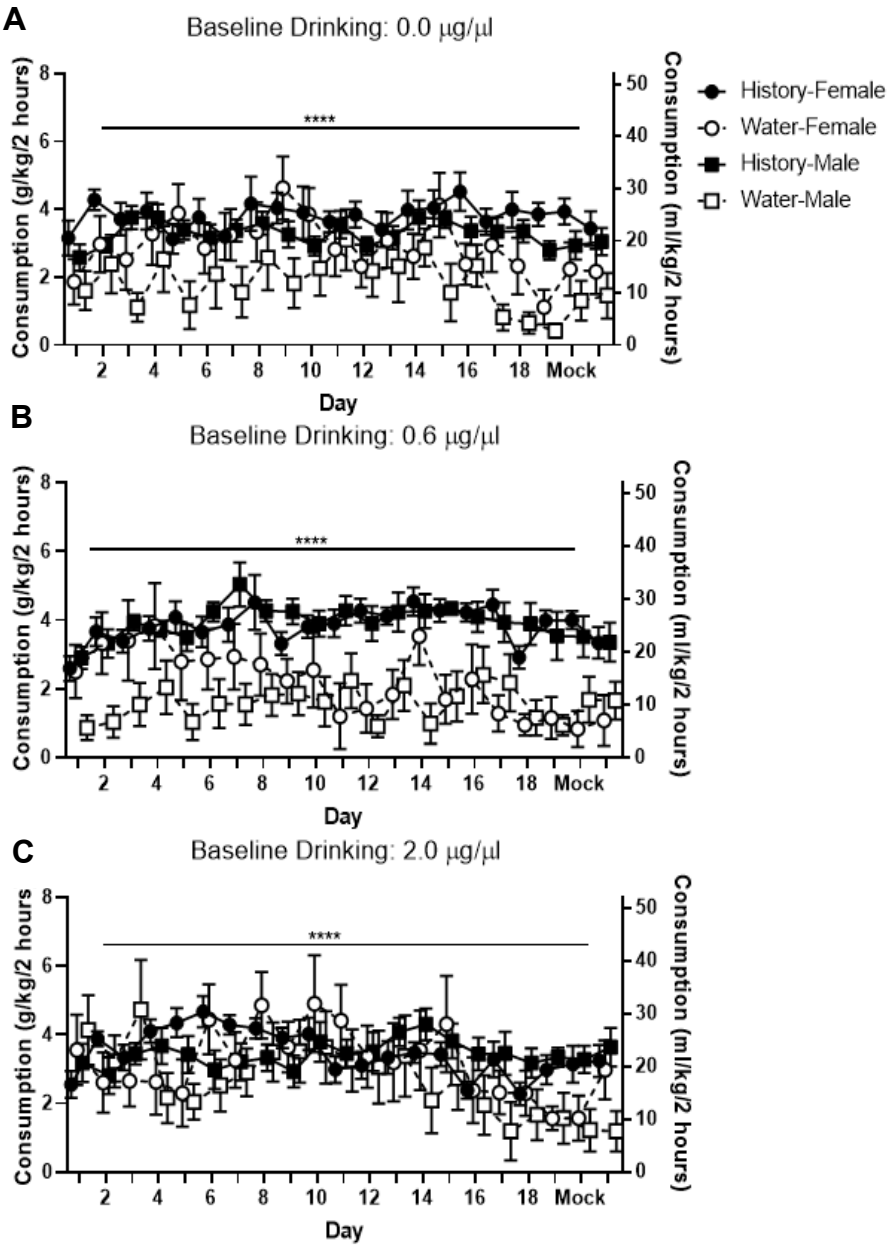

Figure 9. Baseline Drinking from the 21-Days Prior to Infusion.

A. Average daily limited-access alcohol or water consumption across day for each sex and drinking history for the saline infused mice. B. Average daily limited-access alcohol or water consumption across day for each sex and drinking history for the 0.6 µg/µl infused mice. C Average daily limited-access alcohol or water consumption across day for each sex and drinking history for the 2.0 µg/µl infused mice. Repeated-measures ANOVA of sex, history, day, and dose revealed a main effect of day, (****$p < 0.0001$). Data are shown as mean +/- standard error of the mean, n = 7-11 per group.

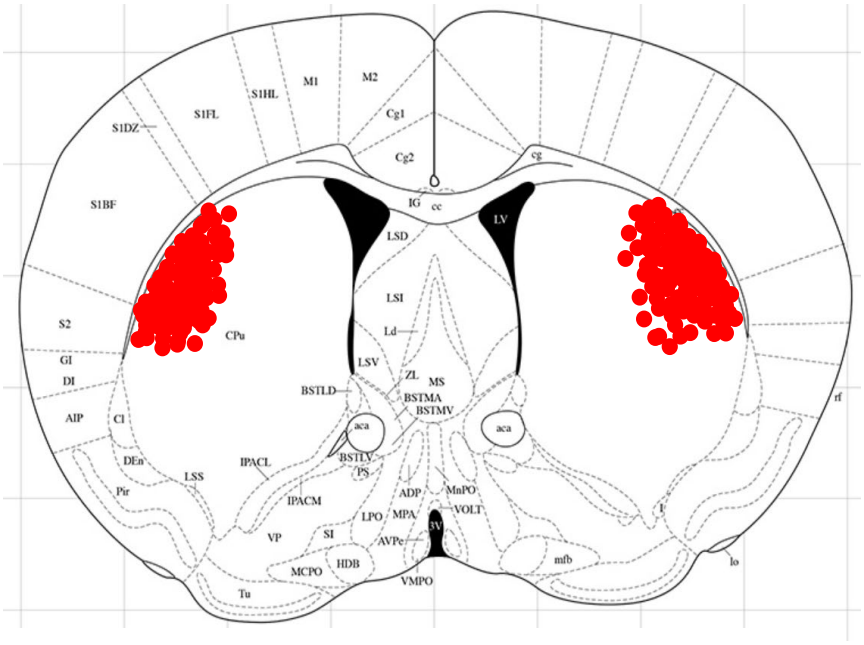

Figure 10. Histological Verification of DLS Bilateral Cannula Placement.

