DETERMINING THE IMPACT OF REPEATED BINGE DRINKING ON CORTICOSTRIATAL THETA SYNCHRONY

by

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ABSTRACT

The development of alcohol use disorder (AUD) is believed to involve functional adaptations in corticostriatal projections which regulate the reinforcing properties of ethanol (EtOH). To further our understanding of how repeated EtOH consumption impacts the corticostriatal circuit, extracellular electrophysiological recordings (local field potentials; LFPs) were gathered from the nucleus accumbens and prefrontal cortex of female and male C57BL/6J mice voluntarily consuming EtOH or water using 'drinking-in-the-dark' (DID) procedures. Mice were given 15 consecutive days of two-hours of access to EtOH (20% v/v), three hours into the dark cycle while LFPs were recorded. To determine the impact of repeated EtOH consumption on neural activity between these brain regions, theta phase-locking value (PLV, a measure of synchrony) was calculated. Specifically, theta PLV was calculated during active drinking periods (bouts) and average PLV during the first bout was compared to the last bout to determine within session changes in synchrony. Results indicated significantly lower PLV during the last bout than the first bout. Additionally, longer bouts predicted lower PLV during the last bout, but not the first bout when mice were consuming EtOH. These results may suggest that alcohol intoxication decreases corticostriatal synchrony over a drinking period. Results considering changes in theta power spectral density (PSD) indicated an increase in PSD when mice were given access to water during the typical EtOH access time following the 15-day EtOH drinking history. This effect was not seen when mice were drinking water prior to EtOH access and may be indicative of a successive negative contrast effect. This work identifies unique functional characteristics of corticostriatal communication associated with binge-like EtOH intake and sets the stage for identifying the biological mechanisms subserving them.

INTRODUCTION

Binge Drinking: Overview of the Problem

Binge drinking is a pattern of problematic drinking resulting in blood alcohol levels of 0.08 g/dL or higher in a timespan of two hours or less (NIAAA, 2004). Binge drinking is also common, with statistical models estimating that one fifth of the global adult population (approximately 1.54 billion individuals) has engaged in at least one episode of binge drinking within the past month (Peacock et al., 2018). The consequences of binge drinking are severe, as longitudinal studies indicate that engaging in multiple binge drinking sessions is a predictor of future development of alcohol use disorder (AUD) (Chassin et al., 2002; Zucker et al., 2006; Dawson et al., 2008). A review of human brain mapping studies highlights that individuals with AUD and those who often binge drink have similar outcomes in regard to brain changes (Petit et al., 2014). Though binge drinking has severe consequences, the neurobiological changes that occur as an individual engages in multiple binge sessions are not entirely understood. The goal of the current study was to identify how corticostriatal power and synchrony are influenced by repeated binge drinking experiences to better understand one aspect of the neurobiological adaptations which occur which may lead an individual to repeatedly binge drink.

Power and Synchrony: Brief Overview

In humans and rodents, neural oscillations (rhythmic patterns of neural activity) are observed at frequency bands ranging from 0.05 to 500 hertz (Hz), where prominent frequency bands include delta (1.5-4 Hz), theta (4-10 Hz), beta (10-30 Hz), and gamma (30-80 Hz) (Buzsaki and Draguhn, 2004). Oscillatory activity occurring within these frequency bands has been hypothesized to be representative of information processing and various cognitive processes (Kopell et al., 2000; Başar et al., 2001; Kahana, 2006). Oscillatory activity can be gathered, often through the acquisition of electroencephalograms (EEG) or local field potentials (LFPs), and assessed to determine the relationship among oscillatory activity, behavior, and environmental variables. Electrophysiological techniques allow for the assessment of synchrony within and between brain regions. Synchrony references two events simultaneously occurring in time, such as neurons firing at the same time (Buzsáki, 2006). Power spectral density, which can

be generated from EEG or LFP data, is inversely proportional to the frequency (f) within the brain, which creates a well-recognized 1/f power relationship (Bak et al., 1987; Buzsaki and Draguhn, 2004; Gao, 2016). This biological organization creates a situation wherein higher frequencies (i.e. gamma) often display lower power than lower frequencies (i.e. delta, theta, beta). Therefore, it is expected that these lower frequencies (delta, theta and beta) will yield more information about synchrony between disparate brain regions. A useful analogy here is that low frequency bands are the 'bass' of the brain, traveling further than higher 'treble' frequencies, allowing the low 'bass' frequencies the possibility of synchronizing activity between non-neighboring brain regions. Therefore, background literature and analyses from the current study will focus on change in corticostriatal synchrony in low power bands, with a focus on theta. The theta band is of specific interest both for this reason, and because previous research has found that alcohol consumption causes alterations in theta power and synchrony.

Alcohol Consumption Causes Alterations in Corticostriatal Theta Power and Synchrony

It is theorized that projections within the corticostriatal circuit undergo functional adaptations that mediate a shift from occasional to high-risk drinking (Belin et al., 2009; Barker et al., 2015). Communication between corticostriatal brain regions, which can be measured as synchronous activity, has been shown to be impaired following alcohol use and abuse (Courtney et al., 2013; Galandra et al., 2019). Synchrony in the corticostriatal circuit is decreased in individuals with AUD, and this synchrony is negatively correlated with time to relapse (Camchong et al., 2013). Further, in both rats and humans, adolescent alcohol exposure is associated with lower cortical theta synchrony than control (Ehlers et al., 2020). Previous research from our laboratory observed reduced theta synchrony between the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) in selectively bred Indiana Alcohol Preferring (P) rats (McCane et al., 2018). These data indicate that either alcohol or the genetic predisposition for excessive drinking is associated with alterations in theta-band synchrony.

Theta power in individuals with a history of binge drinking during response inhibition while performing a NoGo task has been found to negatively correlate with alcohol consumption and number of binge drinking episodes (Holcomb et al., 2019). Resting-state EEGs of individuals with AUD show higher power in the theta band as compared to controls (Pollock et al., 1992; Mumtaz et al., 2017). Similarly, power is also higher in the theta band in individuals

with a history of binge drinking as compared to control (Lopez-Caneda et al., 2017; Affan et al., 2018). It is theorized that increases in theta power are indicative of impairments in cognitive processing (Porjesz and Begleiter, 2003). Alterations within the theta band are largely driven by interactions between interneurons and pyramidal cells (Benchenane et al., 2010; Voloh and Womelsdorf, 2018), which are altered by binge drinking (Salling et al., 2018; Joffe et al., 2020).

Rationale and Hypotheses

Considering this past research, we know that changes in corticostriatal power and synchrony are critical in the development of AUD. However, there remains a need to investigate the specific adaptations within corticostriatal power and synchrony which are induced by multiple alcohol drinking experiences to better understand the functional adaptions which may mediate a shift from occasional to problematic alcohol use. To begin to answer this question, the current study utilized extracellular electrophysiological recordings collected from the NAc and mPFC (corticostriatal brain regions) of mice voluntarily consuming EtOH or water using 'Drinking-in-the-Dark' (DID) procedures (a mouse model of binge drinking). Bouts, episodes of active drinking behavior, were monitored and simultaneously time-locked with electrophysiological recordings, to evaluate how these variables correspond with neural oscillations. To consider how drinking behavior influenced theta band activity, analyses within this document focused on specific epochs of interest during the session: pre-bout, bout, and post-bout, where a bout was defined using the same definition as Darevsky et al. (2018): three or more licks that occur ≤1 sec apart. Previous research suggests that mPFC neuronal activity encodes for the intent to initiate a bout (Linsenbardt et al., 2019). We evaluated this possibility in our data through considering times when mice may have anticipated drinking (the pre-bout epoch), hypothesizing that *average* theta PLV and power would be higher during the pre-bout epoch than the post-bout epoch (Aim 1). Aim 2 considered within session changes in theta power and synchrony by focusing on changes in these measures from the first to last bout. It was hypothesized that these measures would not change acutely, but rather that changes in oscillatory measures would develop progressively over days. Accordingly, Aim 3 considered change in these measures over the days of testing.

