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Ethanol Intake Patterns in Female Mice: Influence of Allopregnanolone and the Inhibition of Its Synthesis

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Abstract

The neurosteroid allopregnanolone (ALLO) is a positive modulator of GABA_A receptors that exhibits a psychopharmacological profile similar to ethanol (i.e., anxiolytic, sedative-hypnotic). Based on research suggesting that manipulation of ALLO levels altered ethanol self-administration in male rodents, the current studies determined whether exogenous ALLO administration or the inhibition of its synthesis *in vivo* modulated ethanol intake patterns in female C57BL/6J mice. Lickometer circuits collected temporal lick records of ethanol (10% v/v) and water consumption during daily 2-hr limited access sessions. Following the establishment of stable ethanol intake, studies examined the effect of an acute ALLO challenge (3.2 – 24.0 mg/kg) or a 7-day blockade of ALLO production with finasteride (FIN; 50 or 100 mg/kg) on ethanol intake in a within-subjects design. In contrast to results in male mice, ethanol dose (g/kg), ethanol preference, and most of the bout parameters were unaltered by ALLO pretreatment in female mice. Ethanol intake in females also was recalcitrant to 7-day treatment with 50 mg/kg FIN, whereas 100 mg/kg FIN significantly reduced the ethanol dose consumed by 35%. The FIN-attenuated ethanol intake was attributable to a significant decrease in bout frequency (up to 45%), with lick patterns indicating reduced maintenance of consumption throughout the 2-hr session. FIN also produced a dose-dependent decrease in brain ALLO levels. In conjunction with data in male mice, the present findings indicate that there are sex differences in the physiological regulation of ethanol intake patterns by GABAergic neurosteroids.

Keywords

alcohol; self-administration; lickometer; neurosteroid; GABA; finasteride

1. Introduction

The neurosteroid allopregnanolone (ALLO, 3 α -hydroxy-5 α -pregnan-20-one) positively modulates type-A γ -aminobutyric acid (GABA_A) receptors, the major inhibitory neurotransmitter receptor in the nervous system. Like ethanol (Grobin et al., 1998), increasing

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concentrations of ALLO induce sedation in laboratory rodents (e.g., Gasior et al., 1999) and humans (Söderpalm et al., 2004; Timby et al., 2006); although in some cases the sedative effect may differ between men and women (van Broekhoven et al., 2007). Neurosteroids have received increased attention in recent years, due to the demonstration that ALLO can modulate sexual behavior (Frye and Rhodes, 2005b), learning and memory (Johansson et al., 2002), maternal behavior (Mann, 2006), psychosis-like behaviors (Ugale et al., 2004), anxiety-like behaviors (Finn et al., 2003, 2006a; Gulinello and Smith, 2003), seizure protection (Frye and Rhodes, 2005a; Finn et al., 2006b) and feeding behaviors (Chen et al., 1996; Reddy and Kulkarni, 1999, but see Fudge et al., 2006).

Given that the GABA_A receptor complex is a shared site for ALLO and some of ethanol's neurobiological effects, it is not surprising that ALLO is implicated in diverse lines of ethanol research. For example, low dose ethanol (i.e., 1.5 g/kg) potentiated the effect of ALLO on motor impairment (Vanover et al., 1999), whereas ethanol-induced loss of righting reflex was potentiated by ALLO (Członkowska et al., 2000). Both ethanol (2 g/kg) and ALLO (10 – 17 mg/kg) produce locomotor stimulation in mice (Palmer et al., 2002), and a quantitative trait locus has been identified on mouse chromosome 2 that contributes to the locomotor-stimulant effects of both ethanol and ALLO (Palmer et al., 2006). Further, recent evidence has demonstrated that ALLO's interaction with the GABA_A receptor may be necessary for some of ethanol's pharmacological effects. ALLO facilitates inhibitory transmission by increasing the decay time of miniature inhibitory post-synaptic currents of neuronal GABA_A receptors, with brain regional differences in sensitivity (e.g., Belelli and Herd, 2003). Additional electrophysiological studies demonstrated that ALLO was necessary for ethanol's enhancement of GABAergic neurotransmission in substantia nigra neurons (Criswell et al., 1999), and that the combination of ALLO and ethanol could enhance GABAergic neurotransmission at concentrations that were ineffective when administered separately (Akk et al., 2007). Thus, data suggest that the interaction between ALLO and ethanol extends beyond the possibility that individual genes exert pleiotropic control over the effects of these substances, but rather that endogenous ALLO levels may exert a permissive action for some of the behavioral (and electrophysiological) effects of ethanol.

An additional consideration is that acute ethanol administration can increase cortical ALLO levels to pharmacologically active concentrations (e.g., Barbaccia et al., 1999; VanDoren et al., 2000), but there are sex and species differences in this effect. For instance, ethanol increases endogenous ALLO levels in male but not female C57BL/6J mice (Finn et al., 2004c), male and female rats (Morrow et al., 1999), and in male and female adolescents (Torres and Ortega, 2003, 2004). However, ethanol decreased ALLO levels in adult men and caused no change in adult women (Pierucci-Lagha et al., 2006). In terms of the physiological relevance, ethanol has both a direct (e.g., Mihic et al., 1997) and an indirect effect on GABA_A receptor function, with the indirect effect involving the biosynthesis of GABAergic neurosteroids (Sanna et al., 2004).

Given these findings, one line of research has sought to determine whether manipulation of ALLO levels altered voluntary ethanol consumption. ALLO and other GABAergic neurosteroids are behaviorally reinforcing, at least under some conditions (Finn et al., 1997; Rowlett et al., 1999; Sinnott et al., 2002a), as is ethanol (e.g., Nizhnikov et al., 2006; Corbit and Janak, 2007; Cunningham and Patel, 2007). And, several results are consistent with the hypothesis that increases or decreases in ALLO levels could produce bi-directional effects on ethanol self-administration. For example, acute administration of ALLO increased operant responding for ethanol in male rats (Janak et al., 1998) and limited access ethanol preference drinking in male but not female C57BL/6 mice (Sinnott et al., 2002b). In contrast, administration of the GABA_A receptor partial agonist/antagonist epipregnanolone (O'Dell et al., 2005) and depletion of 5 α -reduced neurosteroids such as ALLO with finasteride (FIN;

Ford et al., 2005a) decreased ethanol administration in rodents. Taken in conjunction with the finding that the GABA_A receptor agonist muscimol decreased operant responding for ethanol (Janak and Gill, 2003), it is likely that the distinction between the effects of ALLO versus muscimol on ethanol intake reflects differences in activation of GABA_A receptors by a direct agonist (e.g., muscimol) versus a positive allosteric modulator (e.g., ALLO). However, it also should be noted that recent work testing an expanded dose range of ALLO with lickometer circuits demonstrated a bimodal effect of ALLO on operant ethanol self administration (Ford et al., 2007b) and limited access ethanol drinking (Ford et al., 2005b), with low doses increasing ethanol intake [e.g., 50 ng intracerebroventricular (ICV) or 3.2 mg/kg intraperitoneal (IP)] and high doses decreasing ethanol intake (400 ng ICV or 17 and 24 mg/kg IP). Thus, it also is possible that the effects of muscimol on ethanol intake are comparable to the high dose effects of ALLO.