Placements were verified using crysol violet stained brain slices that were examined under a microscope and compared with Paxinos and Watson's Mouse Brain Atlas. DLS coordinates were M/L: +/- 2.5 mm, A/P: + 0.38 mm, D/V: -3.0 mm. A total of 17 mice were excluded due to missed placements (data not shown).

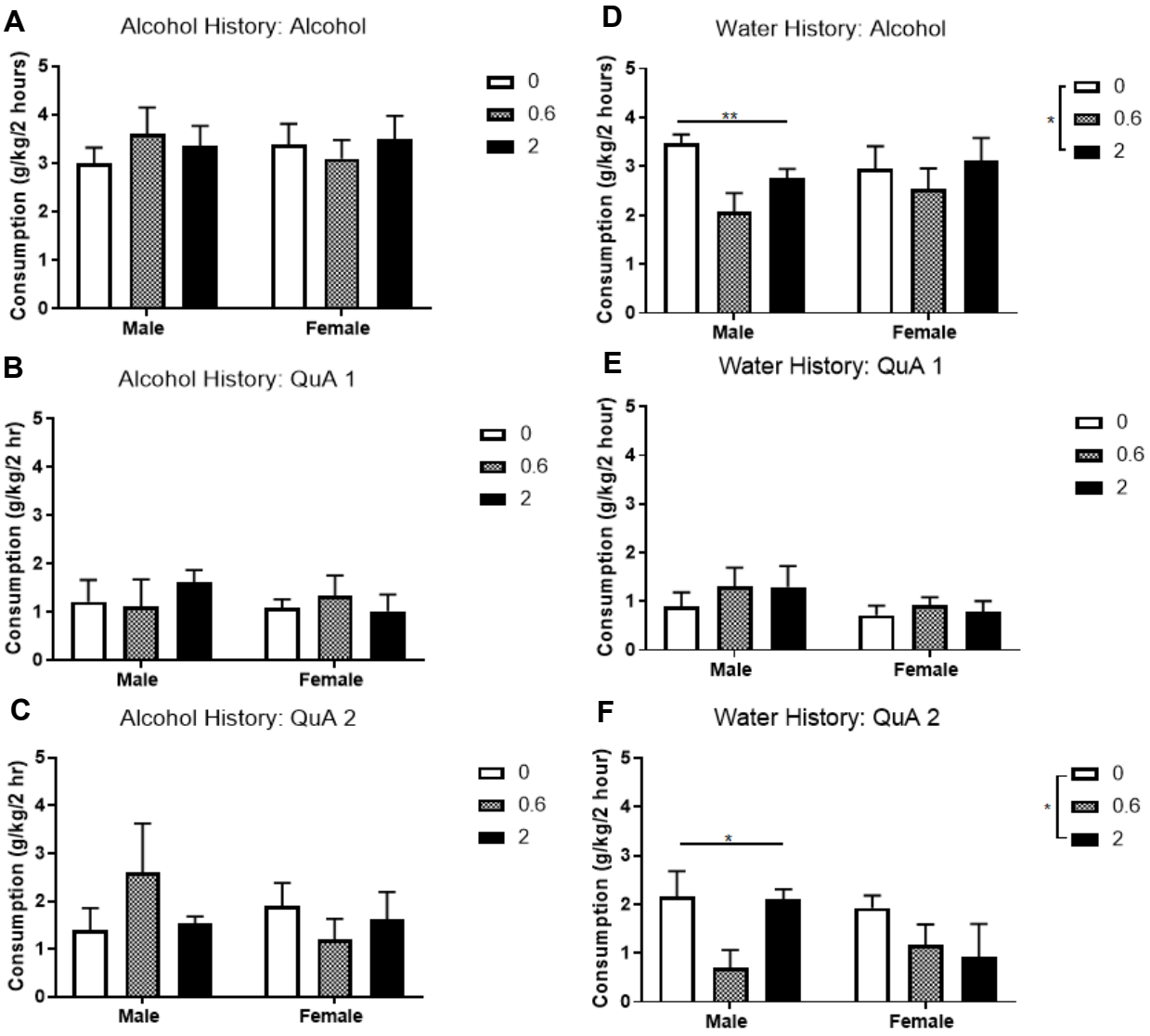

Figure 11. The effect of DLS AMPA Receptor Antagonism on 2-hour Alcohol and QuA Drinking.

A. Alcohol consumption in alcohol history mice is unaffected by NBQX at any concentration. B-C. QuA consumption in alcohol history mice is unaffected by NBQX at any concentration regardless of whether QuA was given first (B) or second (C). D. Alcohol consumption in water history mice. ANOVAs reveal a significant main effect of dose in the male mice (*$p < 0.05$, **$p < 0.01$). E. QuA consumption when QuA was the first solution in water history mice is unaffected by NBQX at any concentration. F. QuA consumption when QuA was the second solution in water history mice. ANOVAs reveal a significant main effect of dose in the male mice (*p's < 0.05). Data are shown as mean +/- standard error of the mean, n = 7-11 for A and D, n = 3-6 for B, C, E, and F.

