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Alteration of Ethanol Drinking in Mice via Modulation of the GABA_A Receptor with Ganaxolone, Finasteride, and Gaboxadol

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Abstract

Background—Neurosteroids and other γ -aminobutyric acid_A (GABA_A) receptor modulating compounds have been shown to affect ethanol intake, although their mechanism remains unclear. The present study examined how patterns of 24-hour ethanol drinking in mice were altered with the synthetic GABAergic neurosteroid ganaxolone (GAN), with an inhibitor of neurosteroid synthesis (finasteride, FIN), or a GABA_A receptor agonist with some selectivity at extrasynaptic receptors (gaboxadol, THIP).

Methods—Male C57BL/6J mice had continuous access to a 10% v/v ethanol solution (10E) or water. Using lickometer chambers, drinking patterns were analyzed among mice treated in succession with GAN (0, 5, 10 mg/kg), FIN (0 or 100 mg/kg) and THIP (0, 2, 4, 8, 16 mg/kg). Results: GAN shifted drinking in a similar but extended manner to previous reports using low doses of the neurosteroid allopregnanolone (ALLO); drinking was increased in hour 1, decreased in hours 2 and 3, and increased in hours 4 and 5 post-injection. THIP (8 mg/kg) and FIN both decreased 10E drinking during the first 5 hours post-injection by 30% and 53%, respectively, while having no effect on or increasing water drinking, respectively. All 3 drugs altered initiation of drinking sessions in a dose-dependent fashion. FIN increased and GAN decreased time to first lick and first bout. THIP (8 mg/kg) decreased time to first lick but increased time to first bout and attenuated first bout size.

Conclusions—The present findings support a role for the modulation of ethanol intake by neurosteroids and GABA_A receptor acting compounds and provide hints as to how drinking patterns are shifted. The ability of THIP to alter 10E drinking suggests that extrasynaptic GABA_A receptors may be involved in the modulation of ethanol intake. Further, the consistent results with THIP to that seen previously with high doses of ALLO suggest that future studies should further examine the relationship between neurosteroids and extrasynaptic GABA_A receptors, which could provide a better understanding of the mechanism by which neurosteroids influence ethanol intake.

Keywords

Neurosteroid; Lickometer; Mice; Alcohol; Extrasynaptic

Introduction

Ethanol has been shown to act on many different receptor types, one of which is the type A γ -aminobutyric acid (GABA_A) receptor (Deitrich et al., 1989; Grobin et al., 1998; Kumar et al., 2009). The GABA_A receptor is a ligand-gated inhibitory receptor, composed of 5 subunits. The specific combination of subunits, together with the sub-cellular localization of the receptor, give it a unique physiology and pharmacology (Mody and Pearce, 2004). Ethanol has a dual pharmacological action on GABA_A receptors; the first is a rapid and direct positive modulation of receptor activity, and the second is an indirect, delayed, positive modulation of the receptor that electrophysiological studies suggest is due to the production of the neurosteroid allopregnanolone (ALLO) following ethanol exposure (Sanna et al., 2004). Supporting this hypothesis, we have shown that ethanol self-administration leads to physiologically relevant increases in ALLO levels in the brains of C57BL/6 (C57) male mice (Finn et al., 1997b; Finn et al., 2004). ALLO is one of the most potent endogenous positive modulators known for GABA_A receptors (Lambert et al., 1995), and it shares physiological characteristics with ethanol in that it has anesthetic, anticonvulsant, sedative-hypnotic, anxiolytic, locomotor-stimulant, and muscle-relaxant properties (Finn et al., 1997b; Gasior et al., 1999). Therefore, clarifying the relationship between neurosteroids and alcohol could be vital to a better understanding of alcohol's mechanism of action.

Physiologically-relevant doses of exogenous ALLO have been shown to dose-dependently increase ethanol drinking in rodents, and mice show conditioned place preference for ALLO, indicating that the production of this neurosteroid may play a role in ethanol reinforcement and reward (Finn et al., 1997a; Finn et al., 1997b; Ford et al., 2008a; Ford et al., 2005b; Ford et al., 2008b; Janak and Gill, 2003; Janak et al., 1998). ALLO is metabolically reduced from progesterone by a two-step reaction, with 5 α -reductase being the rate-limiting enzyme in this reaction. Thus, one way to decrease brain ALLO levels is with finasteride (FIN), a 5 α -reductase inhibitor (Finn et al., 2006). In a 2-hour access study, injections of FIN in C57 male mice have been shown to decrease ethanol drinking, further indicating neurosteroids and other GABA_A acting compounds as tools for altering ethanol intake (Ford et al., 2008a; Ford et al., 2005a; Ford et al., 2008b).

To elucidate how ALLO modulates ethanol intake, previous studies in our lab have used lickometer circuits to examine the temporal patterns of ethanol drinking in 2-hour access studies. Although we have seen transient alterations in ethanol consumption in male mice given administrations of ALLO or FIN (Ford et al., 2005a; Ford et al., 2005b), it is possible that in addition to a simple suppression or elevation in drinking, these drugs could be leading to a shift in the pattern of drinking across 24 hours. The current study used lickometer chambers to examine the drinking patterns of C57 male mice that had continuous access to ethanol and were treated in succession with three different GABA_A receptor modulating drugs: ganaxolone (GAN), FIN, and gaboxadol HCl (THIP).

While we have shown that low doses of ALLO increase ethanol drinking while high doses of ALLO decrease ethanol drinking, intake levels recover after about 80 minutes post-treatment, possibly due to the rapid metabolism of ALLO (Ford et al., 2005b). To overcome the transient nature of these effects, we used GAN, a synthetic analogue of ALLO with an added methyl group, making it more metabolically stable (Hogenkamp et al., 1997). We predicted that GAN would lead to similar changes in ethanol consumption as that seen with ALLO, but because of its slower metabolism, the effect would be more prolonged.

Previous studies have looked at the effects of 7 consecutive days of FIN treatment, but suppression of drinking was transient across the two-hour access period when given at a dose of 50 mg/kg in male mice and non-existent when given to female mice (Ford et al.,

2008a; Ford et al., 2005a). On the other hand, 100 mg/kg FIN was effective at altering ethanol intake in female mice and at impeding acquisition of ethanol drinking in male mice in 2-hour limited access paradigms (Ford et al., 2008a; Ford et al., 2008b). Therefore, we chose to use the higher dose of FIN to examine how effects of this enzyme inhibitor would extend into a 24-hour time frame. In line with the notion that inhibition of ALLO synthesis decreases ethanol intake, we hypothesized that the higher dose of FIN would reveal a more pronounced and prolonged suppression of ethanol drinking than seen previously, and that this would be evident in a paradigm where mice were allowed continuous access to ethanol (Ford et al., 2008a; Ford et al., 2005a; Ford et al., 2005b).