METHODS

Subjects

Data were gathered from adult C57BL/6J (B6) female (*n*=7) and male (*n*=7) mice. All mice were bred in the School of Science vivarium at Indiana University-Purdue University Indianapolis (IUPUI). Breeder mice were supplied from Jackson Laboratories. Mice were group-housed with 3-4 same-sex siblings after weaning. Weaning occurred on post-natal day (PND) 21. Then, one week prior to surgery, mice were transferred from the breeding colony room to one of two other colony rooms within the vivarium which provided staggered light cycles for drinking-in-the-dark (DID). The use of two rooms with separate light cycles enabled efficient data collection as the DID procedure is time sensitive. One room provided an AM cohort with lights off at 0700 and lights on at 1900. The other room provided a PM cohort with lights off at 1100 and lights on 2300. Mice were randomly assigned to live in one of these two rooms. At the time of transfer from the breeding colony, mice were single housed in 12.5 x 7.5 x 7.5-inch cages and allowed to acclimate to their new light cycle and single housing condition for one week prior to surgery. Surgery occurred at PND 84 (±12 days). Mice then had a one-week recovery period before beginning experiments. Please see *Fig. 1* for a timeline of experiments. Other than during EtOH DID testing sessions, mice had *ad libitum* access to water. Standard rodent chow (LabDiet 5001) was always available *ad libitum*, including during DID testing. All procedures were approved by the IUPUI School of Science Animal Care and Use Committee and conformed to the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003).

Surgery

Mice underwent stereotaxic surgery to implant electrodes to record neural activity from the medial prefrontal cortex and nucleus accumbens. Custom-built electrodes were created using eight 25 µm tungsten wires per brain region (California Fine Wires, Grover Beach, CA) inserted into silica tubes. Wires were pinned into a Neuralynx Electrode Interface Board (EIB). Anesthesia was induced with isoflourane at 5% in oxygen, and then maintained with isoflourane at 2% in oxygen. An injection of Ketoprofen (5 mg/kg, subcutaneous (SC), in a volume of 0.1 ml

/ 100g) was administered after anesthesia was induced to reduce post-surgical pain and aide in recovery. A sub-dermal injection of Bupivacaine (2.5 mg/mL, .05 mL) was administered directly over the site where an incision was then made to expose the skull. Next, a craniotomy was made to allow the electrode to be unilaterally implanted into the two corticostriatal brain regions of interest (mPFC: A/P: 1.94, M/L: 0.5, D/V: -2.25; NAc: A/P: 1.10, M/L: 0.8, D/V: -4.25). The side of the brain (left or right) where the electrode was implanted was randomly assigned. Ground wires were attached to the EIB and referenced to two stainless steel screws placed over the cerebellum. The head cap was then completed using dental cement. Mice were monitored closely for one-week following surgery, including daily checks for weight, feeding, and signs of distress. Cephazolin (30mg/kg SC, 1 mg/mL) and/or a topical antibiotic (2% bacitracin/polymixin, as needed) was administered as necessary during this recovery period. Mice were given LabDiet 5015 mixed with water as a high protein 'treat' to encourage post-operative feeding. This treat was available in addition to their standard food (LabDiet 5001) during the first 2-3 days of recovery. After this one-week recovery period, all mice were at or above pre-surgery weight and had fully recovered. Next, local field potentials (LFPs) were recorded using a compatible headstage and OpenEphys software during DID.

EtOH for Drinking-in-the-Dark

EtOH for drinking experiments was prepared by diluting 190 proof EtOH from Pharmco, Inc. (Brookfield, CT) to 20% v/v in tap water. Drinking solution was prepared at the beginning of the experiment and stored in a sealed container. This prepared solution was used to fill ball-bearing sipper tubes during DID procedures described below.

Electrophysiology Recordings During Drinking-in-the-Dark

Mice underwent a home cage binge drinking procedure (drinking-in-the-dark, DID). In the current study, three hours into the dark cycle, mice were given two hours of access to 20% EtOH in the home cage for 15 consecutive days (Linsenbardt and Boehm, 2014, 2015) while neural activity was recorded from the mPFC and NAc. In addition, 2 hours of neural activity was recorded 3 hours into the dark cycle during water drinking for three days before and after the

block of EtOH testing sessions (referred to throughout as water pre-DID and water post-DID, respectively).

On each testing day, mice were transferred from their colony room to the electrophysiology testing room and given an hour and a half to acclimate to the electrophysiology testing room. Mice were not yet tethered and still had access to home-cage water bottles during this acclimation period. It should be noted that electrophysiology recordings occurred while the mice were in their home cage, so during this acclimation period mice remained in their home cage while the home cage was placed on the electrophysiology cart in the testing room (please see *Fig. 2* for a photograph of the electrophysiology recording set up). Mice were then tethered to an OpenEphys system with a compatible headstage and given a 30-minute *baseline* period wherein home-cage water bottles were replaced with standard ball-bearing sippers which always only contained water during the *baseline* period. The first two days consisted of only this *baseline* procedure and were considered 'tether habituation' days. These days were not included in the three water pre-DID days. Once testing (labeled as 'DAYS' in *Fig 1.*) began, the end of this 30 minute *baseline* period was immediately followed by a 2-hour DID testing *session* where the standard *baseline* ball-bearing sippers containing water were replaced with new sippers containing either unsweetened 20% (v/v) EtOH (days 4-18) or water (water pre-DID days 1-3; or water post-DID days 19-21); please see timeline *Fig. 1.* All electrophysiology DID *session* recordings took place in the home cage 3 hours into the mouse's dark cycle. Fluid intake data was recorded as total intake determined from volume left in the sippers at the end of the baseline period (water only) and DID session (water or EtOH, depending on the testing day; please see description above and timeline). Additionally, bouts were recorded using a piezo microphone attached to the sipper tube and connected to the OpenEphys equipment via a Tascam amplifier connected to an I/O board integrated with the OpenEphys acquisition system, allowing the bout times and electrophysiology data to be synchronized using OpenEphys software. Locomotor data was also recorded with AnyMaze and similarly time-locked with electrophysiology recordings.

Immunohistochemistry to Check Electrode Placement

At the end of DID testing, mice were anesthetized with isoflurane as described in the surgery section above and a stimulus isolator was used to create lesions at the site of each of the

wires via sending a 1 mA current through the EIB into each of the wires for one second. Mice then rested for one day following this procedure to allow gliosis to occur, and then were transcardially perfused and brains were removed to check electrode placements. For perfusions, mice were anesthetized using urethane that was administered via intraperitoneal injection at a dose of 1.5 g/kg dissolved in sterile water in a volume of 1.0 mL/kg. After the mouse was not responsive to 3 consecutive toe pinches separated by 1 minute each, the perfusion procedure began. In the event that a mouse was still responsive after 10 minutes, additional urethane was injected at 1.0 ml/kg of the above concentration. Once the mouse had satisfied criteria for full anesthesia, a 2-inch incision was made along the abdomen just below the sternum revealing the diaphragm. The diaphragm was then cut to access the heart and a blunted 19-gauge needle was placed through the left ventricle and into the aorta, where it was then be clamped into place. Next, the right atrium was then cut to allow for drainage and transcardial perfusion of isotonic 0.9% saline began through the aorta followed by 4% paraformaldehyde (PFA). Brains were then be removed and placed in PFA for 4 days and then transferred to sucrose solution (30%) until they sank to the bottom of their respective containers (typically 3 days). Following fixation, standard slicing and staining (DAPI and GFAP) procedures were followed to determine electrode placement. Placements can be seen in *Fig 3.* Two mice (1 F, 1 M) had mPFC placements which were within the corpus callosum. These two mice were removed from all analyses.

RESULTS

Aim 1: Active Bouts Increase Theta Power Spectral Density (PSD) and Phase Locking Value (PLV)

Aim 1 analyses first considered if there was a difference in average fluid intake or average number of bouts across the phases of the experiment. Mixed-effects analyses were calculated separately with experiment phase (water pre-DID, DID, and water post-DID) used as a within subjects factor and each of these two dependent variables (average number of bouts and fluid intake) as separate between subjects factors. Results indicated no difference in average fluid intake, [F (1.98, 19.80) = 0.23, $p > 0.05$; Fig. 4A], or average number of bouts, [F (1.65, 16.51) = 2.34, $p > 0.05$; Fig. 4B] across the experiment phases. This suggests that differences in power and phase-locking value (PLV) would not be due to differences in average intake or average number of bouts. A correlation found that average number of bouts was related to average intake at every phase of the experiment [water pre-DID $R^2 = 0.64$, DID $R^2 = 0.38$, water post-DID $R^2 = 0.71$, p's < 0.05; Fig 4C].