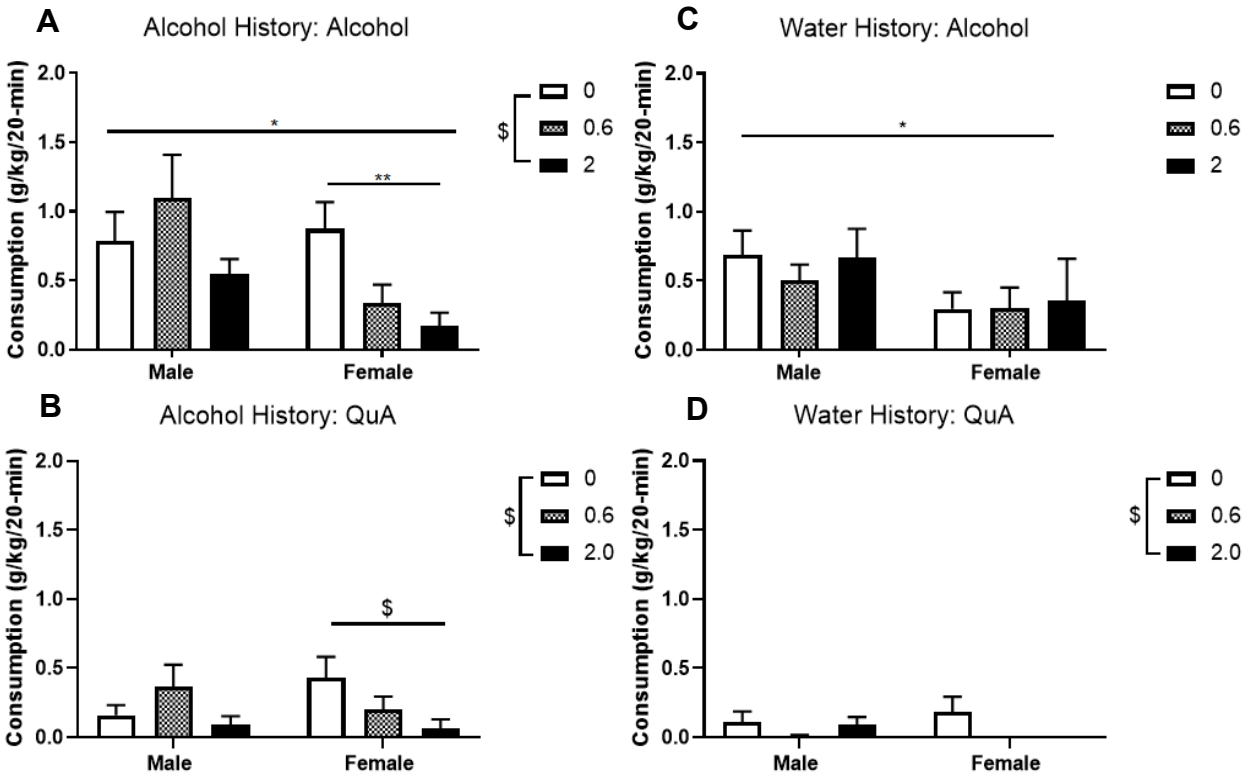

Figure 12. The effect of DLS AMPA Receptor Antagonism on 20-minute Front-Loading of Alcohol and QuA Drinking.

A. Alcohol consumption in alcohol history mice. ANOVA revealed a significant main effect of sex (*$p < 0.05$) and *Kruskal-Wallis* rank sum test revealed a trend toward a main effect of drug concentration, ($p = 0.07$). One-way ANOVA revealed that NBQX significantly reduced alcohol intake in female mice (**$p < 0.01$). B. QuA consumption in alcohol history mice. ANOVA revealed a trend toward a main effect of drug concentration ($p = 0.11$) and a trend toward and interaction of dose and sex ($p = 0.11$). ANOVA revealed that NBQX trended toward significantly reducing QuA intake in female mice, ($p = 0.09$) C. Alcohol consumption in water history mice. ANOVA revealed a significant main effect of sex, (*$p = 0.051$). D. QuA consumption in water history mice. ANOVA revealed a trend toward a main effect of drug concentration ($p = 0.07$). Data are shown as mean +/- standard error of the mean, n = 7-11 per group.