The present study also examined the patterns of ethanol drinking upon administration of THIP, a GABA_A receptor agonist that has some selectivity for receptors containing the α_4/δ subunits (Chandra et al., 2006; Herd et al., 2009). Receptors containing these subunits in combination occur almost exclusively outside of the synapse (Herd et al., 2007). Neurosteroids such as ALLO have a high affinity for extrasynaptic receptors, but it is unclear whether their modulation of ethanol's actions is via these extrasynaptic sites (Brown et al., 2002; Fodor et al., 2005; Mihalek et al., 1999). It is also controversial whether ethanol acts through extrasynaptic receptors (Borghese et al., 2006; Sundstrom-Poromaa et al., 2002). Therefore, we used THIP to examine the role of the extrasynaptic receptors in the modulation of ethanol drinking. We hypothesized that similar to that seen with the GABA_A agonist muscimol, THIP would decrease consumption of ethanol (Hodge et al., 1995; Janak and Gill, 2003; Petry, 1997; Samson and Chappell, 2001). Together, the goal of these experiments was to further examine the manner in which neurosteroids alter the consumption and pattern of ethanol drinking, in order to provide insight regarding a possible mechanism that neurosteroids might be acting through to affect ethanol consumption.

Materials and Methods

Animals

Twenty-four C57 male mice of approximately 8 weeks of age at the start of experiments were used (Jackson Laboratory, Bar Harbor, ME). Mice were individually housed and acclimated to a reverse light/dark schedule (12/12h; lights off at 1500) for 3 weeks prior to the start of experiments. All mice were provided ad libitum access to rodent chow and tap water in lickometer chambers (see below). Mice were weighed and handled daily on all acclimation and experimental days. All procedures were approved by the local Institutional Animal Care and Use Committee and complied with NIH guidelines.

Two-bottle Preference Procedure in Lickometer Chambers

Lickometer chambers were used as previously described (Ford et al., 2008a; Ford et al., 2005a; Ford et al., 2005b; Ford et al., 2008b). Briefly, the chamber was made up of a 4-walled plexiglas insert that was positioned on an elevated wire grid floor inside a shoebox cage with bedding. The insert contained a hinged top and two small holes where the sippers of the drinking bottles entered into the cage. 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). Each time the circuit was completed, a lick was recorded for that individual animal. Lickometers were interfaced to an IBM computer running MED-PC IV software (MED Associates Inc.) to record time-stamped licks for each individual animal and each fluid presented.

Mice had approximately 23 hours access to a 10% (v/v) ethanol solution (10E) in one bottle and tap water in a second bottle for the duration of experiments (except when given only water as noted below). The 10E bottles were counterbalanced between the left and right

sides across lickometer chambers to minimize the influence of side preference. Each individual animal had its ethanol bottle on the same side for the duration of the experiment to allow consistent drinking routines to become established. We have used this strategy consistently in our previous lickometers studies (Ford et al., 2005a, 2005b, 2008a, 2008b).

Drugs

An ethanol solution (10E; Pharmco Products, Brookfield, CT) was prepared by dilution of a 200 proof stock in tap water. GAN was purchased from Dr. Robert Purdy (VA Research Foundation, San Diego, CA) and was solubilized in a solution of 3% (w/v) dimethyl sulfoxide (DMSO, Mallinckrodt Baker, Inc., Paris, KY) and 20% (w/v) 2-hydroxypropyl- β -cyclodextrin (β -cyclodextrin, Cargill Inc., Cedar Rapids, IA) in saline. FIN [1,(5 α)-androstane-4-aza-3-one-17 β -(N-tert-butyl-carboxamide)] was purchased from Steraloids Inc. (Newport, RI) and was solubilized into a 20% (w/v) β -cyclodextrin solution in saline. THIP (4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol hydrochloride) was purchased from Tocris Bioscience (Ellisville, MO) and dissolved in 0.9% saline. Drugs were injected IP in a volume of 0.01 ml/g body weight.

Acclimation

Mice were first acclimated to the lickometer chambers by singly housing them in the chambers with access to food and water for one week. They were then given continuous ethanol access for another week, followed by injections of the 20% β -cyclodextrin solution along with continuous 10E access for another week. This procedure acclimated the mice to the chambers and handling, and it was determined they had reached a stable 10E intake (defined as less than 10% variation for three consecutive days) before experiments began. Over the course of five months, all mice were administered multiple treatments in the following order: GAN, FIN or vehicle, and THIP.

Experiment 1: Effects of GAN on 10E Intake—Each day, bottles were removed and weighed to determine intake, and mice were weighed and injected with 20% β -cyclodextrin to acclimate them to handling. Immediately after injection, mice were placed back in the lickometer cages, and bottles were returned 30 minutes later so that access began immediately prior to the start of the dark cycle.

Once drinking was stable across 3 consecutive days of 20% β -cyclodextrin injections, all mice were given an injection of 3% DMSO/20% β -cyclodextrin (vehicle; 0 mg/kg GAN). There were 4 intervening days with 20% β -cyclodextrin injections, and on the 5th day all mice received 5 mg/kg GAN in 3% DMSO/20% β -cyclodextrin. Following 6 more days of 20% β -cyclodextrin injections, all mice received 10 mg/kg GAN in 3% DMSO/20% β -cyclodextrin. Baseline, which is shown for non-statistical comparative purposes, was calculated by averaging each of the three 20% β -cyclodextrin days that immediately preceded a drug or vehicle day. The current doses were chosen based on a pilot study in male Withdrawal Seizure Resistant mice where a 10 mg/kg dose of GAN produced a 30% decrease in ethanol intake over 24 hours (Finn and Ford, unpublished).

Experiment 2: Effects of FIN and FIN Withdrawal on 10E Intake—Mice were given a 3-week drug washout period where they were given daily vehicle injections (20% β -cyclodextrin) and continuous access to 10E. Mice again showed stable 10E intake with less than 10% variability across three consecutive days. Next, 10E was removed, and mice were given access to food and water for 19 days. The purpose of the 10E washout period was to examine the effect of FIN on the re-acquisition of 10E intake. Baseline was calculated by averaging the intake on the last 3 days prior to removal of 10E, and mice were group-matched for 10E intake on these days so that 14 mice were assigned to the FIN group and 10

mice were assigned to the vehicle group. Each day, bottles were weighed, and access to 10E resumed 24 hours after the first FIN injection (24 hour pretreatment time). The FIN group received daily injections of 100 mg/kg FIN for 7 days, followed by 7 days of vehicle injections (i.e., FIN withdrawal). The vehicle group received vehicle injections daily for the duration of the experiment.

Previous studies from our lab have shown that effects of FIN differ across treatment phase (Ford et al., 2008a; Ford et al., 2005a; Ford et al., 2008b). For this reason, data were split into four treatment phases: baseline (baseline days 1–3), acute FIN (FIN injections days 1–3), chronic FIN (FIN injections days 4–7), and withdrawal (FIN withdrawal days 1–7). In past studies, acute and chronic FIN withdrawal have been treated as separate phases (Ford et al., 2008a; Ford et al., 2005a). In the current study, however, a 2-way ANOVA with withdrawal phase and hour (through hour 6) as repeated measures revealed no significant withdrawal phase by hour interaction [$F(5,65) = 0.763$; $p = 0.580$]. The two withdrawal phases were therefore collapsed for simplicity and are referred to jointly as “withdrawal”.