Endogenous ALLO levels are higher in females than in males, with levels in female rodents fluctuating from 10 – 30 nM during the estrous cycle and increasing to 100 nM during pregnancy (Paul and Purdy, 1992; Finn and Gee, 1994; Concas et al., 1998). With regard to the effects of ethanol and ALLO in female C57BL/6 mice, female mice were resistant to the ability of ethanol injection or consumption to increase brain ALLO levels (Finn et al., 2004c) and to the ability of a narrow dose range of ALLO to modulate limited access ethanol intake (Sinnott et al., 2002b). Based on these findings, we reasoned that higher basal ALLO levels in female mice, in conjunction with the plasticity of GABA_A receptors during the estrous cycle, might render female C57BL/6 mice less sensitive to the modulatory effects of ALLO on ethanol intake (i.e., the dose response curve in females would be shifted to the right of that in males). Thus, the purpose of the present experiments was to further examine the effects of ALLO on ethanol drinking behavior in female C57BL/6 mice by administering an expanded ALLO dose range to increase neural concentrations, and by administering the 5 α -reductase inhibitor FIN (which inhibits production of the ALLO precursor, 5 α -dihydroprogesterone) to decrease its neural concentrations (see Finn et al., 2006a for review). Since we were interested in the effects of these manipulations on the pattern of ethanol intake, an additional goal was to study the microstructure of ethanol bouts with the use of lickometers, an experimental method that allows for the analysis of self-administration behavior at a fine or microstructure level by recording individual licks (e.g., Boughter et al., 2007).

2. Methods

2.1. Animals

Twenty-four female C57BL/6J mice between 8–13 weeks of age were obtained from a colony at the Veterinary Medical Unit of the Portland Veterans Administration Medical Center. This colony is re-established every three generations with mice purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were individually housed in lickometer chambers (see below) and acclimated to a reverse light/dark schedule (12hr/12hr; lights off at 0900 hrs) for 2–3 weeks. All mice were provided *ad libitum* access to rodent chow and tap water. Mice were weighed and handled daily throughout the acclimation and experimental phases of the study. The local Institutional Animal Care and Use Committee approved all procedures in accordance with the guidelines described in the *Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research* (National Research Council of the National Academies, 2003).

2.2. Drugs

An ethanol solution (10% v/v; Pharmco Products, Brookfield, CT) was prepared by dilution of a 200 proof stock in tap water. Finasteride [1,(5 α)-androstan-4-aza-3-one-17 β -(N-tert-butyl-carboxamide); FIN] was purchased from Steraloids Inc. (Newport, RI), and allopregnanolone (3 α -hydroxy-5 α -pregnan-20-one; ALLO) was synthesized by and purchased from Dr. R. H.

Purdy (Veterans Medical Research Foundation, San Diego, CA). Both drugs were solubilized into a 20% w/v 2-hydroxypropyl- β -cyclodextrin (β -cyclodextrin; Cargill, Inc., Cedar Rapids, IA) solution made in saline. All injections were administered IP at a volume of 0.01-ml/g body weight.

2.3. Two-bottle preference procedure in lickometer chambers

Custom lickometer chambers were designed as previously described (Ford et al., 2005a, 2005b; Rhodes et al., 2007). Briefly, the chamber consisted of a four-walled Plexiglas insert with a hinged top, elevated stainless steel wire floor, and two access ports for the insertion of drinking tubes. The raised floor and perforated top permitted sufficient ventilation. Drinking tubes were constructed from stainless steel sippers (Ancare, Bellmore, NY) and polystyrene serological pipettes (VWR, Tualatin, OR), which allowed for volume measurements to the nearest 0.05 ml. The wire floor of the chamber and each sipper formed an open electrical circuit that was connected to a lickometer device (MED Associates, Inc., St. Albans, VT); this circuit permitted the collection of individual cumulative records for the contacts (licks) recorded from each sipper. Lickometers were interfaced to an IBM compatible computer running MED-PC IV software (MED Associates, Inc.) to facilitate data collection.

The 2-hr limited access time (1100 – 1300 hrs; starting 2 hr after lights out) was selected based on previous characterization of a “drinking in the dark” model, whereby limited access ethanol intake was greatest when access began at 2 – 3 hrs into the dark phase (Rhodes et al., 2005, 2007). Prior to each drinking session, mice were removed from their lickometer chambers (homecages), weighed, and placed immediately back into their respective chambers. During the 2-hr preference drinking sessions, mice were given the choice between one sipper tube containing a 10% v/v ethanol solution (10E) and a second sipper tube filled with tap water. The 10E sippers were counterbalanced between the left and right sides across lickometer chambers to minimize the influence of side preference. Upon the establishment of stable 10E intakes, as determined by < 10% variability in ethanol dose over 3 consecutive sessions, the mean $\pm$ SEM of the 10E dose consumed was 3.11 ± 0.10 g/kg. Drinking sessions were conducted 7 days/week.

2.4. Treatment time course for FIN and ALLO administration

Throughout a 3-month period, a single cohort of 24 mice was administered multiple treatment regimens in the following order: sub-chronic (7-day) treatment with 50 mg/kg FIN, acute challenge with multiple ALLO (3.2 – 24 mg/kg) doses, and sub-chronic (7-day) treatment with 100 mg/kg FIN. Prior to testing with FIN, mice were habituated to vehicle injections (VEH; 20% w/v β -cyclodextrin) that were delivered 22-hr prior to the limited access session over a 3-day period. Since ethanol intake remained stable (< 10% variability) across the 3 days of VEH injections, we reasoned that the mice had adapted to the 22-hr pretreatment. The FIN pre-treatment time was selected based on a pilot study conducted in our laboratory which demonstrated that a 50 mg/kg dose decreased plasma and brain ALLO levels in male mice by 66% and 80%, respectively, at a 24-hr post-injection time point (Finn et al., 2004a). Since habituation to VEH injection had no observable effect on stable consumption, these three sessions were consequently collapsed and designated as “baseline.” Mice were then assigned to one of two treatment groups that were balanced for 10E dose (g/kg) consumed. One group received daily injections of FIN (50 mg/kg; $n = 14$) whereas the other group was administered VEH injections ($n = 10$) for 7 days. Following sub-chronic treatment with 50 mg/kg FIN, both groups then received an additional 7 days of VEH injections to assess FIN withdrawal. When the second round of FIN testing (100 mg/kg) was undertaken, group assignments ($n = 13$ FIN-treated; $n = 10$ VEH-treated) for each mouse were the same as that used for the 50 mg/kg dose testing (i.e., mice that received 50 mg/kg FIN also received 100 mg/kg). Notably, the treatment groups remained closely balanced for 10E dose consumption during baseline sessions (see

Results). One mouse was removed from the 100-mg/kg FIN treatment group due to weight loss and lack of 10E consumption during baseline sessions.