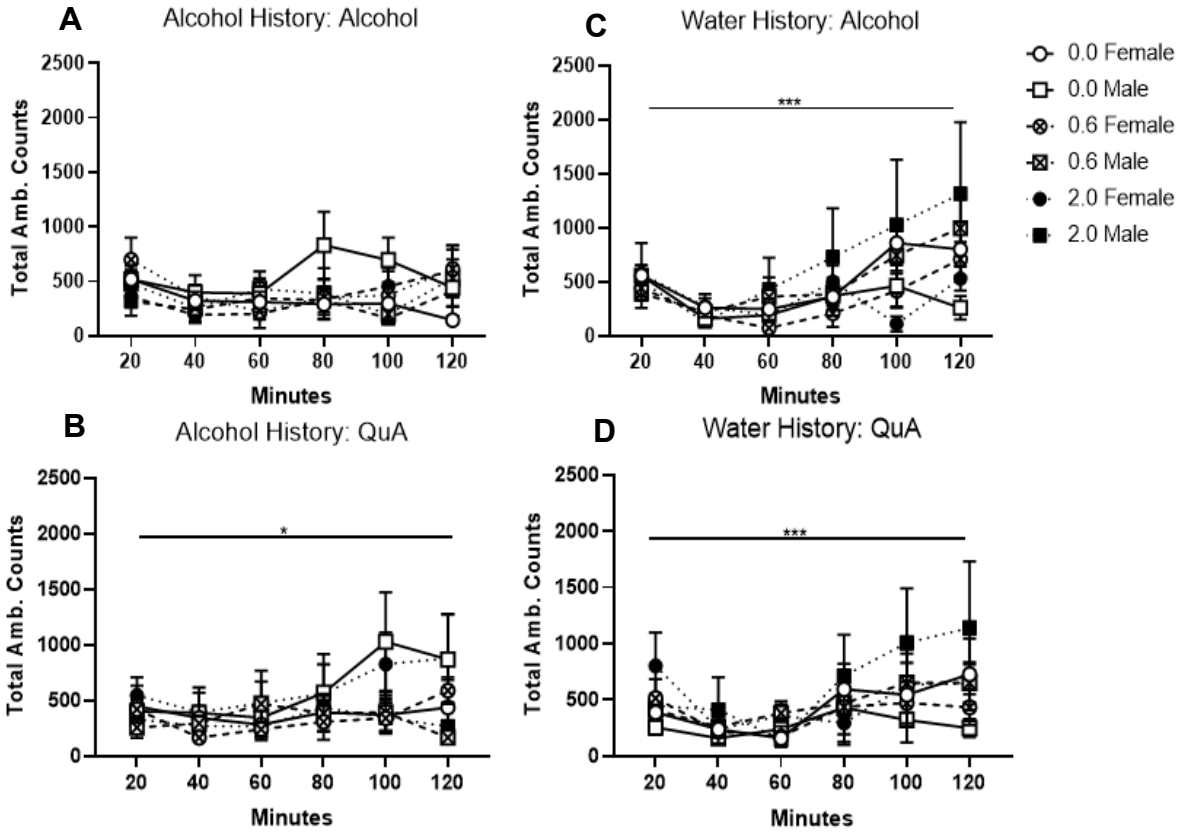

Figure 13. The effect of DLS AMPA Receptor Antagonism on Locomotor Activity During DID on Drug Infusion Days.

A. Locomotor activity during alcohol consumption in alcohol history mice. B. Locomotor activity during QuA consumption in alcohol history mice. ANOVA revealed a significant main effect of locomotor bin (*p = 0.01). C. Locomotor activity during alcohol consumption in water history mice. ANOVA revealed a significant main effect of locomotor bin, (***p < 0.001). D. Locomotor activity during QuA consumption in water history mice. ANOVA revealed a significant main effect of locomotor bin, (***p < 0.001). Data are shown as mean +/- standard error of the mean, n = 7-11 per group.

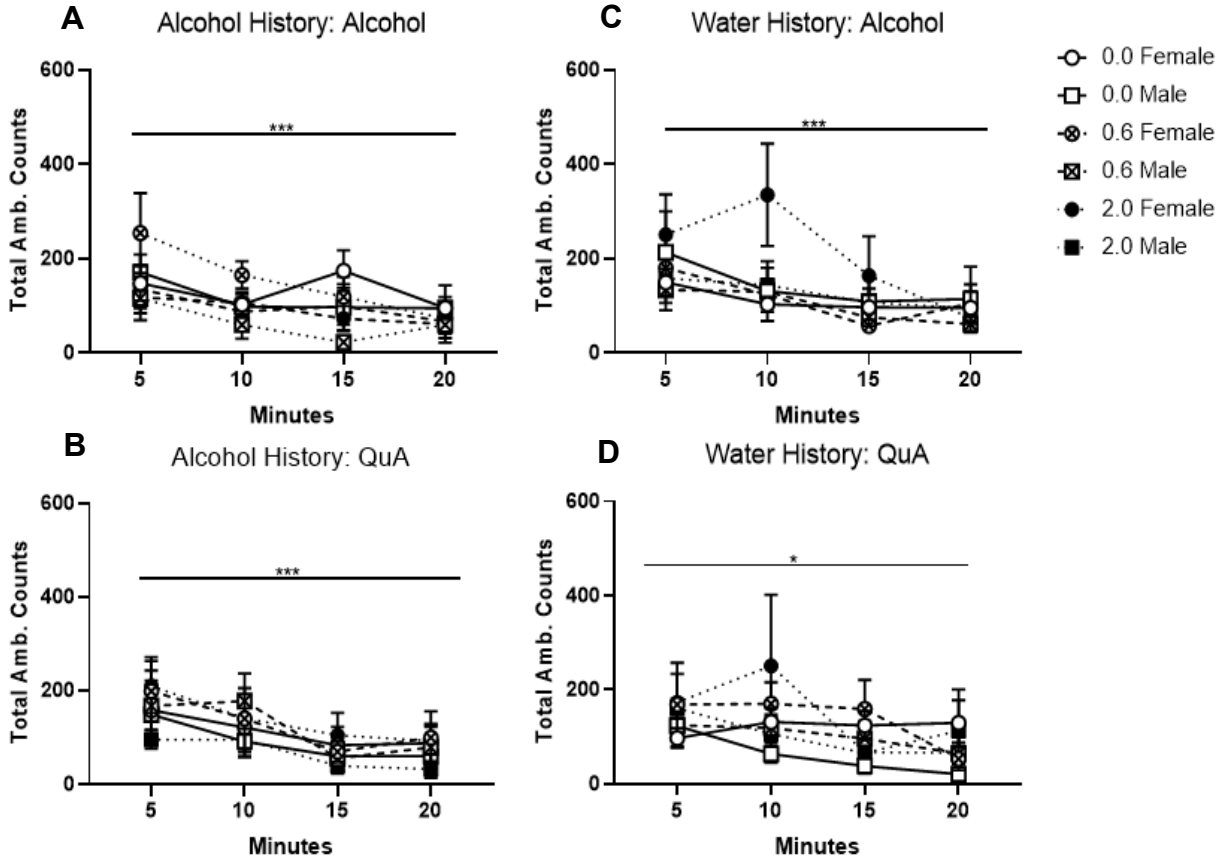

Figure 14. The effect of DLS AMPA Receptor Antagonism on Locomotor Activity During the First 20-Minutes of DID on Drug Infusion Days.