Experiment 3: Effects of THIP on 10E Intake—After FIN treatment, mice were given a 10E washout period with access to food and water for one week. This was followed by a week of continuous 10E access, where animals were weighed and received saline injections daily. Again mice showed stable 10E intake over 24 hours with less than 10% variability across 3 consecutive days prior to any THIP treatment.

As before, bottles were weighed daily. Injections were given 30 minutes prior to the 10E bottles being put back in the chamber so that 10E access began just prior to the start of the dark cycle. A within-subjects design was used for THIP treatment. Mice were balanced for 10E intake and assigned to 1 of 2 groups, and mice previously treated with FIN were split evenly between the two groups. Group 1 received one dose of THIP per week in the following order: 16 mg/kg, 8 mg/kg, 4 mg/kg, 2 mg/kg. Group 2 received one dose of THIP per week in the following order: 8 mg/kg, 16 mg/kg, 2 mg/kg, 4 mg/kg. Mice were injected with saline on all intervening days and data for baseline (0 mg/kg) was calculated by averaging the 4 vehicle days that immediately preceded a treatment day. One mouse was removed at the start of the experiment due to excessive weight loss (vehicle-treated in experiment 2).

Statistical Analysis

The 10E dose (g/kg) consumed was calculated based on the grams of 10E depleted and the mouse's body weight on that day. Total fluid intake was the amount of 10E plus water consumed. Ethanol preference ratios were calculated from the 10E intake divided by total fluid intake. Cumulative and hourly 10E and water licks, as well as bout parameters were recorded using MED-PC IV software, and SoftCR version 4 (MED Associates, Inc.) was used to access the time-stamped data. As defined previously, a 10E bout was defined as a minimum of 20 licks with no more than a 60-s pause between successive licks (Ford et al., 2005b).

All statistical analyses were performed using SYSTAT 11. For GAN treatment, a two-way analysis of variance (ANOVA) was used with dose (0 mg/kg, 5 mg/kg, 10 mg/kg) and hour (1, 2, 3, 4, 5, 6) as repeated measures. For THIP treatment, a two-way ANOVA was used with dose (0 mg/kg, 2 mg/kg, 4 mg/kg, 8 mg/kg, 16 mg/kg) and hour (1, 2, 3, 4, 5, 6) as repeated measures. For FIN treatment, a two-way ANOVA was used with phase (baseline, acute FIN, chronic FIN, and withdrawal) and hour (1, 2, 3, 4, 5, 6) as repeated measures. In all cases, a significant interaction warranted follow-up with one-way ANOVAs at each hour with dose or phase as the repeated measure. In the event of a significant ANOVA, pairwise differences were determined by the Fisher's least significant difference multiple

comparisons procedure against baseline. For all analyses, statistical significance was set at $p \leq 0.05$. Graphs were made using GraphPad Prism 4 for Windows.

Results

Correlation Between Licks and g/kg 10E Consumed

Prior to any drug treatment, animals drank an average of 15.30 ± 0.35 g/kg over 24 hours and showed less than 10% variability between days on 5 consecutive days. As shown in Fig. 1, during this time, a significant positive correlation was found between 10E licks and 10E g/kg consumed ($r(24) = 0.694$; $p < 0.001$) supporting the validity of the lickometer system for estimating fluid intake across time. The average licks/g 10E was also calculated prior to any drug treatment, and data were removed from any lick analysis if an animal's licks/g fell outside 2 standard deviations of the average (1295 ± 976). Generally, discordances occur when an animal leans against the sipper so that liquid leaks but licks are not recorded, or when an animal paws at a piece of the sipper so that many licks are recorded but no liquid is lost.

A three-day average intake (g/kg 10E) was calculated before the start of each new experiment. The pre-drug g/kg 10E intakes for all 24 mice were: GAN baseline= 15.2 ± 0.69 , FIN baseline= 16.6 ± 0.71 , THIP baseline= 13.2 ± 0.73 . These represent normal fluctuations in baseline 24-hour 10E intake in male C57 mice across the five months of the studies. Bodyweight, recorded in grams, increased throughout the duration of the experiments: GAN baseline= 23.2 ± 0.34 , FIN baseline= 24.7 ± 0.35 , THIP baseline= 25.9 ± 0.37 .

Experiment 1: Effects of GAN on 10E Intake—Before GAN treatment, mice exhibited stable 24-hour drinking with less than 10% variability of 24-hour 10E intake over three consecutive days. Table 1 shows results of GAN on 24-hour parameters. All statistical analyses are against the within-subjects vehicle injection, and baseline measurements are provided for non-statistical comparative purposes. There was a significant effect of GAN on 24-hour g/kg 10E intake [$F(2,44) = 6.447$; $p = 0.004$] and 24-hour 10E licks [$F(2,44) = 3.252$; $p = 0.048$]. There was no effect of GAN on overall water licks or preference ratio over 24 hours.

The greatest 10E consumption by mice occurs at the beginning of the dark cycle. This has been previously reported and can be seen graphically in the review by Finn et al. (2010). Visual inspection of our data showed that this was in fact the time period where the greatest differences in drinking occurred in the present study. Therefore, to avoid floor effects from low drinking at the end of the dark cycle and throughout the light cycle, we chose to focus on the drug effects during the first 6 hours of the dark cycle. For the three 20% β -cyclodextrin days prior to the DMSO vehicle or any drug treatments, a 2-way ANOVA with day and hour (through hour 6) as repeated measures revealed no effect of day and no day by hour interaction of drinking prior to GAN or vehicle treatment, indicating stable drinking immediately prior to treatment.

Examining the first 6 hours of the dark cycle during vehicle, 5 mg/kg, and 10 mg/kg GAN treatment, a two-way ANOVA with dose and hour as repeated measures revealed a significant interaction [$F(10,220) = 4.424$; $p < 0.001$]. To examine the interaction, one-way ANOVA's were then performed at each hour with dose as a repeated measure. Figure 2 shows the effects of GAN at each of the first 6 hours of 10E access. Again, all statistical analysis are against the within-subjects vehicle treatment, and baseline values (the average of the three 20% β -cyclodextrin days that immediately preceded a vehicle or drug day) are given for non-statistical comparisons. Statistical analysis showed a dose dependent effect of GAN on 10E licks during hour 1 [$F(2,44) = 5.494$; $p = 0.008$], hour 2 [$F(2,44) = 6.841$; $p =$

0.003], hour 3 [$F(2,44) = 6.328$; $p = 0.004$], hour 4 [$F(2,44) = 3.799$; $p = 0.030$], and hour 5 [$F(2,44) = 4.804$; $p = 0.013$]. The effect of GAN was recovered by hour 6. See Fig. 2A-F for a summary of 10E licks by hour. During hours 1 through 6, there was only a significant effect on water licks during the first hour [$F(2,46) = 9.073$; $p = 0.001$], with 5 mg/kg ($p = 0.017$) and 10 mg/kg GAN ($p = 0.001$) enhancing water licks versus vehicle (data not shown).