For the examination of acute ALLO challenges, mice were habituated to VEH injection administered immediately prior to the 2-hr access periods until stable consumption patterns were observed (i.e., 8 sessions; < 10% variability over 3 consecutive sessions). The longer time frame for habituation injections likely was due to the different pretreatment time (i.e., 30 sec versus 22 hr in FIN studies). Mice then received each ALLO dose (3.2, 10, 14, 17, 20, and 24 mg/kg) once in a within-subject design over a period of 3 weeks. ALLO doses were examined in the following order: 3.2, 10, 17, 24, 20 and 14 mg/kg. VEH injection sessions were conducted between ALLO tests until stable 10E intakes were re-established (typically 2–3 sessions). A collapsed baseline (0 mg/kg ALLO) for all drinking measures was calculated from the average of VEH injections sessions that preceded each ALLO test session. Selection of ALLO doses and pretreatment time (immediately prior to session) was based upon previously examined effects of this neurosteroid on the ethanol consumption patterns in male mice (Ford et al., 2005b).

2.5. Brain ALLO determination following acute FIN challenge

For 3-weeks following the final ethanol drinking session, mice were given a washout period during which no ethanol access was provided. To avoid the potential confound of previous treatment history with FIN, only the vehicle treated animals ($n = 10$) were examined. Mice received an acute injection VEH ($n = 4$), 50 mg/kg FIN ($n = 3$) or 100 mg/kg FIN ($n = 3$) at 22 hrs prior to euthanasia (1000 – 1100 hrs; to match the time frame for the examination of drug effect on ethanol intake). Whole brains were rapidly removed, immediately frozen on dry ice, and stored at -80°C . ALLO was extracted from brain tissue and subsequently measured by radioimmunoassay (RIA), as previously described in detail (Finn and Gee, 1994; Finn et al., 2004c). The RIA used [^3H]ALLO (New England Nuclear; Boston, MA) and a polyclonal antiserum (CoCensys; Irvine, CA), which had minimal cross-reactivity to other steroids (Finn and Gee, 1994). Counts per minute were normalized and fit to a least-square regression equation produced by log-logit transformation of the standards (0.156 – 20 ng). Mass of the samples was calculated by interpolation of the standards and correction for recovery.

2.6. Statistical analyses

The ethanol dose (g/kg) was determined from the 10E volume (ml) consumed and pre-session body weight. Ethanol preference ratios were calculated as the 10E volume consumed divided by total fluid volume (10E plus water). Cumulative lick records on 10E and water sipper tubes were generated by MED-PC IV software. A custom bout analysis program (written for the R Project for Statistical Computing software; version 2.4.0; available at www.r-project.org) was subsequently employed to compile the following consumption endpoints: bout frequency, bout size (licks), bout duration (min), inter-bout interval (IBI; min), bout lick rate (licks/min), and latency to first bout (min). Based upon our previous work in a mouse self-administration model (Ford et al., 2005a, 2005b, 2007a, 2007b), an ethanol bout was experimentally defined as a minimum of 20 licks with no more than a 60-sec pause between successive licks. The reported bout lick rates were derived from the average rate of all bouts expressed and did not reflect time intervals between bouts (IBIs). Throughout the various treatment phases, mice exhibited an average ethanol preference ratio of 0.96 ± 0.01 , with session water lick totals averaging 73 ± 12 . Because the volume of water consumption was typically at or below the level of detection (≤ 0.05 ml), meaningful analyses of bout dynamics and consumption patterns for water were not possible.

All statistical analyses were performed with the SigmaStat version 2.03 software package (SPSS Inc., Chicago, IL). Figures were created with either Sigma Plot 2001 (SPSS Inc.) or

Prism 4 (GraphPad Software, Inc., San Diego, CA). SoftCR for Windows (MED Associates, Inc.) was used to illustrate the temporal distribution of sipper licks across the 2-hr drinking sessions. The influence of FIN on drinking patterns was parsed into treatment phases, based on comparable data in male mice (Ford et al., 2005a), and subsequently analyzed with one-way repeated measures ANOVA [factor = treatment phase: baseline, acute FIN treatment (days 1–3), sub-chronic FIN treatment (days 4–7), early withdrawal (days 1–3), and late withdrawal (days 4–7)]. A one-way repeated measures ANOVA (factor = dose) was similarly used to analyze the effects of ALLO on consumption parameters. The temporal distribution of licks was analyzed with two-way repeated measures ANOVAs [treatment phase (for FIN) or dose (for ALLO) x session interval (20-min bins)]. If a statistically significant interaction was detected, then a subsequent one-way repeated measures ANOVA was conducted for treatment phase within each 20-min interval. A one-way ANOVA was implemented to evaluate brain ALLO concentrations as a factor of FIN treatment dose. When appropriate, pair-wise differences were determined by the Fisher's Least Significant Difference multiple comparisons procedure. During the examination of FIN, the VEH-treated group was also examined for the effects of treatment phase on drinking measures. As values in the VEH-treated group did not change significantly across time (data not shown), collapsed VEH treatment values representative of the entire treatment time course for each drinking pattern variable are presented for non-statistical comparison. For all statistical analyses, significance was set at $P \leq 0.05$.

3. Results

3.1. Predictive validity of cumulative licks for drinking patterns

A significant positive correlation between ethanol dose (g/kg) and total ethanol licks was determined ($r = 0.621$, $P < 0.001$, $n = 284$) throughout the ALLO dose-response time course. A significant correlation between 10E volume depleted and ethanol licks was similarly noted ($r = 0.625$, $P < 0.001$, $n = 284$). The correlative strength between these variables was less than that previously documented in male mice under an identical consumption procedure (see Ford et al., 2005b), suggesting that the predictive validity of cumulative licks might not be as high in female mice. Body weights (mean $\pm$ SEM) were unaffected by ALLO treatment, and ranged between 20.7 ± 0.3 to 21.3 ± 0.2 g during the dose-response assessment.

3.2. Baseline ethanol intake across drug treatment history

Two sub-chronic regimens of FIN and an ALLO dose-response assessment were evaluated within the same animals over a 3-month period. The baseline intakes immediately prior to treatment with 50 and then 100 mg/kg FIN were 3.34 ± 0.09 and 3.25 ± 0.09 g/kg/2-hrs, respectively. The baseline intakes during the intervening ALLO testing were 3.29 ± 0.08 g/kg/2-hrs. These observations indicated that limited access intake was quite stable across an extended number of days, and, importantly, that the various pharmacological manipulations conducted had no residual effect on the re-establishment of a subsequent drinking baseline.

3.3. ALLO dose response assessment

ALLO did not significantly alter the 10E dose (g/kg) consumed during the 2-hr access period (Table 1). However, the cumulative total of 10E licks was significantly altered as a function of ALLO dose [$F(6,137) = 2.63$; $P < 0.05$], with the 10 ($P < 0.05$), 14 and 20 mg/kg doses ($P < 0.01$ for each) reducing licks by 12–13% when compared to baseline. The unexpected disparity between ALLO's influence on 10E licks versus ethanol dose prompted the evaluation of a measure to assess number of licks per unit of volume (licks/ml; see Table 1). A significant main effect of ALLO treatment on the licks/ml value was determined [$F(6,137) = 10.00$; $P < 0.001$], with ALLO doses ranging from 10–20 mg/kg eliciting significant reductions in the

number of licks per ml by 12–18% ($P < 0.01$ or $P < 0.001$). ALLO administration did not significantly alter water licks, total fluid intake, or ethanol preference.