A. Locomotor activity during alcohol consumption in alcohol history mice. ANOVA revealed a significant main effect of locomotor bin, (***$p < 0.001$). B. Locomotor activity during QuA consumption in alcohol history mice. ANOVA revealed a significant main effect of locomotor bin (***$p = 0.001$). C. Locomotor activity during alcohol consumption in water history mice. ANOVA revealed a significant main effect of locomotor bin, (***$p < 0.001$). D. Locomotor activity during QuA consumption in water history mice. ANOVA revealed a significant main effect of locomotor bin, (*$p = 0.03$). Data are shown as mean +/- standard error of the mean, n = 7-11 per group.

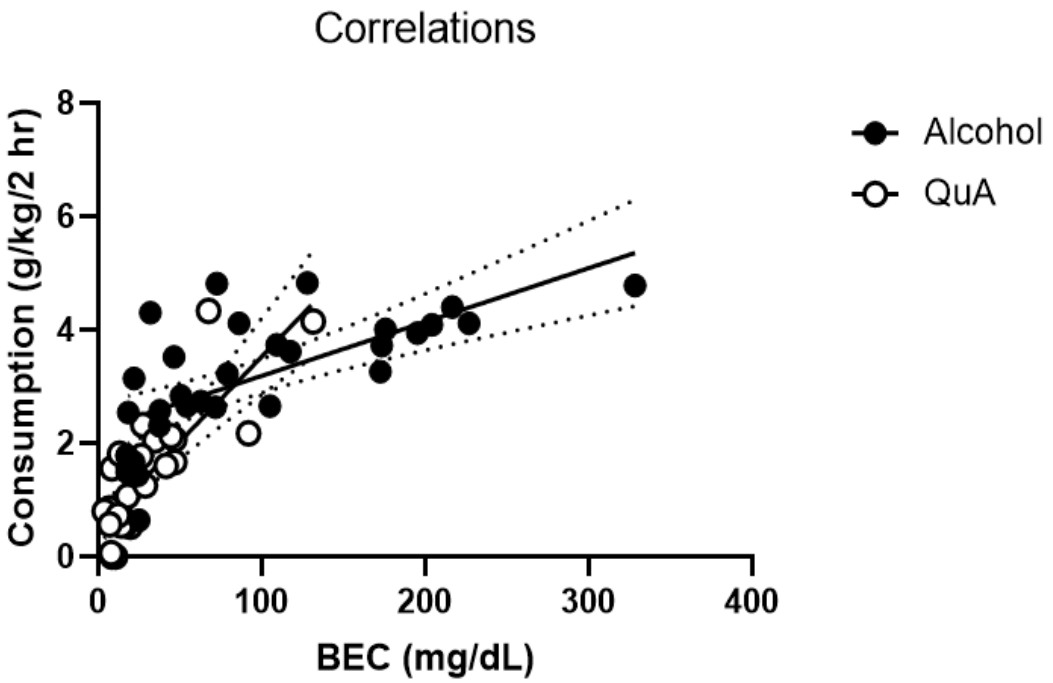

Figure 15. Correlation of Alcohol and QuA Intakes with BECs.

Two-hour alcohol and QuA consumption in alcohol history mice significantly and positively predicted BECs, (p's < 0.000). Data are shown as mean +/- standard error of the mean.

REFERENCES

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (*5th ed.) Washington, DC: Author.

Balleine, B. W., Delgado, M. R., & Hikosaka, O. (2007). The role of the dorsal striatum in reward and decision-making. *Journal of Neuroscience. 27* (31): 8161-8165. doi:10.1523/JNEUROSCI.1554-07.2007

Bamford, N. S., Wightman, R. M., & Sulzer, D. (2019). Dopamine's effects on corticostriatal synapses during reward-based behaviors. *Neuron. 97*(3): 494-510. doi:10.1016/j.neuron.2018.01.006

Banerjee N. (2014). Neurotransmitters in alcoholism: A review of neurobiological and genetic studies. *Indian journal of human genetics*, *20*(1), 20–31. doi: 10.4103/0971-6866.132750

Bauer, M. R., Garcy, D. P., & Boehm, S. L., 2nd (2020). Systemic Administration of the AMPA Receptor Antagonist, NBQX, Reduces Alcohol Drinking in Male C57BL/6J, but not Female C57BL/6J or High Alcohol Preferring (HAP) Mice. *Alcoholism, clinical and experimental research*, 10.1111/acer.14461. Advance online publication. doi.org/10.1111/acer.14461

Becker, J. B., Berkley, K. J., Geary, N., Hampson, E., Herman, J. P., & Young, E. (Eds.). (2007). *Sex differences in the brain: from genes to behavior*. Oxford university press.

Becker J. B. (2009). Sexual differentiation of motivation: a novel mechanism?. *Hormones and behavior*, *55*(5), 646–654. https://doi.org/10.1016/j.yhbeh.2009.03.014

Belin, D., Belin-Rauscent, A., Murray, J. E. & Everitt, B. J. (2013). Addiction: Failure of control over maladaptive incentive habits. *Current Opinion in Neurobiology, 23*: 564-572. doi:10.1016/j.conb.2013.01.025

Rhodes, J. S., Best, K., Belknap, J. K., Finn, D. A., & Crabbe, J. C. (2005). Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. *Physiology & Behavior,* 84: 53-63. doi:10.1016/j.physbeh.2004.10.007

Blegen, M. B., da Silva E Silva, D., Bock, R., Morisot, N., Ron, D., & Alvarez, V. A. (2018). Alcohol operant self-administration: Investigating how alcohol-seeking behaviors predict drinking in mice using two operant approaches. *Alcohol, 67*, 23-36. doi:10.1016/j.alcohol.2017.08.008.