Next, power spectral density (PSD) was calculated over the entire 2-hour session (Fig 5); and then at each epoch (pre-bout, bout, and post-bout) during each of these phases of the experiment. To consider if there were differences in theta power at each of these epochs of interest across the phases of the experiment, the theta band (5-10 Hz) only was extracted from the PSD results. Mixed-effects analyses (with epoch and experiment phase both used as within subjects' factors) were calculated separately for each brain region. Results for the PFC indicated a main effect of epoch, [F (1.65, 18.19) = 8.99, $p < 0.05$], where Tukey's post-hoc tests indicated this was driven by the bout (m = 54.17) epoch having significantly higher theta PSD than the pre-bout (m = 53.36) and post-bout (m = 53.34) epochs; Fig 6A. A main effect of epoch was also found in the NAc, [F (1.87, 20.51) = 5.45, $p < 0.05$], driven by a similar pattern of results where the bout (m = 54.46) epoch had significantly higher theta PSD than the pre-bout (m = 53.95) and post-bout (m = 53.78) epochs; Fig 6B. As there were no main or interaction effects with experiment phase, these findings suggest that active consumption (bout epoch) increases theta PSD regardless of fluid.

Contrary to the hypothesis, pre-bout theta power did not differ from post-bout theta power. Therefore, additional analyses were conducted which utilized pre-bout theta as a

'baseline,' where percent change in theta power was calculated from the pre-bout epoch to further consider how average theta power changed across drinking trials at epochs of interest. In the PFC, there was a main effect of epoch, [F (1, 31) = 13.63, $p < 0.05$], and an experiment phase x epoch interaction, [F (2, 31) = 5.08, $p < 0.05$], where Sidak's multiple comparison's tests indicated that this was driven by significantly higher bout (m = 2.56) than post-bout percent change in theta power (m = -0.66) during the water-post DID phase; Fig 7A. A similar pattern of results was observed in the NAc, with a main effect of epoch, [F (1, 31) = 10.03, $p < 0.05$], and an experiment phase x epoch interaction, [F (2, 31) = 4.53, $p < 0.05$], where Sidak's multiple comparison's tests similarly indicated that this was driven by significantly higher bout (m = 1.98) than post-bout percent change in theta power (m = -0.73) during the water-post DID phase; Fig 7B. Additional one-sample Wilcoxon tests were conducted to determine if percent change in theta PSD significantly differed from zero during the water post-DID phase. Results from these analyses indicated that the bout epoch significantly differed from zero in both brain regions, [PFC: W = 70.00, p < 0.01; Fig 7A; NAc: W=62.00, $p < 0.05$; Fig7B]. There were no differences from zero during the post-bout epoch in either brain region. These results may suggest that when mice have been receiving EtOH for two weeks and are suddenly switched back to water during testing time, power increases during the bout as the result of a negative contrast effect.

Next, theta phase-locking analyses were conducted using a PLV function (Namburi, 2020) and then average percent change in theta PLV at epochs of interest was calculated from pre-bout PLV. A mixed effects analysis (with epoch and experiment phase both as within subjects' factors) was conducted and indicated a main effect of epoch, [F (1, 58) = 6.59, $p < 0.05$], where percent change in bout theta PLV (m = 1.64) was higher than percent change in post-bout theta PLV (m = -2.987); Fig 8. As there were no main or interaction effects with experiment phase, these findings suggest that active consumption (bout epoch) increases theta PLV regardless of fluid.

Aim 2: EtOH Consumption Decreases Within Session Theta PLV

Analyses conducted for Aim 2 considered percent change in theta PSD calculated from pre-bout at each phase of the experiment at each epoch of interest during the first and last bout. Specifically, two-way ANOVAs were calculated separately for each brain region at each phase of the experiment (6 total analyses), with epoch (bout vs. pre-bout) and bout time (first vs. last

bout) both as within subjects' factors. There were no significant effects found for either brain region during the water pre-DID phase (Fig 9A, B) or DID phase (Fig 9 C, D). In the PFC during the water post-DID phase, there was a main effect of epoch, [F (1, 11) = 14.39, $p < 0.05$], and an epoch x bout time interaction [F (1, 11) = 5.312, $p < 0.05$], where Sidak's multiple comparison's post-hoc tests indicated that average percent change in theta power was greater during the bout epoch (m = 6.165) than the post-bout epoch (m = -1.582) during the last bout (Fig 9E). A similar pattern of results was found in the NAc: main effect of epoch, [F (1, 11) = 39.32, $p < 0.05$], and an epoch x bout time interaction, [F (1, 11) = 6.152, $p < 0.05$]. Sidak's multiple comparison's post-hoc tests indicated that average percent change in theta power was greater during the bout epoch (m = 5.649) than the post-bout epoch (m = -3.130) during the last bout (Fig 9F). Additional one-sample Wilcoxon tests were conducted to determine if percent change in theta PSD significantly differed from zero during the water post-DID phase during the first and last bout. Results from these analyses indicated that the bout epoch significantly differed from zero during the last bout in both brain regions, [PFC: W = 32.00, p < 0.05; Fig 9E; NAc: W=64.00, $p < 0.01$; Fig 9F]. There were no differences from zero during the first bout during the bout or post-bout epoch or the last bout during the post-bout epoch in either brain region.

Next, separate two-way ANOVAs were conducted for each phase of the experiment to consider if there were differences in average percent change in PLV between bout and post bout epochs. There were no significant differences in average percent change in PLV between bout and post-bout during water pre-DID (Fig 10A). During DID, there was a main effect of epoch, [F (1, 11) = 24.45, $p < 0.05$], main effect of bout time, [F (1, 11) = 16.93, $p < 0.05$], and an epoch x bout time interaction, [F (1, 11) = 16.66, $p < 0.05$]. Sidak's multiple comparison post-hoc tests indicate that this interaction is driven by significantly higher average percent change in PLV during the first bout (m = 12.19) than the last bout (m = 3.859) during active bouts. Additional one-sample Wilcoxon tests were conducted to determine if percent change in theta PLV significantly differed from zero during the DID phase during the first and last bout. Results from these analyses indicated that the bout epoch significantly differed from zero during the first and last bout, [first bout: W = 78.00, p < 0.001; Fig 10B; last bout: W=70.00, $p < 0.01$; Fig 10B]. There were no differences from zero during the post-bout epoch during the first or last bout. These results suggest that as a mouse becomes intoxicated over a session, PLV decreases (Fig 10B). During water post-DID, there was a main effect of epoch, [F (1, 11) = 12.49, $p < 0.05$],

and an epoch x bout time interaction, [F (1, 11) = 5.821, $p < 0.05$]. Sidak's multiple comparison post-hoc tests indicated that this interaction is similarly driven by significantly higher average percent change in PLV during the first bout (m = 10.21) than the last bout (m = 4.574) during active bouts (Fig 10C). Additional one-sample Wilcoxon tests were conducted to determine if percent change in theta PLV significantly differed from zero during the water post-DID phase during the first and last bout. Results from these analyses indicated that the bout epoch significantly differed from zero during the first and last bout, [first bout: W = 58.00, p < 0.05; Fig 10C; last bout: W=70.00, $p < 0.01$; Fig10C]. There were no differences from zero during the post-bout epoch during the first or last bout. These results suggest that a history of EtOH consumption impacts PLV during consumption at test time.

Next, analyses queried if average change in theta PLV during the bout epoch during first and last bout correlated with average bout size across the phases of the experiment. Results indicated that, during the first bout, there was a relationship between average bout size and percent change in PLV during the water post-DID phase, $R^2 = 0.73$, $p < 0.0001$ (Fig 11A). During the last bout, there was a relationship between average bout size and percent change in PLV during the DID phase, $R^2 = 0.51$, $p < 0.01$ (Fig 11B). The finding that bout length during the first, but not last, bout in the water post-DID phase correlated with theta PLV adds evidence to the theory that alterations in theta PLV during this phase of the experiment may be driven by a negative contrast effect, as this effect would be expected to be highest during the first bout.

Aim 3: Determining How Average Theta Power and Phase-Locking Change Across Drinking Trials at These Epochs Over Days

First, one-way mixed effects analyses were calculated separately for each phase of the experiment with day as a within subject's variable and intake (Fig 12A-C) and bout length (Fig 12D-F), used as between subjects dependent variables. Analyses indicated that at all phases of the experiment, across all dependent variables, there were no main effects of day indicating that intake and bout length are stable over days.

Separate mixed-effect analyses, using day and epoch as within subjects' factors, were conducted for each brain region at each phase of the experiment to determine if there were differences in percent change in theta power. During the water pre-DID phase, there were no significant main or interaction effects observed (Figs 13A, B). During the DID phase in the PFC,

there was a main effect of epoch, [F (1.000, 11.00) = 6.47, $p < 0.05$], where average percent change in theta power was higher during active bouts (m = 1.703) than post-bout (0.7081); Fig 13C. A similar pattern of results was found in the NAc: a main effect of epoch, [F (1.000, 11.00) = 6.26, $p < 0.05$], where average percent change in theta power was higher during active bouts (m = 1.339) than post-bout (0.4426); Fig 13D. No significant effects were found in the PFC in the water post-DID phase, Fig 13E. In the NAc during the water post-DID phase, there was a main effect of epoch, [F (1.000, 11.00) = 8.36, $p < 0.05$], again driven by average percent change in theta power was higher during active bouts (m = 2.202) than post-bout (-0.1920); Fig 13F. These results suggest that EtOH consumption (DID), and a history of EtOH consumption (water post-DID), increased theta PSD during bouts.