There was a significant main effect of ALLO dose on bout frequency [$F(6,137) = 2.77$; $P < 0.05$], but post-hoc tests revealed that no single dose was different from baseline measures. In contrast, the notable influence of ALLO dose on bout size [$F(6,137) = 3.37$; $P < 0.01$] and inter-bout interval [$F(6,137) = 4.20$; $P < 0.001$] both yielded significant pair-wise comparisons. The largest ALLO dose tested significantly diminished both the mean bout size ($P < 0.01$) by 19% and the inter-bout interval ($P < 0.05$) by 21% versus baseline values. Although not reaching statistical significance, a strong trend for 20 mg/kg ALLO to decrease bout size ($P = 0.06$) also was observed.

In order to evaluate ALLO's impact on consumption onset, characteristics of the first drinking bout were analyzed separately (bottom of Table 1). ALLO significantly altered both the size [$F(6,137) = 3.78$; $P < 0.01$] and rate [$F(6,137) = 2.68$; $P < 0.05$] of the first bout, with the 3.2 mg/kg dose augmenting first bout size by 48% ($P < 0.05$) and reducing first bout lick rate by 53% ($P < 0.01$). Investigation into the temporal distribution of licks within 20-min intervals throughout the drinking sessions (Fig. 1A) revealed a significant main effect of ALLO dose [$F(6,132) = 2.63$; $P < 0.05$] and session interval [$F(5,110) = 19.62$; $P < 0.001$], as well as a statistically significant dose $\times$ interval interaction [$F(30,660) = 3.52$; $P < 0.001$]. In order to dissect the lick pattern during consumption onset, the initial 20-min interval (in Fig. 1A) was further divided into two 10-min intervals as illustrated in panel B. This separate analysis likewise detected a significant influence of ALLO dose [$F(6,132) = 4.17$; $P < 0.001$] and a dose $\times$ interval interaction [$F(6,132) = 5.75$; $P < 0.001$]. During drinking onset, the highest ALLO dose (24 mg/kg) significantly blunted 10E licks during the initial 10-min of access ($P < 0.01$) whereas mid-range neurosteroid doses (i.e., 10 & 17 mg/kg) significantly enhanced 10E licks during minutes 10–20 ($P < 0.001$; $P = 0.06$ for the 14 mg/kg dose), when compared to baseline. The enhanced onset of 10E licks observed following 17 mg/kg ALLO extended into the early maintenance phase of the session (20–40 min interval; $P < 0.01$ versus baseline). In contrast, ALLO dose-dependently suppressed drinking throughout the 60–80 min interval ($P < 0.05$ for 14 and 24 mg/kg; $P < 0.001$ for 17 mg/kg), when compared to respective baseline 10E licks.

3.4. FIN dose assessment

Seven days of treatment with 50 mg/kg FIN elicited a significant main effect of treatment session [$F(16,352) = 7.02$; $P < 0.001$] as well as a session $\times$ drug interaction [$F(16,352) = 2.11$; $P < 0.01$] for ethanol dose. However, its influence on g/kg intake was both modest and transient ($P < 0.05$ during treatment day #2; see Fig. 2A) when compared to VEH-treated mice. In contrast, 7 days of treatment with 100 mg/kg FIN produced a more robust and persistent effect on ethanol intake (Fig. 2B), with significant effects of drug [$F(1,21) = 5.79$; $P < 0.05$] and session [$F(16,336) = 4.14$; $P < 0.001$] and a significant drug $\times$ session interaction [$F(16,336) = 7.70$; $P < 0.001$]. Ethanol dose was significantly reduced by 25–34% following 100 mg/kg FIN administration (see treatment days 1–7 in Fig. 2B; $P < 0.05$ in each case) when compared to the ethanol dose consumed by VEH-treated mice. Notably, the FIN-induced decrease in ethanol intake abruptly concluded with treatment cessation, as evidenced by the immediate return to baseline consumption levels on the first day of withdrawal (see withdrawal day 1 in Fig. 2B). Because 100 mg/kg FIN was markedly more efficacious in decreasing ethanol consumption than the 50-mg/kg dose, the remaining analyses reported for drinking bout measures and lick patterns pertain specifically to the 100 mg/kg dose.

Commensurate with the observed reductions in ethanol dose, FIN-treated mice significantly diminished the total number of 10E licks by 32–37% when compared to their within-group baseline level. This statistically significant outcome [$F(4,48) = 39.01$; $P < 0.001$] was apparent

with both acute (treatment days 1–3; $P < 0.001$) and sub-chronic (treatment days 4–7; $P < 0.001$) FIN administration (Table 2). A more subtle, yet significant, decrease in 10E licks ($P < 0.001$) was detected during early withdrawal from FIN, an effect that dissipated during late FIN withdrawal (days 4–7 post-treatment). However, preference for 10E was unaltered by FIN treatment.

Water intakes were typically at or below the level of detection (i.e., 0.05 ml), and were not significantly altered by FIN treatment or its subsequent withdrawal (Table 2). The slight difference in water intake between the VEH control and baseline values, which was at the minimal detectable limit, was likely due to the fact that the FIN and VEH treatment groups were balanced for ethanol (rather than water) intake.

The effect of FIN treatment on total fluid intake (10E plus water) was similar to that for 10E (reported above), no doubt due to the fact that this measure primarily was comprised of 10E intake. Total fluid intake was significantly reduced by FIN treatment [$F(4,48) = 32.28$; $P < 0.001$] during treatment days 1–3 ($P < 0.001$), treatment days 4–7 ($P < 0.001$), and withdrawal days 1–3 ($P < 0.05$) when compared to within-group baseline values.

Based on the suppression of total fluid intake following FIN treatment, we analyzed home cage water intake during the 22 hr between limited access sessions (see Inter-Session Water Intake in Table 2). Inter-session water intake was significantly increased during the 7 days of FIN treatment ($P < 0.001$), suggesting that mice were able to compensate for the decrease in total fluid consumption that occurred during the limited access session. No change in inter-session water intake was observed in VEH-treated mice during FIN treatment (data not shown).

Examination of 10E bout dynamics revealed that the overall decline in drinking that resulted from FIN treatment was primarily attributable to a marked attenuation in bout frequency [$F(4,48) = 44.21$; $P < 0.001$] and the corresponding increase in inter-bout interval [$F(4,48) = 15.62$; $P < 0.001$]. In contrast, mean bout size, bout duration, and lick rate were each unaffected by FIN exposure. First bout dynamics were likewise unchanged by FIN treatment (Table 2), suggesting that the blockade of ALLO biosynthesis selectively impinged on the maintenance, but not the onset, of drinking during the limited access sessions.