First bout characteristics were examined in order to better elucidate the initiation and pattern of drinking (see Table 1). A significant effect of GAN dose on latency to first lick [$F(2,44) = 11.115$; $p < 0.001$] was seen; 5 mg/kg GAN ($p = 0.004$) and 10 mg/kg GAN ($p = 0.002$) were associated with a significant decrease in latency. Analysis also showed a significant effect of GAN dose on latency to first bout [$F(2,42) = 12.351$; $p < 0.001$], with a decrease following 5 mg/kg GAN ($p = 0.005$) and 10 mg/kg GAN ($p = 0.001$). There was no effect of GAN on first bout size.

Experiment 2: Effects of FIN and FIN Withdrawal on 10E Intake—For FIN experiments, all statistical analyses between phases were against the within-subjects baseline. As reported in the methods, a separate group of animals was given vehicle injections throughout the duration of this experiment. The vehicle group allowed us to dissociate the effects of reacquisition from the effects of FIN. There was a transient effect of reacquisition in that the vehicle animals decreased their drinking during the acute FIN phase, when 10E was first returned, during hour 2, and increased it during the withdrawal phase during hour 1 (data not shown). It can be seen from the vehicle error bars in Figure 3 that little variability occurred aside from this during the first 6 hours throughout the rest of the vehicle treatment. Due to the small and transient effects of reacquisition, the vehicle data are averaged for each hour and are shown in Figure 3 for non-statistical comparisons.

During the three baseline days preceding the removal of 10E and prior to any FIN treatment, mice exhibited stable 24-hour drinking with less than 10% variability in 24-hour 10E intake. During hours 1–6 of these days, there was some slight variability. A 2-way ANOVA for the 3 baseline days showed that there was a day by hour interaction. Post-hoc analysis showed that this was due to significantly higher drinking on baseline day 1 hour 1, baseline day 2 hour 5, and baseline day 3 hour 4 when analyzed across FIN animals. Even with these fluctuations, the averages for each hour still were not significantly different than pre-GAN hourly baselines. Given that these fluctuations were not systematic, and because the nature of this study used averages for all phases of the study, we do not believe that our decision to collapse the baseline data changed our interpretation of the study.

See Table 2 for a summary of FIN effects on 24-hour parameters. Among FIN-treated animals, there was a significant effect of phase on 24-hour g/kg 10E consumed [$F(3,39) = 16.535$; $p < 0.001$], 24-hour 10E licks [$F(3,39) = 3.625$; $p = 0.021$], 24-hour 10E preference ratio [$F(3,39) = 15.022$; $p < 0.001$], and 24-hour water licks [$F(3,39) = 29.18$; $p < 0.001$].

Figure 3 shows the effects of FIN phase at each of the first 6 hours of 10E access. A two-way ANOVA examining the first 6 hours of drinking and treatment phase as repeated measures revealed a significant interaction between hour and phase [$F(15,195) = 3.591$; $p < 0.001$]. To elucidate the interaction, one-way ANOVAs were performed at each hour with treatment phase as a repeated measure. There was an effect of phase for FIN-treated mice in hour 1 [$F(3,39) = 6.439$; $p = 0.001$], hour 2 [$F(3,39) = 7.791$; $p < 0.001$], hour 3 [$F(3,39) = 9.61$; $p < 0.001$], hour 4 [$F(3,39) = 3.30$; $p = 0.03$], and hour 5 [$F(3,39) = 4.577$; $p = 0.008$]. The effect was recovered by hour 6. In general, acute FIN treatment decreased 10E licks through hour 4. Chronic FIN treatment decreased 10E licks in hour 1 only, and the

withdrawal phase had no effect on 10E licks at any hour. See Fig. 3A-F for a summary of hourly 10E licks.

During hours 1 through 6, water licks were slightly, though non-significantly, increased in hour 1, and significantly increased in hours 2 ($p = 0.001$), 3 ($p < 0.001$), and 4 ($p < 0.001$) of acute FIN treatment, suggesting a lack of general fluid suppression by FIN (data not shown). There was no effect of treatment group on body weight.

Bout analysis, also shown in Table 2, shows a significant effect of phase on latency to first lick [$F(3,39) = 12.40$; $p < 0.001$] with acute FIN (191%, $p < 0.001$) and chronic FIN (118%, $p < 0.001$) associated with a significant increase in latency. There was also a significant effect of phase on latency to first bout [$F(3,39) = 14.492$; $p < 0.001$] with acute FIN (423%, $p = 0.001$) and chronic FIN (164%, $p < 0.001$) associated with an increase in latency. Importantly, there was no change in latency to first water lick during either of these phases (data not shown), indicating that decreases in 10E consumption were likely not due to general locomotor suppression. There was no effect of phase on first 10E bout size.

Effects of THIP on 10E Intake

Throughout the THIP portion of the experiment, there were 4 animals whose data were excluded from 10E lick analyses due to sensitivity issues with lickometer circuits, which led to a discordance between licks and g/kg 10E consumed. There were two instances of a 10E bottle leak and one instance where a water bottle weight was not recorded. A 2-way ANOVA with day and hour as repeated measures revealed no effect of day and no day by hour interaction for the first 6 hours of drinking for three days prior to THIP treatment, indicating stable drinking immediately prior to THIP treatment.

Because group 1 and group 2 of the THIP experiment received THIP doses in a different order, but the doses were not administered in a perfect pseudo-random design, statistical analysis was done to examine whether there was an order effect of the doses. A three-way mixed model ANOVA with group as a between-subjects factor and dose and hour (through hour 6) as repeated measures revealed no main effect of group [$F(1,17) = 2.314$; $p = 0.147$], no interaction between group and dose [$F(4,68) = 1.948$; $p = 0.112$], no interaction between group and hour [$F(5,85) = 1.601$; $p = 0.169$], and no 3-way interaction of group by dose by hour [$F(20,340) = 0.950$; $p = 0.524$]. This confirmed lack of a group effect, so data were collapsed between groups for all further analysis.

Analysis of the 24-hour data, shown in Table 3, revealed a significant effect of THIP dose on g/kg 10E consumed [$F(4,80) = 9.622$; $p < 0.001$]. Specifically, there was a significant decrease in g/kg 10E consumed with 16 mg/kg THIP versus baseline ($p < 0.001$). There was also a significant effect of dose on 24-hour 10E licks [$F(4,72) = 9.565$; $p < 0.001$]. Post hoc analysis showed an increase in licks with 2 mg/kg ($p = 0.011$) and 4 mg/kg THIP versus baseline ($p = 0.049$) and a decrease in licks with 8 mg/kg ($p = 0.043$) and 16 mg/kg THIP versus baseline ($p < 0.001$). There was a significant effect of 10E preference across doses [$F(4,76) = 4.182$; $p = 0.004$], with 8 mg/kg ($p = 0.013$) and 16 mg/kg ($p = 0.002$) THIP decreasing 10E preference. There was no effect of dose on the 24-hour water licks.