Next, separate mixed-effect analyses, using day and epoch as within subjects' factors, were conducted at each phase of the experiment to determine if there were differences in percent change in theta PLV. Results for water pre-DID indicate a main effect of epoch, [F (1, 6) = 30.24, $p < 0.05$], where percent change in theta PLV during the bout epoch (m = 2.145) was significantly higher than percent change in PLV during the post-bout epoch (m = -0.5943), Fig 14A. Similarly, results for DID indicated a main effect of epoch, [F (1, 11) = 40.90, $p < 0.05$], where percent change in theta PLV during the bout epoch (m = 3.874) was significantly higher than percent change in PLV during the post-bout epoch (m = 1.138), Fig 14B. Lastly, a similar pattern of results was found during water post-DID with a main effect of epoch, [F (1, 8) = 10.86, $p < 0.05$], where percent change in theta PLV during the bout epoch (m = 3.959) was significantly higher than percent change in PLV during the post-bout epoch (m = 0.3197), Fig 14C. As there were no interaction effects with day in any of the experiment phases, these results suggest that theta PLV, regardless of fluid consumed, is higher during active bouts as compared to the post-bout epoch. However, these results must be interpreted with caution, as they are an average of all bouts during each day and do not account for within session changes (i.e. first versus last bout and all bouts in between). Lastly, a correlation was conducted using all bouts between percent change in theta PLV during the bout epoch and bout length on each day. A significant correlation between these variables was found on day 14, [$R^2 = 0.19$, $p < 0.05$], day 15, [$R^2 = 0.07$, $p < 0.05$], and day 16, [$R^2 = 0.09$, $p < 0.05$], suggesting that bout length predicts change in theta PLV during bouts only on later DID testing sessions after an EtOH history has been established.

DISCUSSION

Results from Aim 1 considering percent change in theta PSD from pre-bout indicated that during bouts in the water post-DID phase, average percent change in theta PSD was significantly higher than zero and that of the post-bout epoch in both brain regions (Fig 7A, B). Similar results were found in Aim 2, where analyses considering average percent change in theta PSD from the pre-bout epoch during the first and last bout indicated that during the last bout in the water post-DID phase, average percent change in theta PSD was significantly higher than zero and that of the post-bout epoch of the last bout in both brain regions (Fig 9E, F). These results may suggest that when mice have had access to EtOH for two weeks and are suddenly switched back to water during testing time, power increases during the bout as the result of a negative contrast effect. Additionally, the finding that bout length during the first, but not last, bout in the water post-DID phase correlated with theta PLV adds evidence to the idea that alterations in theta PLV during this phase of the experiment may be driven by a negative contrast effect, as this effect would be expected to be highest during the first bout (Fig 11A). Previous research has found that theta power and synchrony within the mPFC is responsible for the encoding of value of rewarding substances during licks/bouts when rodents are given access to cycling high versus low sucrose concentrations (Amarante et al., 2017; Amarante and Laubach, 2020). Similar findings have been reported during water consumption in water-restricted rats (Horst and Laubach, 2013). Therefore, it is possible that these results found in Aims 1 and 2 are the due to reward loss / negative contrast.

Additional findings for Aim 2 revealed that during DID, average percent change in theta PLV was higher during the first as compared to the last bout (Fig 10B). This effect was unique to the bout epoch (i.e. no differences in first and last bout during the post-bout epoch) and was not found in the water pre-bout phase (Fig 10A). These results suggest that within-session alcohol intoxication acutely decreases theta PLV during EtOH consumption. Interestingly, additional analyses indicated that during the last bout of the session, average bout length correlated with average percent change in theta PLV during the last bout only during the DID phase of the experiment (Fig 11B), a relationship that was not observed between these variables during this phase of the experiment during the first bout (Fig 11A). These results suggest that alcohol intoxication uniquely moderated the relationship between alcohol consumption and

corticostriatal synchrony. Specifically, during the last bout, longer bouts decreased average theta PLV more than shorter bouts indicating that additional alcohol consumption when animals are presumably already encountering the pharmacological effects of alcohol has an impact on corticostriatal synchrony which does not appear as robust when animals are not yet intoxicated (Fig 11A). It should be noted that retro-orbital blood samples were drawn from all mice on Day 17. However, due to the design of the headcap, it was difficult to obtain a reliable sample (i.e. not enough blood to run the Analox EtOH Analyzer and/or unable to make the blood draw quickly enough). Noting this limitation within the current study, previous research has conducted DID with B6 mice using similar procedures (testing over 15 consecutive days) and results indicated similar EtOH intakes with blood ethanol concentrations above 80 g/dL on the last day of testing (Linsenbardt and Boehm, 2014). Thus, based upon the EtOH intakes acquired in the current study, it can be assumed that mice are similarly experiencing the pharmacological effects of EtOH.

Though additional follow-up experiments will be necessary, the finding that PLV is lower during the last versus first bout may represent a potential mechanism by which binge drinking manifests. Specifically, the hypothesis here is that alcohol drinking leads to more drinking modulated by decreases in theta synchrony over a binge. Previous research utilizing magnetoencephalography (MEG) indicates that during a conflict task, alcohol consumption immediately prior to task completion decreases reaction time and theta power within the fronto-parietal network suggesting that theta oscillations may regulate self-control (Kovacevic et al., 2012; Marinkovic et al., 2019). Further, a recent study examining neural activity of binge-drinkers during an attentional performance task indicated that a history of binge drinking is associated with decreased theta power and synchrony within the right inferior frontal cortex, which correlated with reported previous alcohol intake (Correas et al., 2019). Together, these results suggest that both acute alcohol and a history of binge drinking impair cognitive processes which are governed by the theta band within the prefrontal cortex.

Results for Aim 3 indicated that, during DID in both brain regions, average percent change in theta PSD and PLV were higher during the bout epoch as compared to the post-bout epoch (Figs 13C, D; Fig 14B). Further, results indicated that bout length on later DID testing days (days 14, 15, and 16) correlated with percent change in theta PLV during the bout (Fig 15). As there was no main or interaction effect with day, these findings suggest that actively drinking

alcohol may decrease theta PSD and PLV, regardless of day. However, these results should be interpreted with caution. The PSD and PLV analyses presented in Aim 3 consider each animal's average across all bouts on a given day. Thus, the details of how total number of bouts (Fig 12E) and bout length (Fig 12F) are not considered within these specific analyses. Further, there is considerable variability between animals in both average percent change in theta PSD and PLV (Figs 13C, D; Fig 14B) and EtOH intake (Fig 12B). Previous research where male rats were given access to 10% EtOH in an intermittent access paradigm and then LFPs were subsequently recorded from the mPFC and NAc to consider the impact of alcohol consumption on corticostriatal neural oscillations indicated that low and high drinkers could be predicted from oscillations. Specifically, 4/5 features within the prediction model implicated gamma power and synchrony as necessary to distinguish high versus low drinkers (Henricks, 2019). A similar study from this group utilizing female and male rats indicated that a model with corticostriatal neural oscillations as inputs could successfully predict alcohol consumption of male but not female rats (Henricks et al., 2019). To this point, future analyses should consider both how individual alcohol experiences (intake, number of bouts, bout length) impact theta PSD and PLV over days and how these measures change dynamically across different frequency bands. Additionally, analyses should consider if and how sex impacts these measurements, as current analyses did not break subjects by sex.

In summary, the results also indicated that changes in theta PSD and PLV during the water post-DID phase may be indicative of a negative contrast effect induced from a loss of EtOH reward (Fig 7A, B; Fig 9E, F; Fig 11A). To explicitly test this hypothesis, future analyses should compare percent change in theta PSD during the bout epoch on day 18 (the last day of DID; EtOH access) to day 19 (the first day of water-post DID). Current analyses for both Aim 1 and Aim 2 do not offer an explicit comparison of these two days of interest (where a negative contrast effect would be most apparent and expected) as they represent an average over all days. In summary, the results herein provide evidence for changes in theta PLV during EtOH consumption which are presumably modulated by intoxication (Aim 2; Fig 10B, Fig 11B). This may be an important factor when considering why some individuals repeatedly engage in binge drinking or drink more than intended in a given session.

