Published in final edited form as:

Behav Brain Res. 2007 May 16; 179(2): 265–272.

Allopregnanolone influences the consummatory processes that govern ethanol drinking in C57BL/6J mice

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Abstract

Although systemic allopregnanolone (ALLO; a positive modulator of GABA_A receptors) has been shown to enhance ethanol-reinforced responding and to modulate drinking patterns in rodents, the effects of centrally administered ALLO on ethanol intake are not known. The current work examined the effects of intracranial ALLO on operant ethanol self-administration in food- and water-satiated mice, with a procedure designed to estimate ALLO's influence on appetitive versus consummatory processes. Male C57BL/6J (B6) mice were trained to press an ethanol-appropriate lever by being reinforced with 30-min of continuous access to a 10% ethanol solution. Following surgical implantation of a guide cannula aimed at the lateral ventricle and subsequent habituation to vehicle infusions, ALLO (50–400 ng; ICV) was delivered immediately prior to session start. ALLO doses of 100 and 400 ng were further evaluated for their effects on locomotor behavior within activity chambers. ALLO selectively modulated ethanol intake patterns associated with the onset and maintenance of self-administration, while leaving appetitive (i.e., ethanol seeking) measures unaltered. The effects of ALLO on drinking patterns were dissociable from changes in locomotor behavior, as evidenced by the absence of ALLO's influence on response frequency and horizontal distance traveled. These findings support the premise that manipulations in brain ALLO levels may influence the regulatory processes governing ethanol consumption.

Keywords

Animal model; Alcohol intake; Operant; Neurosteroid; Reinforcement; Lickometer; Drinking patterns; Locomotor activity

1. Introduction

Allopregnanolone (ALLO; 3 α -hydroxy-5 α -pregnan-20-one) is a potent positive modulator of GABA_A receptors, and its allosteric activity at GABA_A receptors is thought to underlie ALLO's profile as an anticonvulsant, anxiolytic, locomotor stimulant, and hypnotic [2]. These behavioral effects of ALLO are similar to those exhibited by ethanol, which exerts some of its effects through an interaction with GABA_A receptors (for review, see [18]). Furthermore,

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Supported in part by the Department of Veteran Affairs and grants AA10760, AA12439, AA07468, AA015234 and DA07262.

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ALLO is capable of substituting for ethanol's stimulus effects within drug discrimination procedures employing rodents and primates [3,17,19]. This indicates that the complex interoceptive cue elicited by ethanol (see [16]) demonstrates considerable overlap with ALLO. Like ethanol, ALLO and structurally related neurosteroids possess rewarding properties as determined by conditioned place preference in mice [9], 2-bottle choice preference in rats [41], and intravenous self-administration in rhesus monkeys [33].

We previously demonstrated that systemic administration of ALLO increased limited access ethanol preference drinking in male C57BL/6J (B6) mice in a dose- and time-dependent manner [42]. Furthermore, a subsequent study using lickometer circuits to assess ethanol consumption patterns of B6 mice revealed that ALLO dose-dependently altered ethanol intake patterns by promoting the onset of ethanol drinking (increased first bout size) and blunting the maintenance of consumption during the later half of a 2-hr session [14]. In contrast, acute treatment with the 5 α -reductase enzyme inhibitor finasteride, which blocks the biosynthesis of endogenous ALLO, suppressed the onset of ethanol self-administration [13]. Additional reports by other investigators have reported that ALLO dose-dependently modulates ethanol-reinforced responding in male rats [20,21] and reinstates ethanol-appropriate responding following extinction [26]. Collectively, these findings suggest that ALLO may influence the regulatory mechanisms underlying ethanol intake as well as modulate ethanol's reinforcing effects.

Several reports have documented behavioral and neurochemical manifestations of intracranially-administered ALLO. For instance, ALLO infusion into the nucleus accumbens (NAc) was observed to fully substitute for the discriminative stimulus effects of systemically administered ethanol in rats [19]. Additional studies demonstrated that ALLO administered via the intracerebroventricular (ICV) route accentuated the hypnotic effect of ethanol in mice [7] and dose-dependently increased DA levels within the NAc in rats [32]. Collectively, these findings point to a centrally-mediated involvement of ALLO in ethanol discrimination and hypnosis.

To date, data are not available on the effects of centrally administered ALLO on ethanol-reinforced responding in rodents. One concern stemming from systemic drug administration is the possibility that the exogenous agent exhibits peripheral effects that are unrelated to its centrally-mediated influence in modulating the behavioral endpoint of interest. For instance, ALLO elicits a localized peripheral analgesia, as indicated by an increased paw withdrawal latency from a thermal challenge, following doses as low as 10 ng [28]. Therefore, one goal of the present study was to assess the influence of centrally-administered ALLO on ethanol-reinforced responding and self-administration in male B6 mice to circumvent potential confounds associated with systemic application. A second goal was to assess ALLO's effects on mice that were uniquely trained in an instrumental conditioning procedure that separated the appetitive and consummatory phases of self-administration, as previously demonstrated in a rat model [37,38]. Furthermore, unlike previous studies with ALLO, cumulative drinking records and bout micro-architecture were monitored in the current work to determine the impact of this neurosteroid on drinking patterns throughout the operant sessions.