Figure 4 shows the effects of THIP at each of the first 6 hours of 10E access. A 2-way ANOVA performed over the first six hours of the dark cycle, with treatment dose and hour as repeated measures, revealed a significant interaction [$F(20,360) = 2.252$; $p = 0.002$]. To elucidate the interaction, a one-way ANOVA was performed at each hour with dose as a repeated measure until drinking returned to baseline. There was a significant effect of dose at hour 1 [$F(4,72) = 6.953$; $p < 0.001$], hour 2 [$F(4,72) = 19.902$; $p < 0.001$], hour 3 [$F(4,72) = 17.467$; $p < 0.001$], hour 4 [$F(4,72) = 8.257$; $p < 0.001$], and hour 5 [$F(4,72) =$

2.951; $p = 0.026$]. The effect of dose was absent at hour 6 (Fig. 4F). See Fig. 4A-F for a summary of 10E licks by hour. Water licks were decreased by the 16 mg/kg dose of THIP at hours 1 and 2, reflective of a sedative effect of this dose. Water licks were unaffected in hours 1 and 2 and enhanced during hours 3 and 4 with the 8 mg/kg dose THIP, indicating a selectivity for decreasing 10E intake during this time. Water intake during hours 1–6 was unaffected after 2 or 4 mg/kg THIP (data not shown).

Analysis of first bout characteristics, shown in Table 3, revealed an effect of dose on latency to first lick [$F(4,84) = 18.856$; $p < 0.001$], with 4 mg/kg (72%, $p = 0.001$) and 8 mg/kg (53%, $p = 0.001$) associated with a decrease and 16 mg/kg (134%, $p < 0.001$) associated with an increase compared to baseline. There was also a significant dose effect on latency to first bout [$F(4,80) = 16.215$; $p < 0.001$], with 8 mg/kg (103%, $p = 0.002$) and 16 mg/kg (317%, $p < 0.001$) associated with an increase. Finally, a significant dose effect on first bout size was revealed [$F(4,80) = 3.322$; $p = 0.018$]. Post-hoc analysis showed that first bout size was reduced following 8 mg/kg THIP (33%, $p = 0.001$).

Discussion

Growing evidence suggests that manipulation of neurosteroid levels, or other modulations at the GABA_A receptor, can influence voluntary 10E intake. Here we show that in a 24-hour two-bottle choice paradigm, C57 male mice drank significantly less in the first 6 hours of 10E access following FIN or THIP administration. This is in accordance with previous data from our lab showing that high doses of neurosteroids, or the inhibition of their synthesis, decreases ethanol intake in mice in a limited access session (Ford et al., 2008a; Ford et al., 2005a; Ford et al., 2005b). In addition, GAN shifted 10E intake so that drinking was increased in the first hour, decreased in the next two hours, and increased in hours 4 and 5, illustrating a similar, but extended pattern to that seen with low doses of ALLO (Sinnott et al., 2002). GAN, FIN, and THIP also altered first lick and first bout latency, and THIP decreased first bout size. Importantly, some human studies have indicated that total ethanol consumption is an insufficient predictor of the successfulness of therapeutic strategies (Bobak et al., 2004; Gmel et al., 2001); rather, the efficacy of treatment is reliant on drinking patterns (Anton et al., 2004; O'Malley et al., 2002). The differences in pharmacology between these drugs may underlie their differential effects on the bout parameters and patterns of consumption and may improve therapeutic approaches to alcohol use.

GAN Alters Ethanol Intake Patterns

GAN is a synthetic analog of the neurosteroid ALLO, but contains an added methyl group, making it more resistant to metabolism than ALLO (Hogenkamp et al., 1997). Previous work with neurosteroids has shown a bimodal effect of ALLO on alcohol intake in mice: low doses of ALLO that produce physiological concentrations of plasma ALLO increased ethanol intake in a 2-hr access procedure, while high doses of ALLO that produce supra-physiological plasma concentrations decreased ethanol intake (Finn et al., 1997b; Ford et al., 2005b). Specifically, the findings with GAN are consistent with data showing that low doses of ALLO dose-dependently increase ethanol intake in the first hour of access, and dose dependently decrease ethanol intake in the second hour of access (Sinnott et al., 2002). This increase in ethanol intake in the first hour is also consistent with data showing that low doses of ALLO increase operant lever presses for ethanol in a 30-minute limited access session and increase reinstatement for 10E in rodents in an operant procedure (Finn et al., 2008; Janak and Gill, 2003; Janak et al., 1998; Nie and Janak, 2003).

The effects of GAN in the present study lasted 5 hours into ethanol access in contrast to effects with ALLO, which persisted for about 80 minutes (Ford et al., 2008a; Ford et al., 2005b). Given that injections of ALLO and GAN when given at 1, 3, or 10 mg/kg resulted

in comparable behavioral and anticonvulsant properties on a battery of tests, it is possible that these doses of GAN and ALLO exhibit comparable doses on other indexes such as their effect on ethanol (Beekman et al., 1998; Gasior et al., 1997). If this is indeed the case, it would not be surprising that GAN affected 10E drinking in a similar but extended manner as has been shown for low doses of ALLO (Sinnott et al., 2002). It is important to keep in mind, however, that differences in pretreatment times and drug administration in relation to the dark cycle may complicate direct comparisons between the effects of GAN with those seen with ALLO in previous studies.

The effects of GAN on locomotor activity were not examined in the present study, however, Besheer et al., (2010) found no change in locomotion with the dose reported to increase self-administration in rats (1 mg/kg), and sedation only at a dose (30 mg/kg) much higher than that used in the present study and which decreased drinking in their study (Besheer et al., 2010).

FIN Decreases Ethanol Intake

Consistent with the drinking suppression seen in the 2-hour access studies that examined FIN effects on ethanol maintenance and acquisition (Ford et al., 2005a; Ford et al., 2008b), acute FIN administration in the current study decreased 10E intake in male mice by 53% during the first five hours of reacquisition to 10E continuous access. No effects of FIN withdrawal on 10E intake were observed during the 6 hourly measurements (Figure 3) in the present study, consistent with results in female C57 mice with the 100 mg/kg FIN dose (Ford et al., 2008a). It should be noted that the overall effects of acute FIN on 10E reacquisition were significant when compared with the animals' baseline values (Table 2) or versus values in the vehicle controls (not shown), which were slightly lower than baseline. Thus, even though the vehicle group did not exhibit an alcohol deprivation effect upon 10E reacquisition, the significant suppression of ethanol intake upon reacquisition indicate that FIN may be a useful therapeutic tool to prevent relapse among alcoholics and should be examined further.