Bocarsly, M. E., da Silva E Silva, D., Vuderman, K. D., Shashikiran, S., Rubinstein, M., Sibley, D. R., Dobbs, L. K., & Alvarez, V. A. (2019). A mechanism linking two known vulnerability factors for alcohol abuse: Heightened alcohol stimulation and low striatal dopamine D2 receptors. *Cell Reports, 29*(5):1147-1163.e5. doi: 10.1016/j.celrep.2019.09.059

Cai, Y., & Ford, C. P. (2018). Dopamine Cells Differentially Regulate Striatal Cholinergic Transmission across Regions through Corelease of Dopamine and Glutamate. *Cell reports*, *25*(11), 3148–3157.e3. doi: 10.1016/j.celrep.2018.11.053

Cannady, R., Fisher, K. R., Graham, C., Crayle, J., Besheer, J., Hodge, C. W. (2017) Potentiation of amygdala AMPA receptor activity selectively promotes escalated alcohol self-administration in a CaMKII-dependent manner. *Addiction Biology, 22*(3): 652-664. doi: 10.1111/abd.12357

Corbit, L. H., Nie, H., & Janak, P. H. (2014). Habitual responding for alcohol depends upon both AMPA and D2 receptor signaling in the dorsolateral striatum. *Frontiers in Beh Neuro. 8*(301):1-9. doi:10.3389/fnbeh.2014.00301

Cox, J., Witten, I., B., (2019). Striatal circuits for reward learning and decision-making. *Nat Rev Neurosci* 20, 482–494. doi: 10.1038/s41583-019-0189-2

Darevsky, D., Gill, T. M., Vitale, K. R., Hu, B., Wegner, S. A., & Hopf, F. W. (2019). Drinking despite adversity: behavioral evidence for a head down and push strategy of conflict-resistant alcohol drinking in rats. *Addiction biology*, *24*(3), 426–437. doi: 10.1111/adb.12608

Deroche-Gamonet, V., Belin, D., & Piazza, P. V. (2004). Evidence for addiction-like behavior in the rat. *Science (New York, N.Y.)*, *305*(5686), 1014–1017. doi: 10.1126/science.1099020

Dev, K. K., Petersen, V., Honoré, T. & Henley, J. M. (1996). Pharmacology and regional distribution of the binding of 6-[3H]nitro-7-sulphamoylbenzo[f]-quinoxaline-2,3-dione to rat brain. *J Neurochem. 67*(6):2609-12. doi:10.1046/j.1471-4159.1996.67062609.x

DiFeliceantonio, A. G., & Berridge, K. C. (2016). Dorsolateral neostriatum contribution to incentive salience: opioid or dopamine stimulation makes one reward cue more motivationally attractive than another. *The European journal of neuroscience*, *43*(9), 1203–1218. doi: 10.1111/ejn.13220

Ding, Z. M., Rodd, Z. A., Engleman, E. A., Bailey, J. A., Lahiri, D. K., & McBride, W. J. (2013). Alcohol drinking and deprivation alter basal extracellular glutamate concentrations and clearance in the mesolimbic system of alcohol-preferring (P) rats. *Addiction biology*, *18*(2), 297–306. doi: 10.1111/adb.12018

Doig, N. M., Magill, P. J., Apicella, P., Bolam, J. P., & Sharott, A. (2014). Cortical and thalamic excitation mediate the multiphasic responses of striatal cholinergic interneurons to motivationally salient stimuli. *Journal of Neuroscience*, *34*(8), 3101-3117. 10.1523/JNEUROSCI.4627-13.2014

Everitt, B. J. & Robbins, T. W. (2006). Neural systems of reinforcement for drug addiction: From actions to habits to compulsion. *Nature Neuroscience, 8*: 1481-1489. doi:10.1038/nn1579

Everitt, B. J., Belin, D., Exonomidou, D., Pelloux, Y., Dalley, J. W., & Robbins, T. W. (2008). Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. *Philospohical Transactions of the Royal Society,* 363:3125-3135. doi:10.1098/rstb/2008.0089.

Everitt, B. J. & Robbins, T. W. (2013). From the ventral to the dorsal striatum: Devolving views of their roles in drug addiction. *Neuroscience & Biobehavioral Reviews, 37*(9):1946-1954. doi: 10.1016/j.neubiorev.2013.02.010

Everitt, B. J. & Robbins, T. W. (2016). Drug addiction: Updating actions to habits to compulsions ten years on. The *Annual Review of Psychology, 67*: 23-50. doi:10.1146/annurev-psych-122414-033457

Finn, D. A., Hashimoto, J. G., Cozzoli, D. K., Helms, M. L., Nipper, M. A. M., Kaufman, M. N., Wiren, K. M., Guizzetti, M. (2018) Front. Genet. 12. doi:10.3389/fgene.2018.00325

Fulenwider, H. D., Nennig, S. E., Price, M. E., Hafeez, H., & Schank, J. R. (2019). Sex differences in aversion-resistant ethanol intake in mice. *Alcohol and Alcoholism, 54*(4):345-352. doi:10.1093/alcalc/agz022

Gardoni, F., & Bellone, C. (2015). Modulation of the glutamatergic transmission by Dopamine: a focus on Parkinson, Huntington and Addiction diseases. *Frontiers in cellular neuroscience*, *9*, 25. doi: 10.3389/fncel.2015.00025

Gill, R., Nordholm, L., & Lodge, D. (1992). The neuroprotective actions on 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) in a rat focal ischaemia model. *Brain Research, 580:* 35-43. doi:10.1016/0006-8993(92)09924-X

Giuliano, C., Peña-Oliver, Y., Goodlett, C. R., Cardinal, R. N., Robbins, T. W., Bullmore, E. T., Belin, D., & Everitt, B. J. (2018). Evidence for a Long-Lasting Compulsive Alcohol Seeking Phenotype in Rats. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *43*(4), 728–738. doi: 10.1038/npp.2017.105