Significant overall effects of FIN treatment phase [$F(4,48) = 36.65$; $P < 0.001$] and session interval [$F(5,60) = 70.18$; $P < 0.001$], and a statistically significant phase $\times$ interval interaction [$F(20,240) = 2.07$; $P < 0.01$] were determined for the temporal distribution of 10E licks throughout the drinking sessions (Figure 3A). Subsequent simple main effect analyses for each session interval revealed a significant influence of FIN treatment phase on all 20-min intervals [$F(4,48) = 3.38 - 10.07$; $P < 0.05$ for 20–40, $P < 0.01$ for 60–80 min, and $P < 0.001$ for 0–20, 40–60, 80–100 min intervals], except 100–120 min, when compared to baseline measures. Despite the significant main effect of FIN treatment phase during the initial 20-min of 10E access, post-hoc comparisons of each treatment phase versus baseline values were not significant (Fig. 3A). Furthermore, there was no significant effect of FIN treatment phase on 10E licks when the initial 20-min of access was further delineated and analyzed as two 10-min bins (Fig. 3B). Consistent with the absence of detectable differences in first bout dynamics as a function of FIN treatment and withdrawal (Table 2), these temporal lick observations collectively suggested that FIN exhibited little impact on drinking onset. In contrast, FIN treatment days 1–3 (and treatment days 4–7 to a lesser degree) reliably suppressed licking behavior during minutes 20–100 versus baseline, resulting in a blunted maintenance of consumption. Just as the g/kg dose consumed exhibited a rapid recovery to baseline levels upon FIN withdrawal (Fig. 2B), lick patterns were almost completely restored by withdrawal days 4–7 (Fig. 3A).

3.5. Brain ALLO levels following acute FIN challenge

As noted above, this analysis was conducted in FIN naive mice, to avoid the potential confound of treatment history on the ability of an acute FIN challenge to suppress ALLO levels. In this subset of mice ($n = 10$), ALLO concentrations were 5.11 ± 0.57 ng/g ($n = 4$), 3.61 ± 0.45 ng/g ($n = 3$), and 2.80 ± 0.24 ng/g ($n = 3$) for the animals pretreated with VEH, 50 mg/kg FIN, or 100 mg/kg FIN, respectively. A one-way ANOVA for ALLO concentrations revealed a significant influence of FIN dose [$F(2,7) = 6.20$; $P < 0.05$]. Acute treatment with 100 mg/kg FIN significantly attenuated brain ALLO levels by 45% ($P < 0.05$) whereas 50 mg/kg FIN elicited a strong trend for a decrease in ALLO by 31% ($P = 0.06$) when compared to VEH-treated mice.

4. Discussion

4.1. Summary of findings

Recent work in the laboratory has validated the use of lickometers to examine the microarchitecture of ethanol drinking patterns following exogenous administration of ALLO (Ford et al., 2005b, 2007b) or FIN (Ford et al., 2005a) in male C57BL/6 mice. Significant dose- and time-dependent effects were revealed that were not readily apparent when only total 2-hr ethanol intake was considered. Thus, we reasoned that use of lickometers in the present studies would allow us to systematically examine whether female mice would respond differently than male mice to the modulatory effects of manipulations in endogenous ALLO levels on ethanol intake. Consistent with preliminary findings (Sinnott et al., 2002b), the results documented that female C57BL/6 mice were less sensitive than male mice (Ford et al., 2005a, 2005b) to manipulations of endogenous ALLO levels on ethanol intake and consumption patterns. Specifically, acute administration of exogenous ALLO in a wide range of doses (3.2 – 24 mg/kg) or sub-chronic administration of the 50-mg/kg dose of FIN did not significantly alter the overall 2-hr 10E dose consumed. However, 10E licks were suppressed by mid – high ALLO doses (10, 14 & 20 mg/kg), and bout size was decreased by the highest ALLO dose (24 mg/kg). These results suggest that ALLO produced some subtle changes in the microarchitecture of ethanol drinking. More robust effects on 10E intake were observed following sub-chronic administration of a higher FIN dose (100 mg/kg), which significantly decreased ethanol intake in the female mice. Notably, the 100-mg/kg FIN dose significantly decreased endogenous ALLO levels, whereas the 50-mg/kg dose tended to decrease ALLO levels. Collectively, the results suggest that there are sex differences in the physiological regulation of ethanol intake patterns by GABAergic neurosteroids.

4.2. Implications of increasing endogenous ALLO levels on ethanol consummatory behavior

Analysis of the temporal distribution of 10E licks indicated that ALLO administration produced transient changes in the pattern of ethanol consumption (Figure 1), a finding that was consistent with the lack of effect of ALLO on the 2-hr ethanol dose consumed (g/kg). ALLO administration also did not affect ethanol preference or water consumption. And, with the exception of a significant decrease in bout size following the 24 mg/kg dose of ALLO, the remainder of mean bout parameters were unaffected by ALLO pretreatment (Table 1). Likewise, first bout parameters, taken as an index of the onset of ethanol self-administration, were not altered by ALLO pretreatment. Collectively, these findings suggest that an exogenous ALLO challenge does not alter the onset or maintenance of limited access ethanol self-administration in female C57BL/6 mice.

A curious finding was that there was a significant overall decrease in 10E licks following administration of the 10, 14, and 20 mg/kg ALLO doses, while the dose of ethanol consumed was unaltered. Upon closer inspection of the data, this decline in 10E lick frequency was due to a significant reduction in the number of licks per unit of volume (i.e., lick/ml in Table 1), a

finding that may have contributed to the difference in correlation between 10E licks and ethanol dose in female ($r = 0.62$) versus male ($r = 0.87$; Ford et al., 2005b) mice. Although an ALLO treatment effect on ethanol lick volume was not observed in male C57BL/6 mice (Ford et al., 2005b), it is possible that ALLO may induce subtle, yet fundamental, changes in the drinking patterns of female mice that are not reliably apparent with the traditional bout microarchitecture analysis performed in the present study. Nonetheless, 10E licks and ethanol dose consumed were correlated in the female mice, which would argue that an effect of ALLO on ethanol lick volume did not confound the overall interpretations of the present findings.

The decreased sensitivity of female mice to the modulatory effect of ALLO on ethanol intake patterns contrasts sharply with previous results in male mice (Ford et al., 2005b, 2007b). For comparative purposes, systemic administration of 3.2 g/kg ALLO increased 2-hr ethanol intake by approximately 20% in male mice, whereas the 17 and 24 mg/kg ALLO doses decreased 2-hr ethanol intake by approximately 10 and 30%, respectively. These effects in male mice were predominantly due to a concomitant change in bout frequency. Taken in conjunction with evidence for the involvement of the GABA_A receptor system in the discriminative stimulus properties of ethanol (Hodge and Aiken, 1996; Grant et al., 1997; Bowen et al., 1999; Engel et al., 2001; Hodge et al., 2001; Besheer et al., 2003; Hodge et al., 2006) as well as in ethanol self-administration (Rassnick et al., 1993; Hodge et al., 1995, 1996; Hyttiä and Koob, 1995; Petry, 1997; Janak et al., 1998; June et al., 1998a, 1998b; Nowak et al., 1998; Söderpalm and Hansen, 1998; Samson and Chappell, 2001; Sinnott et al., 2002b), the contribution of GABAergic neurotransmission to the regulatory processes governing ethanol drinking, reinforcement and/or reward (Koob et al., 1998; Laviolette and van der Kooy, 2001) may differ in male and female mice.