Although we did not directly measure ALLO levels in these animals, previous work in our lab has shown that 100 mg/kg FIN decreases endogenous plasma levels of ALLO by over 80% in naïve C57 male mice when given at a 24-hour pre-injection time (Finn, unpublished). A decrease in ethanol intake with FIN is therefore consistent with data showing an increase in ethanol intake with exogenous 3.2 mg/kg ALLO administration, which results in elevated but still physiologically relevant plasma levels (Finn et al., 1997b; Ford et al., 2005b). However, in our recent examination of the effects of FIN on the acquisition of 10E intake, we found that ALLO levels were suppressed by 28% following the 7th FIN injection (50 mg/kg) and the 2 hour 10E drinking session, and that 10E intake was suppressed by 43% (Ford et al., 2008b). We did not observe a similar relationship with the 100 mg/kg FIN dose, but it should be noted that interpretation of these data from Ford et al. (2008b) has the potential confound that ALLO measurements were conducted after limited access 10E intake, which has been shown to significantly increase brain ALLO levels in male C57 mice (Finn et al., 2004). Thus, the putative involvement of GABAergic neurosteroids in the FIN-induced suppression of 10E intake is likely more complex than simply due to a concomitant suppression of ALLO levels following FIN treatment and will require additional studies that can more accurately measure several GABAergic steroid levels at various periods during the timecourse of FIN treatment and ethanol consumption.

In order to have a 24-hour pre-injection time, animals were injected immediately prior to the preceding drinking session. FIN at this dose causes noticeable sedative effects that one could argue are underlying the decrease in 10E intake observed (Gabriel et al., 2004). However, the substantial increase in water licks during these same time points (almost a twofold

increase in hour 1 and a 5–6-fold increase in hours 2, 3, and 4; data not shown) suggests that sedative effects were not the primary factor in decreasing 10E intake. Additionally, although FIN increased latency to first 10E lick, it had no effect on latency to first water lick, indicating that FIN administration did not cause an inability to locomote to the bottles. It is possible that the increase in water intake during FIN treatment was mediated by FIN's decrease of deoxycorticosterone (DOC) metabolism, another substrate of 5 α -reductase, as high concentrations of DOC have been shown to increase water intake in rats (Cooney and Fitzsimons, 1996). Future studies are necessary to determine the manner by which FIN increased water intake.

There was no difference in weight gain between FIN and vehicle treated animals, suggesting a lack of general food and fluid suppression and also suggesting there were not differences in general metabolism between groups. It is also unlikely that FIN's effects are a result of changes in ethanol metabolism; a previous study showed that FIN had no effect on blood ethanol concentrations following injections of ethanol in mice (Gorin-Meyer et al., 2007).

THIP Decreases Ethanol Intake

Currently, the effect of THIP on ethanol drinking in rodents is not clear, with some labs reporting an increase (Boyle et al., 1993) and others reporting a decrease (Moore et al., 2007). We reasoned that if THIP affected ethanol drinking in a similar manner to ALLO or GAN, it could provide clues for a possible mechanism explaining how neurosteroids alter ethanol drinking behavior. Specifically, THIP was used in the present study to reveal the influence of extrasynaptic GABA_A receptors on ethanol drinking. Although ethanol is known to modulate GABA_A receptors (Deitrich et al., 1989; Harris and Mihic, 2004), the localization and subunit specificity of the receptors acted on by ethanol remains highly controversial. A majority of *in vitro* studies showing potentiation at GABA_A receptors by ethanol only see this effect when using doses on the order of 100 mM to 300 mM, 2–3 times what is seen in humans even with extreme intoxication (Borghese et al., 2006; Casagrande et al., 2007; Mehta et al., 2007). Recent reports have revealed a subclass of GABA_A receptors located exclusively in the extrasynaptic membrane that are responsive to doses of ethanol on the magnitude of 1–30 mM, in the range seen for humans after alcohol consumption (Olsen et al., 2007; Sundstrom-Poromaa et al., 2002; Wallner et al., 2003). These extrasynaptic receptors are thought to be predominantly composed of a δ subunit in combination with α_4 or α_6 and β_2 or β_3 subunits and are responsible for tonic GABA_A receptor-mediated inhibition (Farrant and Nusser, 2005; Nusser et al., 1998; Stell et al., 2003). This is in contrast to synaptic receptors, which are responsible for phasic GABAergic inhibition and are predominantly composed of γ_2 along with β and α_1 , α_2 , or α_3 subunits (Farrant and Nusser, 2005). The sensitivity to low doses of ethanol make extrasynaptic receptors a plausible pharmacological target of ethanol (Kumar et al., 2009). The potentiation of these receptors with small doses of ethanol, however, is not consistently seen between labs (for a review on the subject see Borghese and Harris, 2007). Similar to ethanol, neurosteroids have been shown to have a high affinity for the δ subunit of the GABA_A receptor, raising the intriguing possibility that endogenous neurosteroids could be modulating ethanol intake via extrasynaptic GABA_A receptors (Brown et al., 2002; Fodor et al., 2005; Mihalek et al., 1999). Using THIP, we show that a drug that acts selectively on extrasynaptic receptors was able to affect ethanol drinking, suggesting that this is a functionally relevant pathway to explore further.

In the present study, the highest dose of THIP (16 mg/kg) greatly suppressed 10E intake but also led to a very noticeable sedative effect and suppressed water intake. It is possible that this high dose activated synaptic receptors as well as extrasynaptic receptors. On the other hand, 8 mg/kg THIP (see Figure 4) selectively decreased 10E drinking by 30% during the first 5 hours of access. Importantly this dose of THIP caused a decrease in latency to first

10E lick (Table 3) and first water lick (not shown), indicating a lack of sedative effects confounding the decreases in drinking. It also appeared that the 2 mg/kg and 4 mg/kg dose of THIP did not sedate the animals, as these doses caused no change and a decrease, respectively, in latency to both first water lick and first 10E lick.

It has been shown that rats injected with a 10 mg/kg subcutaneous dose of THIP had peak CNS concentrations of 2.9 μ M and that this concentration of THIP selectively activated extrasynaptic GABA_A receptors (Cremers and Ebert, 2007). Additionally, the peak concentration was reached 30 minutes post injection (Cremers and Ebert, 2007). Given that we had a pre-injection time of 30 minutes, that we used a lower dose (8 mg/kg) than Cremers and Ebert (2007), and that the injection took place in a species with a faster metabolism than rats, the peak levels of THIP in the current study were likely to be less than 2.9 μ M, implicating a role for extrasynaptic GABA_A receptors in modulating ethanol intake. Our dose-response effect also is consistent with a study showing that a 10 mg/kg injection of THIP in GABA_A receptor α_4 subunit knockout mice failed to induce sedative, analgesic, and ataxic behaviors seen in wild type mice after THIP injection (Chandra et al., 2006). The lack of effect of the 10 mg/kg dose of THIP in the knockout mice suggests that at this dose, THIP was affecting these behaviors exclusively via extrasynaptic receptors. On the other hand, the same study found that a 15 mg/kg injection of THIP caused ataxia, analgesia, and sedation in both wild type and knockout mice, consistent with the idea that our 16 mg/kg dose of THIP likely activated more than just extrasynaptic receptors (Chandra et al., 2006). Interestingly, the low doses of THIP increased 24-hour 10E licks while high doses decreased licks, mimicking the biphasic effect seen with neurosteroids.