Giuliano, C., Belin, D., & Everitt, B. J. (2019). Compulsive alcohols seeking results from a failure to disengage dorsolateral striatal control over behavior. *Journal of Neuroscience, 39*(9): 1744-1754. doi:10.1523/JNEUROSCI.2615-18.2018

Henley, J. M., & Wilkinson, K. A. (2016). Synaptic AMPA receptor composition in development, plasticity and disease. *Nature reviews. Neuroscience*, *17*(6), 337–350. https://doi.org/10.1038/nrn.2016.37

Goodwani, S., Saternos, H., Alasmari, F., & Sari, Y. (2017). Metabotropic and ionotropic glutamate receptors as potential targets for the treatment of alcohol use disorder. *Neuroscience and biobehavioral reviews*, *77*, 14–31. doi: 10.1016/j.neubiorev.2017.02.024

Green, A., Neff, D., Giuliano, G., Lee, N., Turchin, R., & Kunkel, E. J. (2018). Surrogate alcohol or nonbeverage alcohol consumption: the Surrogate Alcohol Questionnaire (SAQ). *Psychosomatics*, *59*(4), 349-357. doi:10.1016/j.psym.2018.01.001

Henley, J. M., & Wilkinson, K. A. (2013). AMPA receptor trafficking and the mechanisms underlying synaptic plasticity and cognitive aging. *Dialogues in clinical neuroscience*, *15*(1), 11–27.

Hopf, F. W. & Lesscher, H. M. (2014). Rodent models for compulsive alcohol intake. *Alcohol. 48*(3): 253-264. doi:10.1016/j.alcohol.2014.03.001

Kasten, C. R. & Boehm, S. L. (2014). Intra-nucleus accumbens shell injections of R(+)- and S(-)-baclofen bidirectionally alter binge-like ethanol, but not saccharin, intake in C57Bl/6J mice. *Behav Brain Res.* 272: 238-247. doi: 10.1016/j.bbr.2014.07.011

Kawa, A., B. & Robinson, T., E., (2019). Sex differences in incentive-sensitization produced by intermittent access cocaine self-administration. *Psychopharmacology* 236, 625–639. doi: 10.1007/s00213-018-5091-5

Koob, G. F. & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology*, 35: 217-238. doi: 10.1038/npp.2009

Lachenmeier, D. W., Rehm, J., & Gmel, G. (2007). Surrogate alcohol: what do we know and where do we go?. *Alcoholism: Clinical and Experimental Research*, *31*(10), 1613-1624. doi: 10.1111/j.1530-0277.2007.00474.x

Lagström, O., Danielsson, K., Söderpalm, B., Ericson, M., & Adermark, L. (2019). Voluntary Ethanol Intake Produces Subregion-Specific Neuroadaptations in Striatal and Cortical Areas of Wistar Rats. *Alcoholism: Clinical and Experimental Research*, *43*(5), 803-811.

Lerner, T. N., Shilyansky, C., Davidson, T. J., Evans, K. E., Beier, K. T., Zalocusky, K. A., Crow, A. K., Malenka, R. C., Luo, L., Tomer, R., & Deisseroth, K. (2015). Intact-Brain Analyses Reveal Distinct Information Carried by SNc Dopamine Subcircuits. *Cell*, *162*(3), 635–647. doi: 10.1016/j.cell.2015.07.014

Lei, K., Wegner, S. A., Yu, J. H., Simms, J. A., Hopf, F. W. (2016). A single alcohol drinking session is sufficient to enable subsequent aversion-resistant consumption in mice. *Alcohol.* 55*:* 9-16. doi: 10.1016/j.alcohol.2016.07.008

Lesscher, H. M., Van Kerkhof, L. W., & Vanderschuren, L. J. (2010). Inflexible and indifferent alcohol drinking in male mice. *Alcoholism: Clinical and Experimental Research*, *34*(7), 1219-1225. doi: 10.1111/j.1530-0277.2010.01199.x.

Linsenbardt, D. N., & Boehm, S. L., 2nd (2014). Alterations in the rate of binge ethanol consumption: implications for preclinical studies in mice. *Addiction biology*, *19*(5), 812–825. doi: 10.1111/adb.12052

Linsenbardt, D. N., & Boehm, S. L., 2nd (2015). Relative fluid novelty differentially alters the time course of limited-access ethanol and water intake in selectively bred high-alcohol-preferring mice. *Alcoholism, clinical and experimental research*, *39*(4), 621–630. doi: 10.1111/acer.12679

Lüscher, C., Robbins, T.W. & Everitt, B.J. (2020) The transition to compulsion in addiction. *Nat Rev Neuroscience* 21, 247–263. doi:10.1038/s41583-020-0289-z

Ma, T., Barbee, B., Wang, X., & Wang, J. (2017). Alcohol induces input-specific aberrant synaptic plasticity in the rat dorsomedial striatum. *Neuropharmacology*, *123*, 46–54. https://doi.org/10.1016/j.neuropharm.2017.05.014

Ma, T., Cheng, Y., Hellard, E. R., Wang, X., Lu, J., Gao, X., Huang, Y. C. C., Wei, X., Ji, J., & Wang, J. (2018). Bidirectional and long-lasting control of alcohol-seeking behavior by corticostriatal LTP and LTD. *Nature Neuroscience.* 21: 373-383. doi:10.1038/s41593-018-0081-9

Malenka, R. C., & Bear, M. F. (2004). LTP and LTD: an embarrassment of riches. *Neuron*, *44*(1), 5–21. https://doi.org/10.1016/j.neuron.2004.09.012