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FIGURES

Figure 1. Timeline of Experiments

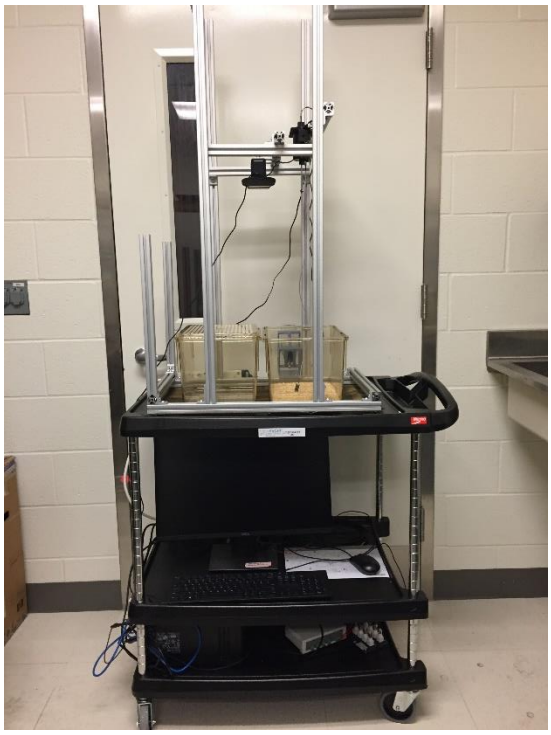

Figure 2. Photo of Electrophysiology Cart

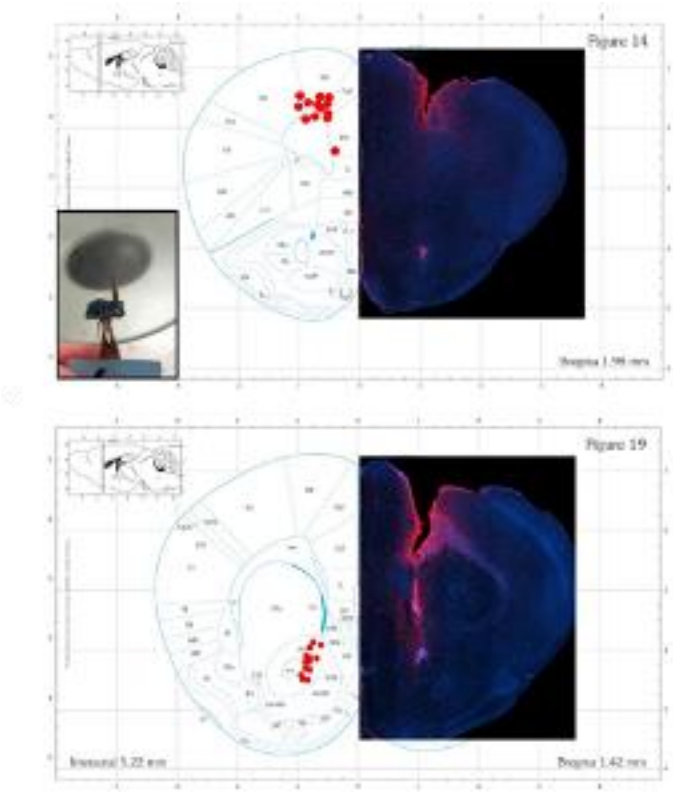

Figure 3. Electrode Placements

Placements are shown for the mPFC (top) and NAc (bottom). Both images feature placements for all animals displayed as a red dot on the left side of the image with an example representative image which was used to determine placements shown on the right. Additionally, the mPFC image features an image of the electrodes which were used in the bottom left-hand corner. The two mice which had placements within the corpus callosum (mPFC) were removed from all analyses.

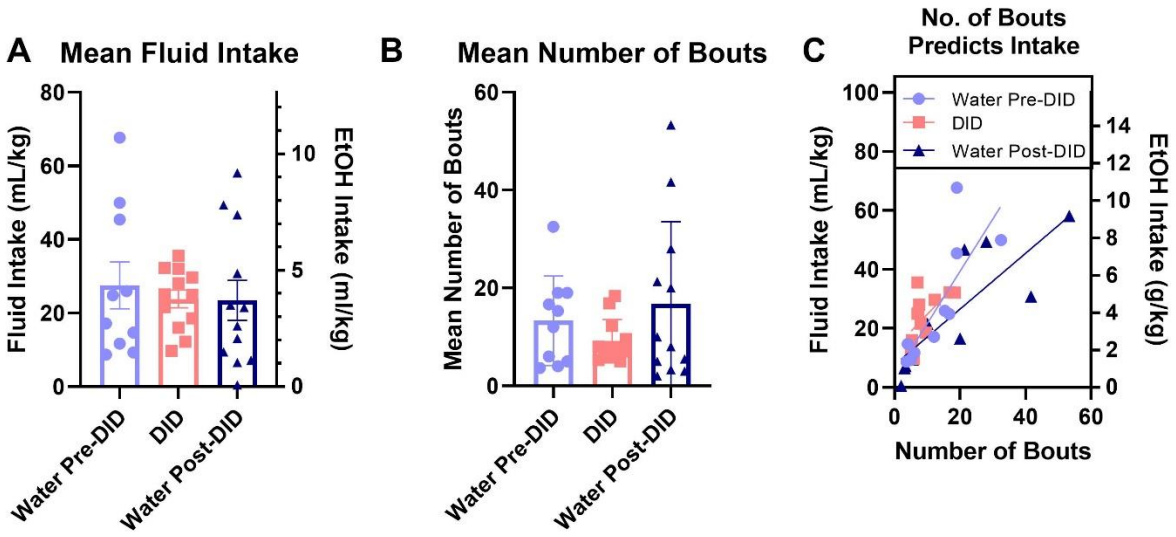

Figure 4. Intake

There were no significant differences in mean fluid intake (A) or mean number of bouts (B) across the different phases of the experiment. At each phase of the experiment, number of bouts was predictive of fluid intake (C).

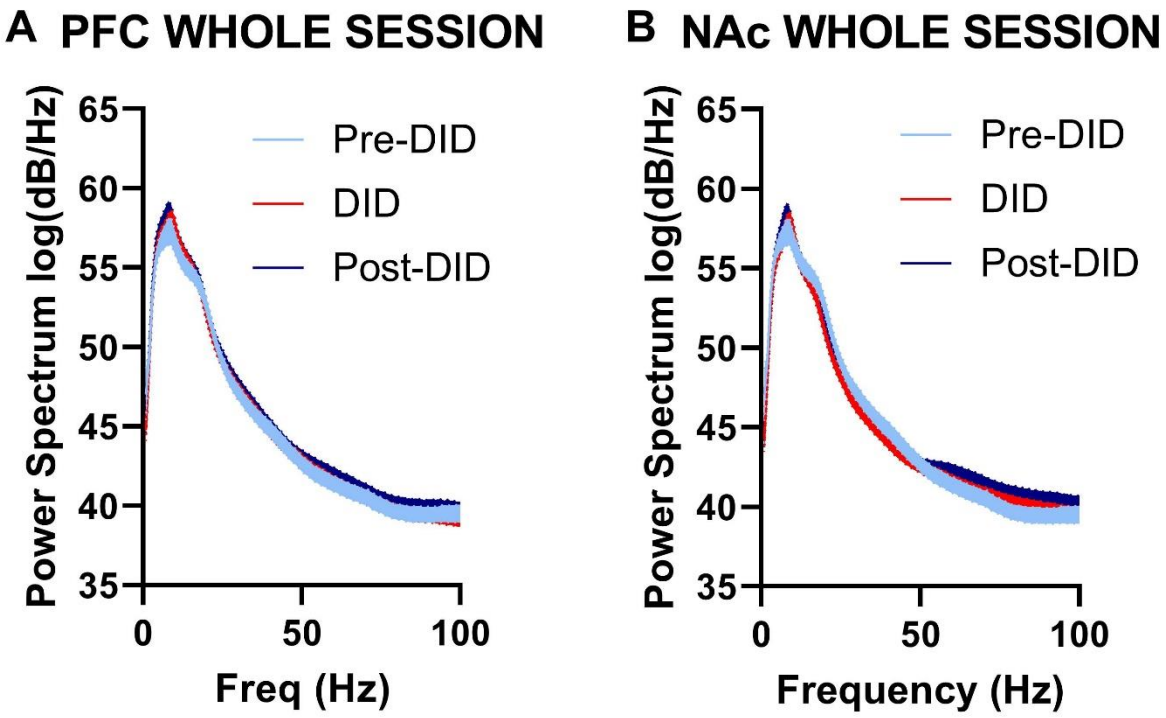

Figure 5. Power Spectral Density Overview

Power spectral density (PSD) graphs are displayed for the entire 2-hour testing session in the PFC (A) and the NAc (B).