Although the study design did not allow for the determination of ALLO levels following injection of ALLO, our earlier work in male C57BL/6 mice indicate that administration of ALLO doses ranging from 1 – 32 mg/kg produced a dose-dependent and significant increase in plasma ALLO levels (Finn et al., 1997). For a comparison with doses used in the present study, plasma ALLO levels (average in parentheses) were significantly increased over values in vehicle controls following administration of 10 mg/kg (~100 ng/ml), 17 mg/kg (~200 ng/ml), and 32 mg/kg (~500 ng/ml) ALLO. Injection of 3.2 mg/kg ALLO increased plasma ALLO levels to 34 ng/ml, which might be physiologically relevant. Since injection of ALLO in this dose range (i.e., 3.2 – 32 mg/kg) produced endogenous ALLO levels in the high physiological to supra-physiological range of concentrations, we presume that similar levels would be achieved in female mice, as ALLO injection would increase this endogenous neurosteroid to levels that exceed any fluctuations in an intact female animal.

Since female mice have been reported to be more sensitive than male mice to the locomotor stimulant effects of the 10 and 17- mg/kg ALLO doses (Palmer et al., 2002, 2006), it is possible that an ALLO-induced change in activity level contributed to the present findings. In the present study, female mice were observed to be visually ataxic following injection of the 24 mg/kg ALLO dose for approximately 30 – 60 min post injection, with the majority of their alcohol consumption occurring during the 2nd hour of the limited access session. Taken in conjunction with the observation that the locomotor stimulant effects of ALLO occur primarily during the first 30 min post injection, one must consider that an effect of ALLO on activity level could impact the pattern of alcohol consumption during the 2 hr limited access session in female mice.

4.3. Implications of decreasing endogenous ALLO levels on ethanol consummatory behavior

When the 5 α -reductase inhibitor FIN was administered to indirectly decrease endogenous ALLO levels, ethanol intake was recalcitrant to sub-chronic (i.e., 7 day) treatment with 50 mg/kg FIN. Whereas this dose decreased ethanol intake by approximately 22% in the acute

treatment and early withdrawal phases in male mice (Ford et al., 2005a), ethanol-drinking behavior in female mice was relatively insensitive to manipulation at this dose. A higher dose of FIN was required (100 mg/kg), and this dose decreased ethanol intake by 35% in the female mice. Notably, the doses of FIN that significantly decreased ethanol intake primarily reduced the maintenance of ethanol self-administration throughout the 2-hr limited access session, as indicated by the temporal lick patterns, but these effects occurred via different mechanisms in the male and female mice. FIN decreased bout size and length in the male mice, whereas it decreased bout frequency in the female mice.

It is unlikely that the decrease in 10E and total fluid intake during the limited access session was due to a general suppression of activity. Although recent work documented that FIN administration suppressed locomotor activity during the first 30 min post-injection (Gabriel et al., 2004), mice were pretreated with FIN approximately 22 hrs prior to the limited access session in the present study. Additionally, between-session water intake (i.e., during the 22 hr immediately after FIN injection and prior to the next day's limited access session) was significantly increased during the 7 days of FIN treatment. These results indicate that the decrease in fluid intake during the limited access session was selective for ethanol and that it was not due to a non-specific effect of FIN on activity.

Measurement of endogenous ALLO levels after acute challenge with the 50 and 100 mg/kg doses of FIN confirmed that these FIN doses produced dose-dependent decreases in brain ALLO levels. Although the experimental design precluded our assessment of ALLO levels following sub-chronic FIN administration, it is likely that the effect would be similar to (or perhaps slightly greater than) that seen following a single injection. Nonetheless, the ALLO data suggests that endogenous ALLO levels must be suppressed by approximately 45% in order to observe significant decreases in 10E consumption. This idea is consistent with results in male C57BL/6 mice, whereby a 50-mg/kg dose of FIN, which decreases endogenous ALLO levels by approximately 50% (Finn, unpublished), also decreased 10E consumption. Since endogenous ALLO levels in female C57BL/6 mice are higher than in males (Finn et al., 2004c), it is reasonable to presume that a higher FIN dose would be required to produce a comparable reduction in endogenous ALLO levels to that seen in males. Thus, endogenous ALLO tone in females (as with recent findings with males) may be important in the regulatory processes that participate in the onset and maintenance of drinking episodes.

4.4. Sex differences in GABAergic neurosteroid tone and ethanol consummatory behavior: Potential mechanisms

Comparison of the present findings in female mice with recent results in males (Ford et al., 2005a, 2005b) provides a hint for sex differences in ethanol bout parameters following ALLO or FIN challenges. In terms of sex differences in ethanol intake patterns and self-administration, the ethanol dose consumed under limited access or continuous access 2-bottle choice procedures typically is higher in female than in male C57BL/6 mice (Middaugh et al., 1999; Finn et al., 2004c). Also, there is some anecdotal evidence to suggest that female rodents acquire operant self-administration of ethanol more slowly than males (Roberts et al., 1998; Wiren et al., 2006). However, once the lever-pressing behavior was acquired, female C57BL/6 mice tended to respond more for ethanol solutions when the instrumental response contingency was lower [e.g., fixed ratio (FR)1 – FR4] and access was longer, whereas the male C57BL/6 mice responded more when behavioral demands were higher and access time was shorter (Middaugh and Kelley, 1999; Wiren et al., 2006). Although these findings suggest that subtle changes in experimental design can impact the degree to which sex differences in ethanol self-administration are expressed, we were careful to keep all experimental parameters consistent across the lickometer studies in male and female C57BL/6 mice in order to facilitate comparisons of the ALLO and FIN effects.

Stage of estrous cycle was not monitored in the present study, based on earlier reports that estrous cycle-related changes in operant ethanol self-administration were observed in intact female rats that had their cycles synchronized, but not in rats that were allowed to cycle freely (Roberts et al., 1998). Consistent with the notion that the regulatory processes that underlie ethanol consumption patterns are undetected when total intake is the sole measure evaluated (Samson and Hodge, 1996), a recent study found no change in total daily intake, but significant differences in bout parameters, across the estrous cycle in female rats (Ford et al., 2002). The authors suggested that cyclical fluctuations in ovarian hormones or other neuromodulators contributed to the estrous cycle-related changes in the microstructural components of ethanol intake. Related to the idea that variations in a neuromodulator could have a physiological and behavioral consequence, fluctuations in endogenous GABAergic neurosteroid levels during the estrous cycle in female C57BL/6 mice were found to modulate GABA_A receptor-mediated tonic inhibition, seizure susceptibility and anxiety level (Maguire et al., 2005). However, we did not observe a systematic change in ethanol intake levels or more importantly, in ethanol bout parameters, across the weeks of baseline consumption measurements or in the VEH-treated groups in the present studies. Thus, we feel that it is unlikely that an estrous cycle-related change in the microanalysis of ethanol intake in the control conditions confounded interpretation of the data following ALLO or FIN pretreatment.