There is evidence for extrasynaptic localization of GABA_A receptors in the thalamus, cerebellum, dorsal and ventral striatum, olfactory bulb, and cerebral cortex of the rat, and in the hippocampus, primarily in the dentate gyrus, of humans and rats (Brooks-Kayal et al., 1999; Fodor et al., 2005; Pirker et al., 2000). The localization in the nucleus accumbens (ventral striatum) is especially interesting as this area has well-documented GABAergic involvement in ethanol self-administration (Hodge et al., 1995; Hyttia and Koob, 1995). Additionally, ALLO infused into the nucleus accumbens fully substitutes for ethanol, suggesting that neurosteroids present in this region may be important for some of the subjective effects of ethanol (Hodge et al., 2001). A recent report also showed that deletion of GABA_A receptor α_4 subunits in the nucleus accumbens shell decreased ethanol consumption in rats (Rewal et al., 2009). Given the combination of these data, it is possible that neurosteroids acting at extrasynaptic GABA_A receptors in the nucleus accumbens may be contributing to some of ethanol's reinforcing effects.

The hippocampus also may play an important role in ethanol consumption, and ethanol consumption has been shown to be modulated by neurosteroids in this region. Voluntary consumption of ethanol is highly correlated with a change in c-fos expression in the hippocampus of C57 mice (Ryabinin et al., 2003). Additionally, infusions of ALLO into this brain region decreased voluntary ethanol consumption in rats (Martin-Garcia et al., 2007). Further, there is evidence that voluntary ethanol consumption might be controlled in part by extrasynaptic receptors in this region; administration of a benzodiazepine inverse agonist selective for the α_5 subunit, which is thought to be primarily confined to the hippocampus, decreased ethanol intake when given systemically or infused directly into the hippocampus (Cook et al., 2005; June et al., 2001). Future studies should further examine the relationship between neurosteroids and extrasynaptic GABA_A receptors, which could provide a better understanding of both the mechanism and the brain regions involved in neurosteroid modulation of alcohol intake.

Bout Analysis

Bout characteristics were differentially affected by GAN, FIN, and THIP in the current study. As noted earlier, elucidating patterns of drinking may be an important component to better understanding and treating alcohol-related disorders. Similar to what was seen in previous studies, FIN decreased overall voluntary ethanol intake in mice (Ford et al., 2008a; Ford et al., 2005a; Ford et al., 2008b). As has been reported, high doses of ALLO and FIN both decreased alcohol drinking, but did so by different mechanisms. Earlier work showed that whereas ALLO dose-dependently altered bout frequency, FIN decreased bout size (Ford et al., 2005a; Ford et al., 2005b). In the current study, first bout characteristics were assessed to provide clues as to how mice were initiating alcohol sessions. Here, FIN increased latency to first lick and latency to first bout, consistent with a delay in the initiation of ethanol drinking. GAN also affected first bout characteristics; 5 and 10 mg/kg GAN decreased both latency to first lick and latency to first bout, suggesting that GAN facilitated the initiation of ethanol drinking. Interestingly, 8 mg/kg THIP decreased time to first lick, while increasing time to first bout. First bout size was only affected by 8 mg/kg THIP, which led to a decrease. This indicates that the 8 mg/kg THIP dose might be affecting the initiation of drinking quite differently than either of the two other drugs. Namely, it appears that with this dose, animals were taking their first lick sooner than they had with vehicle, but their first completed bout was smaller and more delayed. The way these drugs work to alter bout parameters warrants further investigation, as they could increase our knowledge of how alcohol drinking patterns are initiated and maintained.

Limitations and Potential Confounds

Although all mice received an acute 5 mg/kg dose of GAN one week prior to an acute dose of 10 mg/kg GAN, and all mice received GAN before FIN or THIP treatment, we do not believe there was a pharmacological order effect of the drugs. Previous data show no difference in plasma levels after an acute versus chronic injections of 7 mg/kg GAN in rats, suggesting that no accumulation of GAN occurred (Reddy and Rogawski, 2000). To minimize confounds of previous treatment, treatment groups were matched for 10E intake at the beginning of testing each new drug. Additionally, animals treated with FIN and vehicle were balanced across the THIP groups. As mentioned, baseline 10E intake prior to the initiation of testing each drug did not differ from the normal fluctuations in 24 hour 10E consumption that we observe in male C57 mice across time, making it unlikely that drug carryover effects altered subsequent ethanol intake.

With 24 hour access procedures, it is difficult to pick a time to measure blood ethanol concentrations without knowing patterns of ethanol intake. Since the goal of these studies was to measure patterns of intake, we did not introduce the disruption of measuring blood ethanol concentration (BEC) during a particular hour post-injection. With limited access procedures, we have already shown that administration of ALLO and FIN did not alter ethanol metabolism, as the change in BEC corresponded to the change in ethanol intake in these studies (Ford et al., 2005a, 2005b, 2008a). Thus, it is unlikely that the effects of FIN on ethanol intake in the present study were due to an indirect effect on ethanol metabolism. Nonetheless, because we did not measure BECs in the present studies, we cannot rule out that the effect of GAN and THIP on 10E intake was due to an indirect effect on ethanol metabolism.

The present experiments were performed only in C57 male mice. Previous work in our lab has shown that female C57 mice were less sensitive to the behavioral and pharmacological effects of ALLO and FIN administration on ethanol intake (Finn et al., 2010; Ford et al., 2008a). For this reason, we decided to assess the robustness of the effects in males first, with

the goal of extending the findings in female mice given continuous 10E access in future experiments.

Although the present study illustrates selectivity of these GABA_A receptor acting compounds for ethanol versus water, their effects on other sweet or calorie-containing solutions is currently unknown. There have been mixed reports of ALLO's effects on sucrose or saccharin intake, which may reflect a species difference or procedural differences, such as concurrent versus separate access to the sweetened solution and ethanol (Sinnot et al., 2002; Janak and Gill, 2003). A general effect of GAN, FIN, and THIP on calorie-containing or sweet solutions therefore cannot be ruled out and remain the subject of ongoing investigation in our lab.

An additional consideration is that progesterone, testosterone and deoxycorticosterone are all metabolized by 5 α -reductase. Since FIN can block the conversion of these three steroids to their GABAergic neurosteroid derivatives, there may be a complex interaction between FIN, ethanol, and their subsequent interaction at GABA_A receptors (Finn et al., 2006). Therefore, we cannot rule out the contribution of other 5 α -reduced steroids to the alterations in 10E consumption observed with FIN.

Conclusions

Even in light of the caveats mentioned above, the characterization of the alterations in 10E intake patterns with GAN, FIN, and THIP strongly support a role for the modulation of ethanol intake by neurosteroids and GABA_A receptor-active compounds. A better understanding of the manner in which neurosteroids and GABA_A receptor active compounds affect drinking patterns may greatly increase our understanding and ability to treat alcohol-related disorders. Future studies should continue to look at this relationship and further characterize how extrasynaptic receptors may be involved.

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Figure 1.