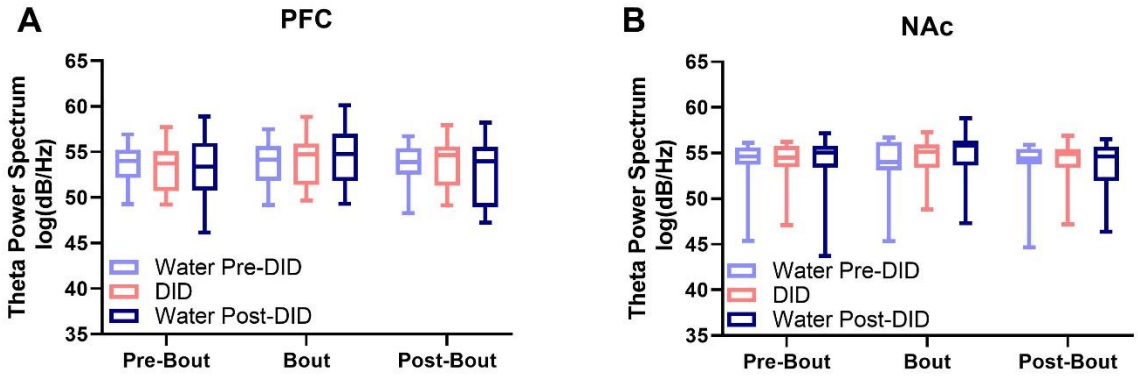

Figure 6. Consumption (Bouts) Increased Theta Power

In both brain regions, theta power (5-10 Hz) was extracted from the PSD analyses and plotted at epochs of interest (pre-bout, bout, and post-bout) at each phase of the experiment (water pre-DID, DID, and water post-DID). In both brain regions, analyses indicated a main effect of epoch driven by theta power during active bouts being higher than pre and post-bout theta power.

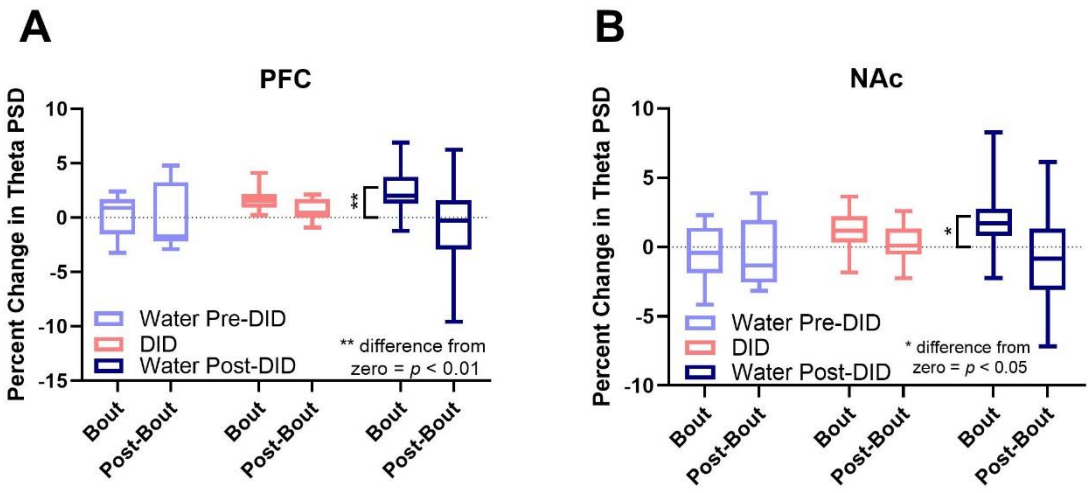

Figure 7. Theta Power is Higher During Water Bouts Following an EtOH History

During water post-DID, in both brain regions, there is significantly higher percent change in average theta PSD during the bout epoch as compared to post-bout. This may suggest a negative contrast effect.

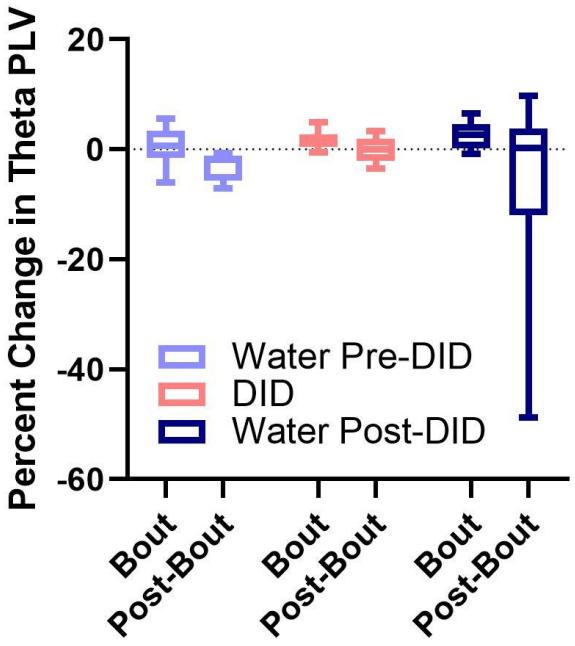

Figure 8. Consumption (Bouts) Increased Theta PLV

Analyses indicate a main effect of epoch, where bouts have higher change in theta PLV than post-bout.

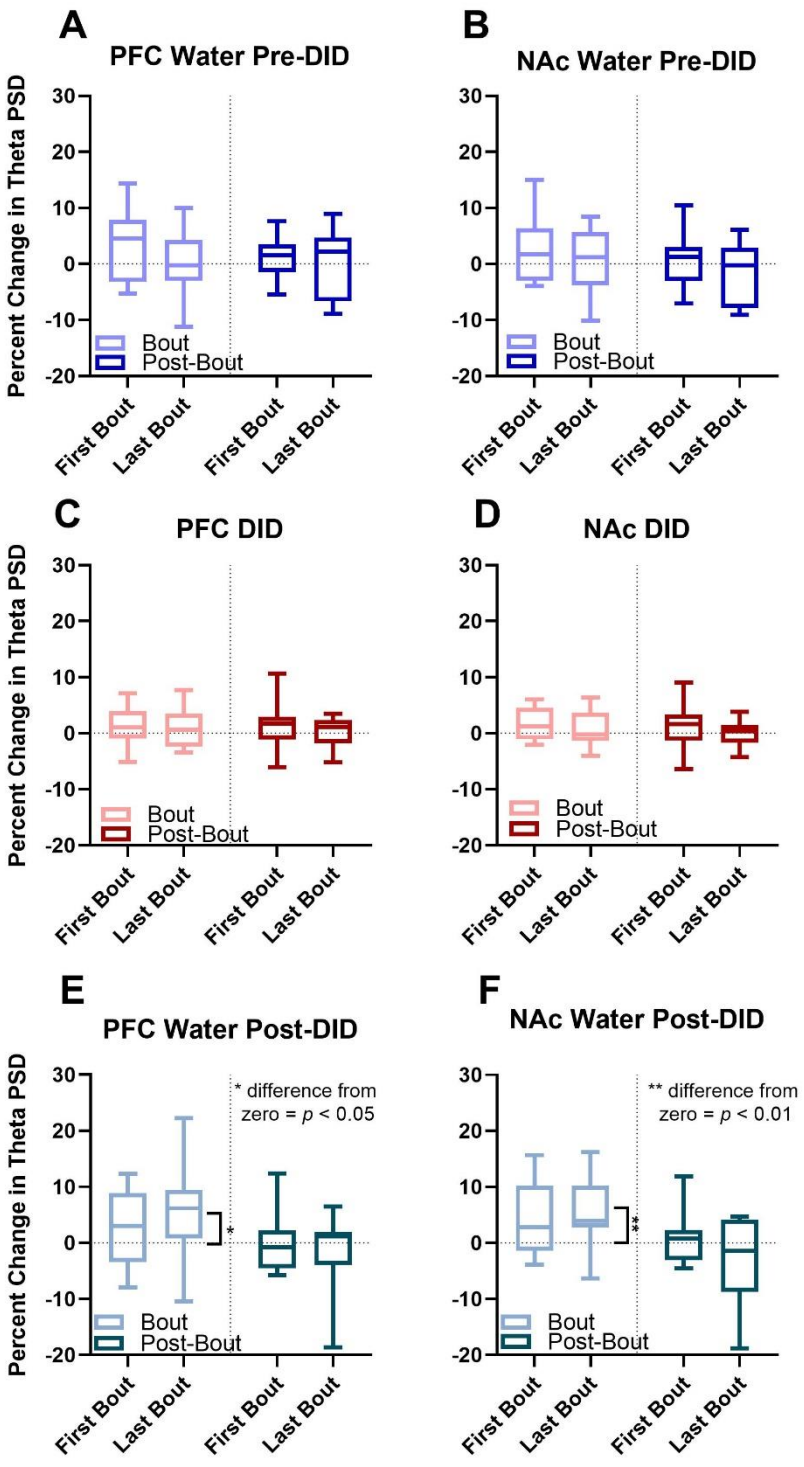

Figure 9. Theta Power is Higher During the Last Bout of Water Following an EtOH History

An interaction effect of bout time by epoch in both brain regions during the water post-DID phase indicates that on the last bout, percent change in theta PSD is higher during the bout than activity during post-bout epoch and zero change. This may suggest a negative contrast effect.