It should be noted that basal endogenous levels of ALLO are higher in female than in male C57BL/6 mice and that 2-hr ethanol intake produced a significant increase in brain ALLO levels only in the male mice (Finn et al., 2004c). With this in mind, it is notable that all the data to date for positive modulatory effects of ALLO on ethanol reinstatement (Nie and Janak, 2003), limited access ethanol intake (Sinnott et al., 2002b; Ford et al., 2005b) and ethanol self-administration (Janak et al., 1998; Janak and Gill, 2003; Ford et al., 2007b) have been generated in male rodents. Thus, the present findings in females, in conjunction with the results in males, suggest that there is a complex interaction between the changes in endogenous ALLO levels following ethanol consumption, the putative effect of these changes on GABA_A receptor function and plasticity, and the impact of ALLO on subsequent self-administration. Consistent with this notion, a higher dose of FIN was required to modulate ethanol intake in the female mice.

It is possible that prior treatment with 50 mg/kg FIN may have altered the sensitivity of the female C57BL/6 mice to subsequent treatment with either acute ALLO challenge or sub-chronic 100 mg/kg FIN exposure. However, several observations suggest that initial exposure to 50 mg/kg FIN did not confound subsequent treatment assessments. First, the ethanol dose consumed during the baseline phases prior to each treatment regimen were exactly matched, and bout microarchitecture as well as the temporal distribution of licks reverted to baseline patterns after each treatment schedule. Second, the lower sensitivity of female mice to exogenous ALLO challenge was similarly noted in an earlier preliminary drinking study that tested fewer doses (Sinnott et al., 2002b). Lastly, administration of 100 mg/kg FIN (but not 50 mg/kg) to progesterone-treated female mice mimicked the effect of progesterone withdrawal by increasing immobility time in the forced swim test (FST; Beckley and Finn, 2007), thereby indicating that a similar dose threshold for FIN efficacy in female mice was independently observed for another behavioral endpoint. Importantly, the pretreatment time for the 100 mg/kg FIN dose (2 – 4 hrs in FST study, 22 hrs in present study) as well as the fact that water intake was increased following FIN injection in the present study (discussed earlier), would argue that the behavioral effects of FIN on FST immobility and ethanol intake were not confounded by motoric effects.

Another consideration is that manipulation of ALLO levels can modulate some, but not all, of ethanol's pharmacological effects (reviewed in Finn et al., 2006a) and that there can be sex differences in this interaction. Pretreatment with FIN (single dose of 50 mg/kg) produced

opposite effects on acute ethanol withdrawal in male and female C57BL/6 mice (Gorin-Meyer et al., 2007), increasing withdrawal severity in the female mice and decreasing withdrawal severity in the male mice. And, sub-chronic FIN (4 doses of 50 mg/kg) significantly decreased chronic ethanol withdrawal in male C57BL/6 mice without significantly altering chronic withdrawal in the female mice (Finn et al., 2004b). However, pre-treatment with ALLO produced a comparable anticonvulsant effect in male and female rats (Devaud et al., 1995) and mice (Finn et al., 2006b; Beckley et al., 2008) when they were ethanol naive as well as during ethanol withdrawal, with one report for an estrous cycle-related change in sensitivity to the anticonvulsant effect of ALLO in female rats (Finn and Gee, 1994). Collectively, these findings suggest that females are sensitive to some modulatory effects of ALLO. One possible explanation for the current observations is that the plasticity of GABA_A receptors in neurocircuits relevant to ethanol drinking behavior versus seizure susceptibility and alcohol withdrawal may differ in males and females.

Ovariectomy (OVX), which decreases brain ALLO levels by 50 – 70% (depending on the brain region examined; Bernardi et al., 2002), has been reported to alter sensitivity to the anxiolytic (Laconi et al., 2001) and anticonvulsant (Alele and Devaud, 2007) effects of ALLO in female mice. Whereas OVX reduced sensitivity to the anxiolytic effect of ALLO in female rats, it increased sensitivity to the anticonvulsant effect of ALLO during ethanol withdrawal so that the anticonvulsant effect in OVX female rats was similar to that in male rats. Thus, it is possible that OVX might render exogenous ALLO injections more effective, as they were in male mice. Future studies will investigate this possibility further.

5. Conclusions

Female C57BL/6 mice were less sensitive to the modulatory influence of exogenous ALLO (3.2 – 24 mg/kg) or FIN (50 mg/kg) on ethanol drinking patterns, when compared with recent findings in male rodents. Differences in ALLO modulation within female versus male mice suggest that the physiological regulation of ethanol intake and underlying consumption patterns are sex-specific, and that variations in therapeutic intervention (i.e., GABAergic manipulation) according to sex may be necessary for the treatment of alcohol abuse.

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Figure 1. Effects of exogenous ALLO challenge on the temporal distribution of ethanol (10E) licks
 The frequency of 10E licks recorded throughout the 2-hr drinking session is depicted in 20-min bins (**panel A**). Lick patterns during the initial 20-min of access were further examined as 10-min intervals (**panel B**) to investigate consumption onset. A collapsed mean of the baseline days that immediately preceded each ALLO test session is shown as 0 mg/kg. Vertical bars represent the mean $\pm$ SEM of licks for 24 mice. * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$ versus within-subject baseline (i.e., 0 mg/kg) for the respective session interval.

Figure 2. Effects of sub-chronic FIN administration and its subsequent withdrawal on daily ethanol dose (g/kg) consumed

Two doses of FIN were assessed: 50 mg/kg (**panel A**) and 100 mg/kg (**panel B**). Each data point represents the mean $\pm$ SEM ethanol intake for 13–14 FIN-treated or 10 VEH-treated mice. * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$ versus VEH-treated mice (i.e., 0 mg/kg) within a given drinking session.

Figure 3. Effects of FIN treatment (100 mg/kg) and withdrawal on the temporal distribution of ethanol (10E) licks

The frequency of 10E licks recorded throughout the 2-hr drinking session is depicted in 20-min bins (**panel A**). Lick patterns during the initial 20-min of access were further examined as 10-min intervals (**panel B**) to investigate consumption onset. Vertical bars represent the mean $\pm$ SEM of licks for 13 FIN-treated mice. * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$ versus within-subject baseline for the respective session interval.

Table 1

Effects of ALLO on ethanol intake and bout microarchitecture

Baseline (0 mg/kg) values represent the within-group collapsed mean $\pm$ SEM of sessions immediately preceding each ALLO treatment. Each measure represents the mean $\pm$ SEM for 24 mice.