Positive correlation between 24-hour 10E intake (g/kg) and licks on the 10E bottle. Data points show the average 10E licks and 10E g/kg consumed for each mouse ($n=24$) for the five baseline days preceding the first drug treatment. Solid line represents linear regression of the data. $p < 0.001$ as determined by a Pearson bivariate correlation analysis. 10E = 10% (v/v) ethanol solution

Figure 2. Effects of GAN on the first 6 hours of ethanol access. The number of licks of 10E with each dose of GAN are shown for hour 1 (A), hour 2 (B), hour 3 (C), hour 4 (D), hour 5 (E), and hour 6 (F). A collapsed mean of the baseline days immediately preceding a drug day are shown for non-statistical comparative purposes. One animal's data was removed from all 10E lick analysis due to a discordance between licks and g/kg consumed. Vertical bars represent the mean $\pm$ SEM for 23 mice. $+p \leq 0.10$, $*p \leq 0.05$, $**p \leq 0.01$ versus within subjects vehicle (0 mg/kg). 10E=10% (v/v) ethanol solution

Figure 3.

Effects of FIN on the first 6 hours of ethanol access. Total 10E licks during each phase of FIN (100 mg/kg) treatment are shown for hour 1 (A), hour 2 (B), hour 3 (C), hour 4 (D), hour 5 (E), and hour 6 (F). A collapsed mean of the last three 10E-access days before FIN treatment are shown as baseline. Vehicle treated animals' data were collapsed across treatment phase and are graphed for non-statistical comparative purposes. Vertical bars represent the mean $\pm$ SEM for 14 mice (FIN) or 10 mice (vehicle). + $p \leq 0.10$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ versus within subjects baseline. FIN=finasteride; 10E = 10% (v/v) ethanol solution

Figure 4.

Effects of THIP on the first 6 hours of ethanol access. The number of licks of 10E with each dose of THIP are shown for hour 1 (A), hour 2 (B), hour 3 (C), hour 4 (D), hour 5 (E), and hour 6 (F). A collapsed mean of the vehicle days immediately preceding a drug day are shown as 0 mg/kg. Four animals were removed from all 10E lick analysis due to discordance between licks and g/kg 10E consumed on a drug or baseline day. Vertical bars represent the mean $\pm$ SEM for 19 mice. $+p \leq 0.10$, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$ versus 0 mg/kg. THIP = gaboxadol HCl; 10E = 10% (v/v) ethanol solution

Table 1

Effect of GAN on 24-hour Measures and Bout Parameters

	Baseline	Vehicle	5 mg/kg GAN	10 mg/kg GAN
10E dose (g/kg)	14.55 ± 0.67	15.59 ± 0.76	13.98 ± 0.75**	14.18 ± 0.81*
10E licks	3983 ± 200	3981 ± 212	3942 ± 198	4308 ± 235*
Preference ratio	0.81 ± 0.04	0.82 ± 0.05	0.81 ± 0.05	0.82 ± 0.05
Water licks	1046 ± 229	972 ± 222	1068 ± 264	1059 ± 214
Latency to first lick (sec)	612 ± 340	1245 ± 341	174 ± 86**	48 ± 16**
Latency to first bout (sec)	1848 ± 363	2177 ± 532	445 ± 199**	48 ± 16***
First bout size	87 ± 7	102 ± 9	105 ± 13	107 ± 12

Each mouse received all GAN doses. Baseline was calculated by averaging each of the three days that immediately preceded a drug or vehicle day and are shown for non-statistical comparative purposes. One animal's data was removed from any 10E lick analysis because there was discordance between his licks and g/kg 10E. Values represent the mean ± SEM for 23 mice.

*
 $p \leq 0.05$,

**
 $p \leq 0.01$,

***
 $p \leq 0.001$ versus within subjects vehicle treatment. GAN = ganaxolone; 10E = 10% (v/v) ethanol solution

Table 2

Effect of FIN and FIN Withdrawal on 24-hour Measures and Bout Parameters.

	Vehicle	Baseline	Acute FIN	Chronic FIN	FIN withdrawal
10E dose (g/kg)	14.48 ± 1.48	16.62 ± 0.91	10.08 ± 1.46***	12.48 ± 1.48**	13.22 ± 1.36*
10E licks	4025 ± 357	4254 ± 276	3195 ± 446*	3775 ± 445	3627 ± 391
Preference ratio	0.73 ± 0.08	0.84 ± 0.06	0.47 ± 0.08***	0.56 ± 0.07**	0.64 ± 0.07*
Water licks	1338 ± 372	734 ± 346	3233 ± 536***	2847 ± 492***	1870 ± 386***
Latency to first lick (sec)	1925 ± 563	1911 ± 383	5563 ± 756***	4163 ± 405***	3042 ± 446
Latency to first bout (sec)	3079 ± 965	2225 ± 392	11636 ± 2196***	5866 ± 698***	4016 ± 755
First bout size	95 ± 9	95 ± 9	85 ± 12	100 ± 20	91 ± 8

Mice were given 7 days of 100 mg/kg FIN or vehicle followed by 7 days of vehicle (i.e. FIN withdrawal). Values represent the mean ± SEM for 14 mice for FIN treatment and 10 mice for vehicle treatment. Data for the vehicle-treated group were collapsed across all phases and are shown for non-statistical comparison.

* $p \leq 0.05$,

** $p \leq 0.01$,

*** $p \leq 0.001$ versus within subjects baseline. FIN = finasteride; 10E = 10% (v/v) ethanol solution

Table 3

Effect of THIP on 24-hour Measures and Bout Parameters.

	0 mg/kg THIP	2 mg/kg THIP	4 mg/kg THIP	8 mg/kg THIP	16 mg/kg THIP
10E dose (g/kg)	13.01 ± 0.80	13.28 ± 0.91	13.05 ± 0.94	12.20 ± 0.93	10.21 ± 0.96***
10E licks	3921 ± 233	4294 ± 295*	4378 ± 310*	3498 ± 290*	3077 ± 277***
Preference ratio	0.79 ± 0.04	0.76 ± 0.05	0.76 ± 0.05	0.71 ± 0.05*	0.68 ± 0.06**
Water licks	1182 ± 236	1356 ± 348	1095 ± 249	1296 ± 243	1363 ± 294
Latency to first lick (sec)	2267 ± 293	1900 ± 490	624 ± 329***	1048 ± 233***	5311 ± 687***
Latency to first bout (sec)	2949 ± 371	2093 ± 515	2208 ± 674	5996 ± 776**	12306 ± 2220***
First bout size	85 ± 4	100 ± 10	70 ± 10	57 ± 7***	72 ± 11

Each mouse received all THIP doses. Baseline (0 mg/kg) was calculated by averaging each of the four vehicle treatment days that immediately preceded a drug day. Four animals were removed from all 10E lick analysis due to discordance between licks and g/kg 10E consumed on one day. Values represent the mean ± SEM for 23 mice (10E dose; preference ratio; water licks), 19 mice (10E licks; latency to first lick; latency to first bout; first bout size).

* $p \leq 0.05$,

** $p \leq 0.01$,

*** $p \leq 0.001$ versus 0 mg/kg. THIP = gaboxadol HCl [4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol hydrochloride]; 10E = 10% (v/v) ethanol solution