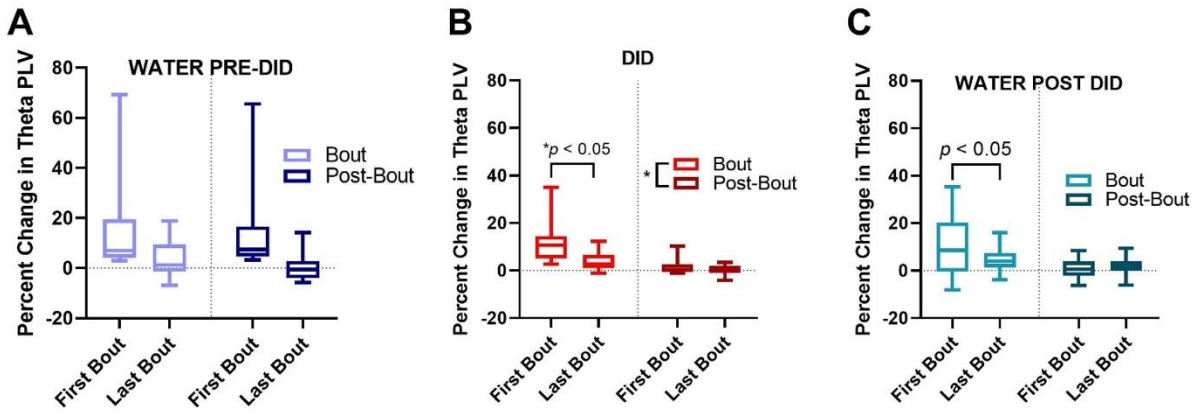

Figure 10. EtOH Consumption Decreases Within Session Theta PLV

During DID, percent change in PLV was higher during active bouts during the first bout than the last bout (B), suggesting that intoxication over the DID session decreases theta PLV. A similar pattern of results was found during the water post-DID phase of the experiment (C).

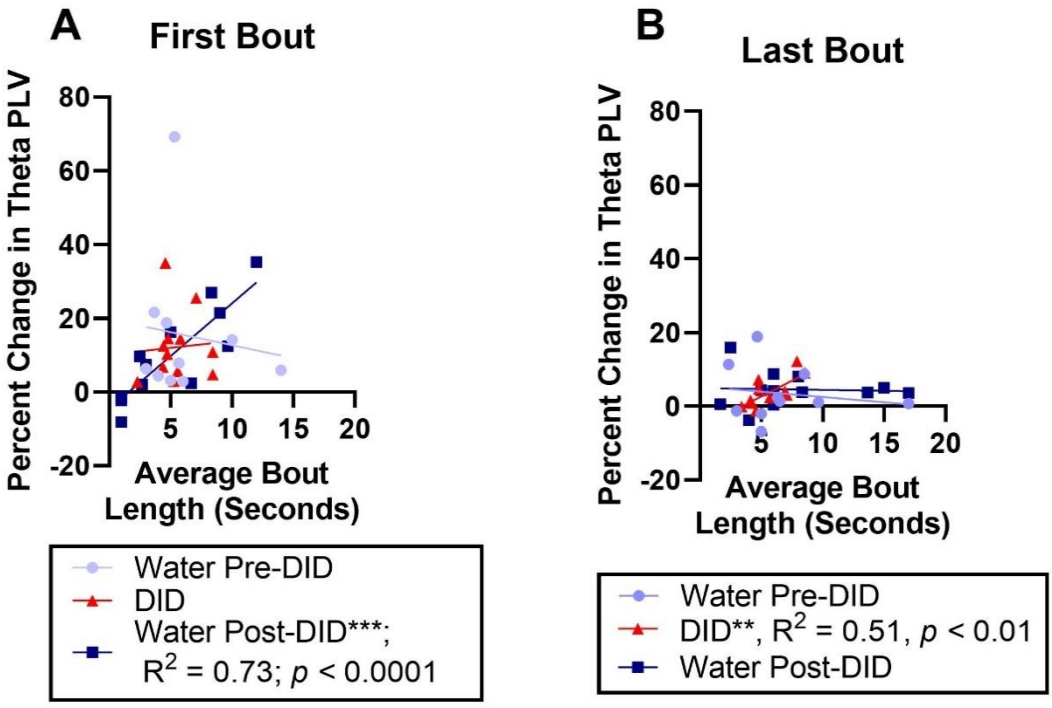

Figure 11. Intoxication Moderates the Relationship Between Bout Length and Theta PLV

There is a relationship between average bout size and percent change in PLV during the first bout of the water post-DID phase (A), and during the last bout of the DID phase (B). The finding that bout length during the first, but not last, bout in the water post-DID phase correlated with theta PLV adds evidence to the theory that alterations in theta PLV during this phase of the experiment may be driven by a negative contrast effect, as this effect would be expected to be highest during the first bout. Findings in Figure B suggest that alcohol intoxication uniquely moderated the relationship between alcohol consumption and corticostriatal synchrony.

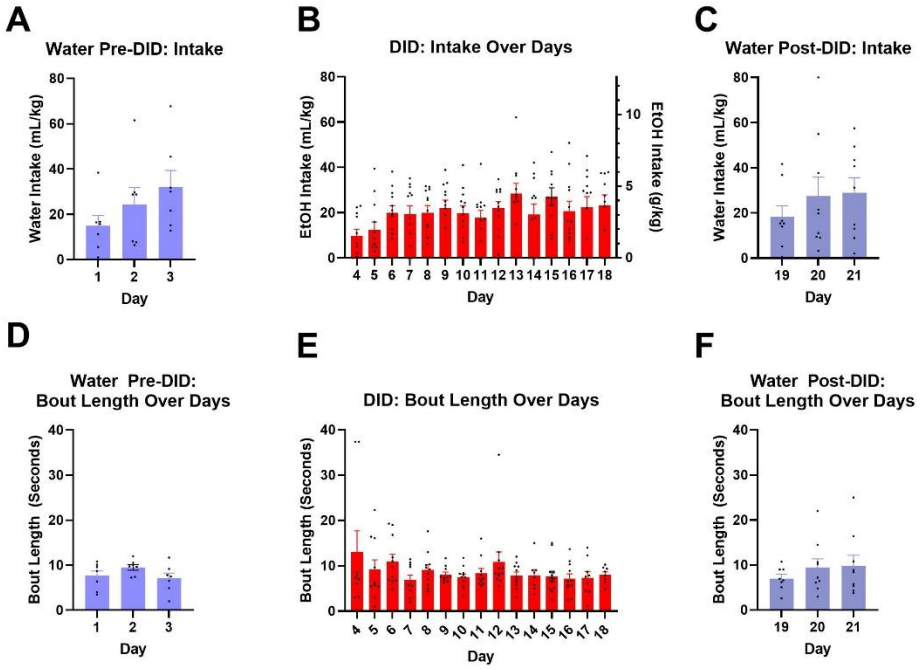

Figure 12. Intake Over Days

To better understand animal's drinking experience over days, intake and bout length are displayed at each phase of the experiment across each day.