	Allopregnanolone Dose (mg/kg; i.p.)					
	Baseline	3.2	10	14	17	24
General Measures						
10E Dose (g/kg)	3.29 $\pm$ 0.08	3.26 $\pm$ 0.12	3.32 $\pm$ 0.16	3.42 $\pm$ 0.10	3.49 $\pm$ 0.10	3.34 $\pm$ 0.18
10E Licks	963 $\pm$ 38	936 $\pm$ 40	858 $\pm$ 46*	835 $\pm$ 39**	886 $\pm$ 37	896 $\pm$ 45
Licks/ml 10E	1106 $\pm$ 30	1095 $\pm$ 27	977 $\pm$ 26***	911 $\pm$ 36***	945 $\pm$ 27***	1024 $\pm$ 39*
Water Licks	84 $\pm$ 21	93 $\pm$ 25	111 $\pm$ 37	101 $\pm$ 44	157 $\pm$ 63	196 $\pm$ 64
Total Fluid Intake (ml)	0.94 $\pm$ 0.02	0.95 $\pm$ 0.05	0.99 $\pm$ 0.07	1.03 $\pm$ 0.05	1.06 $\pm$ 0.06	1.02 $\pm$ 0.07
Preference Ratio	0.95 $\pm$ 0.02	0.93 $\pm$ 0.03	0.94 $\pm$ 0.03	0.92 $\pm$ 0.03	0.93 $\pm$ 0.03	0.90 $\pm$ 0.04
Total Session 10E Bout Dynamics						
Bout Frequency	11.9 $\pm$ 0.5	10.8 $\pm$ 0.6	11.0 $\pm$ 0.7	10.2 $\pm$ 0.7	11.6 $\pm$ 0.6	13.0 $\pm$ 0.7
Bout Size (licks)	83 $\pm$ 3	91 $\pm$ 6	78 $\pm$ 3	81 $\pm$ 7	78 $\pm$ 6	67 $\pm$ 4**
Inter-Bout Interval (min)	10.3 $\pm$ 0.7	11.1 $\pm$ 0.7	10.8 $\pm$ 0.8	12.1 $\pm$ 1.2*	9.0 $\pm$ 0.5	8.2 $\pm$ 0.4*
Bout Duration (min)	0.76 $\pm$ 0.07	0.86 $\pm$ 0.09	0.78 $\pm$ 0.08	0.83 $\pm$ 0.09	0.82 $\pm$ 0.07	0.69 $\pm$ 0.06
Lick Rate (licks/min)	307 $\pm$ 16	301 $\pm$ 21	293 $\pm$ 19	290 $\pm$ 19	275 $\pm$ 20	297 $\pm$ 22
First 10E Bout Dynamics						
Latency to 1 st Bout (min)	7.2 $\pm$ 2.4	10.5 $\pm$ 5.1	6.0 $\pm$ 1.5	7.6 $\pm$ 1.7	5.8 $\pm$ 1.3	11.4 $\pm$ 3.2
Size of 1 st Bout (licks)	96 $\pm$ 9	143 $\pm$ 19*	123 $\pm$ 18	87 $\pm$ 12	110 $\pm$ 17	64 $\pm$ 9
Rate of 1 st Bout (licks/min)	233 $\pm$ 25	109 $\pm$ 14	195 $\pm$ 35	271 $\pm$ 49	180 $\pm$ 39	246 $\pm$ 44

* $P \leq 0.05$

** $P \leq 0.01$

*** $P < 0.001$ versus within-subject baseline value.

Table 2

Effects of FIN treatment (100 mg/kg) and withdrawal on ethanol intake and bout microarchitecture

All values represent the mean $\pm$ SEM of each treatment phase for $n = 13$ FIN-treated mice. Collapsed values of VEH-treated mice ($n = 10$) are depicted for non-statistical comparison.

	VEH Control	Baseline	Treatment Phase			
			Treatment Days 1–3	Treatment Days 4–7	Withdrawal Days 1–3	Withdrawal Days 4–7
General Measures						
10E Licks	971 $\pm$ 53	970 $\pm$ 51	609 $\pm$ 42 ***	657 $\pm$ 45 ***	804 $\pm$ 38 ***	899 $\pm$ 27
Water Intake (ml)	0.013 $\pm$ 0.006	0.059 $\pm$ 0.027	0.054 $\pm$ 0.026 ***	0.032 $\pm$ 0.025 ***	0.033 $\pm$ 0.018 ***	0.038 $\pm$ 0.016
Total Fluid Intake (ml)	0.89 $\pm$ 0.03	0.96 $\pm$ 0.01	0.71 $\pm$ 0.03 ***	0.72 $\pm$ 0.03 ***	0.87 $\pm$ 0.03 *	0.93 $\pm$ 0.02
Preference Ratio	0.99 $\pm$ 0.01	0.95 $\pm$ 0.03	0.92 $\pm$ 0.04 ***	0.94 $\pm$ 0.05 ***	0.98 $\pm$ 0.02	0.96 $\pm$ 0.02
Inter-Session Water Intake (ml)	5.88 $\pm$ 0.41	6.30 $\pm$ 0.32	7.24 $\pm$ 0.37 ***	7.60 $\pm$ 0.32 ***	6.73 $\pm$ 0.39	6.54 $\pm$ 0.36
Mean 10E Bout Dynamics						
Bout Frequency	9.6 $\pm$ 0.3	12.5 $\pm$ 0.7	6.7 $\pm$ 0.5 ***	7.5 $\pm$ 0.5 ***	9.2 $\pm$ 0.6 ***	10.4 $\pm$ 0.6 ***
Bout Size (licks)	104 $\pm$ 6	79 $\pm$ 5	99 $\pm$ 9 ***	95 $\pm$ 7 ***	95 $\pm$ 8 *	89 $\pm$ 6
Inter-Bout Interval (min)	13.1 $\pm$ 0.6	9.5 $\pm$ 0.7	22.4 $\pm$ 2.7 ***	19.3 $\pm$ 1.7 ***	14.8 $\pm$ 1.4 *	12.3 $\pm$ 0.8
Bout Duration (min)	0.86 $\pm$ 0.09	0.83 $\pm$ 0.10	0.84 $\pm$ 0.07	0.76 $\pm$ 0.05	0.83 $\pm$ 0.09	0.75 $\pm$ 0.06
Lick Rate (licks/min)	300 $\pm$ 21	298 $\pm$ 22	291 $\pm$ 14	312 $\pm$ 10	302 $\pm$ 21	317 $\pm$ 14
First 10E Bout Dynamics						
Latency to 1 st Bout (min)	0.7 $\pm$ 0.2	1.8 $\pm$ 0.8	1.0 $\pm$ 0.1	6.1 $\pm$ 4.6	0.6 $\pm$ 0.1	0.7 $\pm$ 0.1
Size of 1 st Bout (licks)	209 $\pm$ 18	153 $\pm$ 18	153 $\pm$ 15	168 $\pm$ 19	191 $\pm$ 20	183 $\pm$ 20
Rate of 1 st Bout (licks/min)	138 $\pm$ 13	157 $\pm$ 24	129 $\pm$ 14	136 $\pm$ 15	130 $\pm$ 19	156 $\pm$ 17

* $P \leq .05$

** $P < .01$

*** $P < .001$ versus within-group baseline values for FIN-treated mice.