Palmiter R. D. (2008). Dopamine signaling in the dorsal striatum is essential for motivated behaviors: lessons from dopamine-deficient mice. *Annals of the New York Academy of Sciences*, *1129*, 35–46. doi: 10.1196/annals.1417.003

Pascoli, V., Hiver, A., Van Zessen, R., Loureiro, M., Achargui, R., Harada, M., Flakowski, J., & Lüscher, C. (2018). Stochastic synaptic plasticity underlying compulsion in a model of addiction. *Nature*, *564*(7736), 366–371. doi: 10.1038/s41586-018-0789-4

Roberto, M., Schweitzer, P., Madamba, S. G., Stouffer, D. G., Parsons, L. H., Siggins, G. R. (2004) Acute and chronic ethanol alter glutamatergic transmission in rat central amygdala: an *in vitro* and *in vivo* analysis. *The Journal of Neuroscience, 24*(7):1594-1603, doi: 10.1523/JNEUROSCI.5077-03.2004

Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain research. Brain research reviews*, *18*(3), 247–291. doi: 10.1016/0165-0173(93)90013-p

Robinson, T. E., & Berridge, K. C. (2008). Review. The incentive sensitization theory of addiction: some current issues. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *363*(1507), 3137–3146. doi: 10.1098/rstb.2008.0093

Berridge, K. C., & Robinson, T. E. (2016). Liking, wanting, and the incentive-sensitization theory of addiction. *The American psychologist*, *71*(8), 670–679. doi: 0.1037/amp0000059

Ruda-Kucerova, J., Babinska, Z., Luptak, M., Getachew, B., & Tizabi, Y. (2018). Both ketamine and NBQX attenuate alcohol drinking in male Wistar rats. *Neurosci Lett. 14*(666):175-180. doi: 10.1016/j.neulet.2017.12.055

Seif, T., Chang, S. J., Simms, J. A., Gibb, S. L., Dadgar, J., Chen, B. T., Harvey, B. K., Ron, D., Messing, R. O., Bonci, A., & Hopf, F. W. (2013). Cortical activation of accumbens hyperpolarization-active NMDARs mediates aversion-resistant alcohol intake. *Nature Neuroscience*, *16*(8), 1094–1100. doi: 10.1038/nn.3445

Seif, T., Simms, J. A., Lei, K., Wegner, S., Bonci, A., Messing, R. O., & Hopf, F. W. (2015). D-Serine and D-Cycloserine Reduce Compulsive Alcohol Intake in Rats. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, *40*(10), 2357–2367. doi: 10.1038/npp.2015.84

Sheardown, M. J., Nielsen, E. 0., Hansen, A. J., Jacobsen, P., and , Honoré, T. (1990) 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline: a neuroprotectant for cerebral ischemia. *Science. 247(*4942):571-4. doi: 10.1126/science.2154034

Siciliano, C. A., Noamany, H., Chang, C. J., Brown, A. R., Chen, X., Leible, D., Lee, J. J., Wang, J., Vernon, A. N., Vander Weele, C. M., Kimchi, E. Y., Heiman, M., & Tye, K. M. (2019). A cortical-brainstem circuit predicts and governs compulsive alcohol drinking. *Science (New York, N.Y.)*, *366*(6468), 1008–1012. doi: 10.1126/science.aay1186

Shiflett, M. W., Balleine, B. W. (2011). Molecular substrates of action control in cortico-striatal circuits. *Prog Neurobiol. 95 (*1): 1-13. doi: 10.1016/j.pneurobio.2011.05.007

Sneddon, E. A., White, R. D., & Radke, A. K. (2019). Sex Differences in Binge-Like and Aversion-Resistant Alcohol Drinking in C57 BL/6J Mice. *Alcoholism: clinical and experimental research*, *43*(2), 243-249.

Stephens, D. N. & Brown, G. (1999) Disruption of operant oral self-administration of ethanol, sucrose, and saccharin by the AMPA/kainite antagonist, NBQX, but not the AMPA antagonist, GYKI 52466. *ACER. 23*(12)1914-20. doi: 10.111/j.1530-0277.1999tb04091.x.

Tiwari, V., Veeraiah, P., Subramaniam, V., & Patel, A. B. (2014). Differential effects of ethanol on regional glutamatergic and GABAergic neurotransmitter pathways in mouse brain. *Journal of neurochemistry*, *128*(5), 628–640. doi: 10.1111/jnc.12508

Wang, J., Hamida, S. B., Darcq, E., Zhu, W., Gibb, S. L., Fe Lanfranco, M., Carnicella, S., & Ron, D. (2012). Ethanol-mediated facilitation of AMPA receptor function in the dorsomedial striatum: Implications for alcohol drinking behavior. *Journal of Neuroscience. 32* (43): 15124-15132. doi:10.1523/JNEUROSCI.2783-12.2012

Wegner, S. A., Hu, B., De Oliveira Sergio, T., Darevsky, D., Kwok, C. C., Lei, K., & Hopf, F. W. (2019). A novel NMDA receptor-based intervention to suppress compulsion-like alcohol drinking. *Neuropharmacology*, *157*, 107681. doi: 10.1016/j.neuropharm.2019.107681

Wilcox, M. V., Cuzon Carlson, V. C., Sherazee, N., Sprow, G. M., Bock, R., Thiele, T. E., Lovinger, D. M., & Alvarez, V. A. (2014). Repeated binge-like ethanol drinking alters ethanol drinking patterns and depresses striatal GABAergic transmission. *Neuropsychopharmacology, 39*(3), 579–594. doi:10.1038/npp.2013.230

Yin, H. H. & Knowlton, B. J. (2006). The role of the basal ganglia in habit formation. *Nature Reviews Neuroscience, 7*: 464-476. doi:10.1038/nrn1919