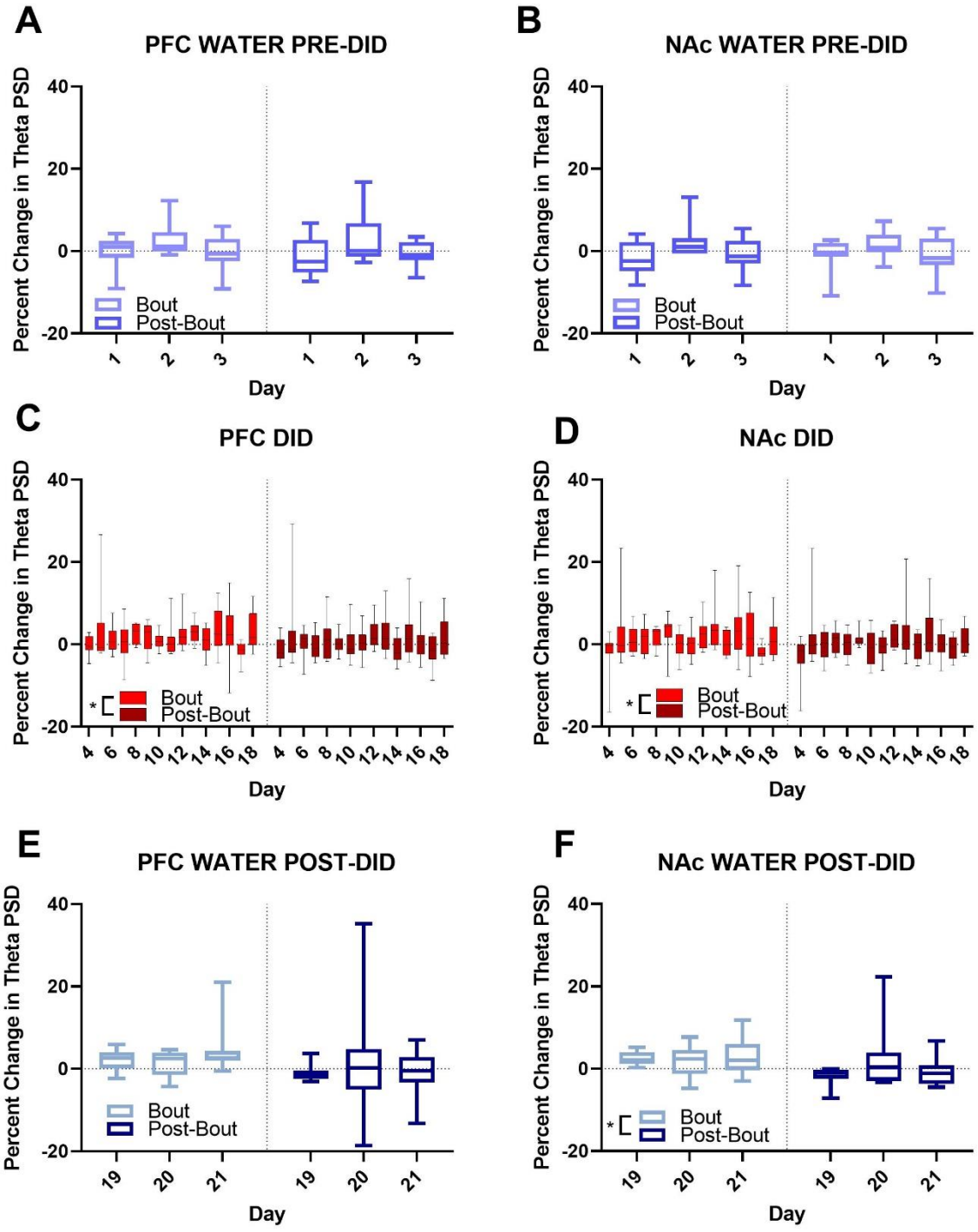

Figure 13.EtOH Consumption Increases Theta Power During Bouts

A main effect of bout during DID and during water-post DID suggest that EtOH consumption (DID; B, C), and a history of EtOH consumption (water post-DID, F), increased percent change in theta PSD during bouts.

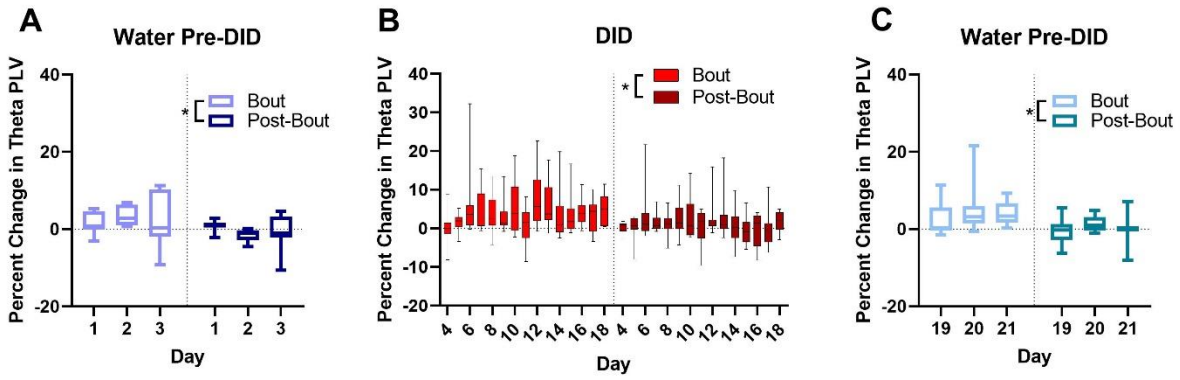

Figure 14. Consumption (Bouts) Increased Theta PLV During Later DID Sessions

A main effect of bout at all phases of the experiment indicates greater percent change in theta PLV during the post than post bout epoch at all phases of the experiment. As there were no interaction effects with day in any of the experiment phases, these results suggest that theta PLV, regardless of fluid consumed, is higher during active bouts as compared to the post-bout epoch. However, these results must be interpreted with caution, as they are an average of all bouts during each day and do not account for within session changes (i.e. first versus last bout and all bouts in between).

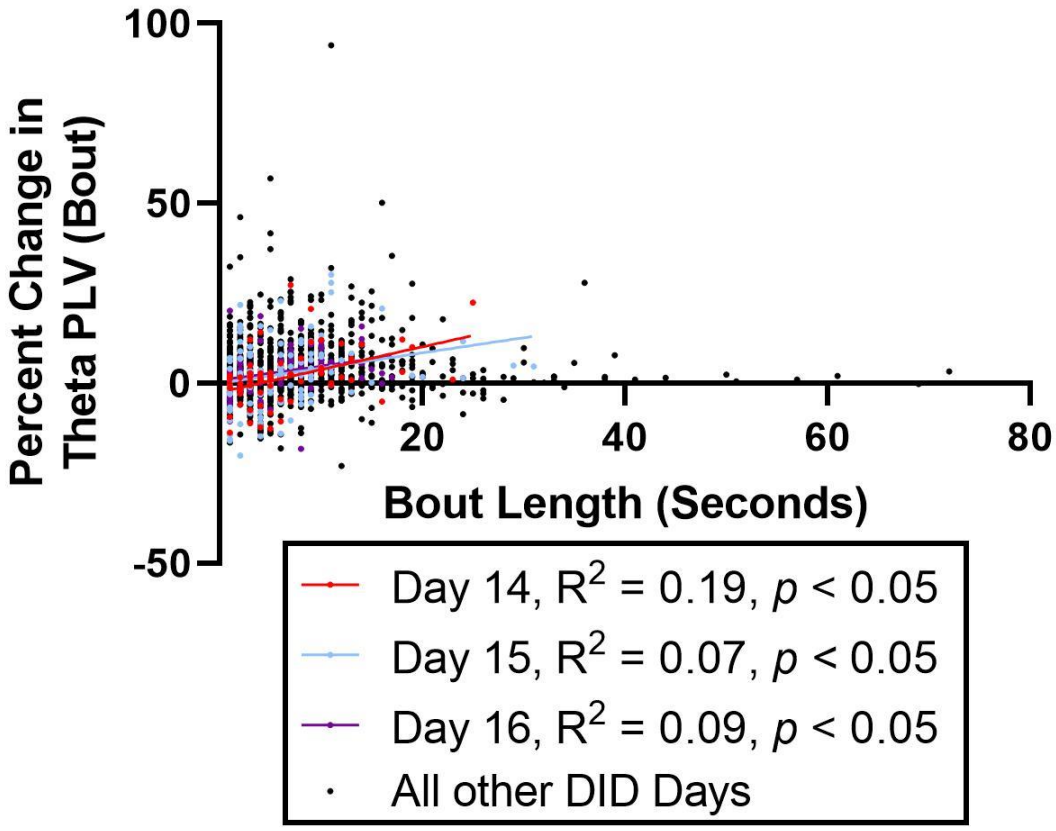

Figure 15. Bout Length is Only Related to Theta PLV During Later DID Sessions

A correlation between bout length and percent change in theta PLV during bouts was found on days 14, 15, and 16, suggesting that bout length predicts change in theta PLV during bouts only on later DID testing sessions after an EtOH history has been established.