2. Materials and methods

2.1. Animals

Male B6 mice (6 weeks of age) were purchased from The Jackson Laboratory (Bar Harbor, ME). Each mouse was individually housed, acclimated to a 12hr/12hr light/dark cycle (lights on at 0600 hrs), and provided with *ad libitum* access to food and water (except when noted below). Animals were weighed and handled daily. 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* [25].

2.2. Apparatus

Self-administration sessions were carried out in eight modular mouse chambers ($21.6 \times 17.8 \times 12.7$ cm) with stainless steel grid floors (Med-Associates Inc., St Albans, VT). Each chamber was outfitted with a house light, 2 ultra-sensitive retractable levers, 2 stimulus lights, and a retractable sipper apparatus for extending/retracting a 10 ml graduated pipette with a double ball-bearing metal sipper tube. A lickometer circuit was connected to each metal sipper to monitor lick patterns. Circuits were interfaced to an IBM compatible computer operated by MED-PC software (Med-Associates Inc.). One wall of the chamber contained the levers located at opposite extremes (with a stimulus light positioned directly above each lever). The opposing wall included the access portal for the sipper tube. The chamber top accommodated a drug infusion apparatus (Instech Laboratories; Plymouth Meeting, PA), which included a multi-axis lever arm and low-resistance swivel that permitted mice to move freely within the chamber. The drug infusion swivel was attached to a 100 μ l gastight syringe (Hamilton Company; Reno, NV) mounted on a syringe pump (model PHM-100-1RPM; Med-Associates) by PE-20 tubing (polyethylene; Becton Dickinson and Company; Sparks, MD). Each chamber was housed within a sound-attenuating cabinet to control lighting and to exclude external noise.

2.3. Drugs

Saccharin solutions (w/v) were composed of sodium saccharin (Sigma-Aldrich Company; St. Louis, MO) in tap water. Ethanol solutions (v/v) were similarly made by the addition of ethyl alcohol (200 proof; Pharmco Products, Inc; Brookfield, CT) to tap water. Artificial cerebrospinal fluid (aCSF) was adjusted to pH 7.3 and was composed of 128 mM NaCl, 3.9 mM KCl, 1.2 mM CaCl_2 , 1.0 mM MgCl_2 , 25 mM NaHCO_3 , and 0.3 mM NaH_2PO_4 . ALLO was solubilized in a 20% w/v 2-hydroxypropyl- β -cyclodextrin (CDX; Cerestar USA, Inc., Hammond, IN) stock solution, which was then diluted 1:10 in aCSF to yield a working ALLO solution in 2% CDX. ALLO was synthesized by and purchased from Dr. R. H. Purdy (Veterans Medical Research Foundation, San Diego, CA).

2.4. Sipper and lever acquisition training phases

Instrumental response training was conducted with modifications to previously described procedures [23]. Mice were initially trained to consume a 0.2% w/v saccharin solution (0.2S) from a retractable sipper during two 20-min trials per day for 7 days. Continuous sipper access was permitted during the first 2 days. The remaining 5 days of training involved a gradual increase in the frequency of limited access opportunities as follows: multiple 4-min access periods interspersed by 1-min time outs resulting in 4 access opportunities per trial (4'-1'×4) on day 3, 3'-2'×4 on day 4, 2'-2'×5 on day 5, 1'-2'×7 on day 6, and 0.5'-2'×8 on day 7.

Mice were subsequently trained to respond on an appropriate lever to obtain 30-sec access to a sipper containing 0.2S on a fixed ratio 1 (FR1) reinforcement schedule throughout 30-min sessions. Upon completion of each FR1 requirement, both levers retracted, the house light turned off and a stimulus light illuminated for a 5-sec period, and the sipper was extended into the chamber. Following 30-sec of access, the sipper retracted and both levers were extended back into the chamber. Responding on the inappropriate lever had no scheduled consequence. To account for lever side preference, the ethanol-appropriate lever was counterbalanced between the left and right sides across subjects. Over successive training sessions the sipper access time was reduced from 30- to 15-sec to encourage the mice to elevate the frequency of responding. Twelve sessions were required to achieve a minimum criterion of ten 15-sec sipper presentations (reinforcers) per session. To enhance acquisition of responding, mice were water restricted for 16-hrs prior to each sipper training session and during the first 9 lever acquisition sessions, but thereafter were provided water and food *ad libitum*. No significant weight loss was observed during water restriction.

2.5. Saccharin fading procedure and reinforcement schedule manipulation

A modified saccharin fading procedure was used to initiate ethanol self-administration [23, 35]. Briefly, the ethanol concentration was increased within the 0.2S solution [for two sessions each at 0.2% saccharin/3% ethanol (0.2S/3E), 0.2S/6E, 0.2S/9E, and 0.2S/12E], and then saccharin was subsequently faded out in a step-wise manner to yield a 10% v/v ethanol (10E) solution [for 5 sessions each at 0.1S/12E, 0.05S/12E, 0.025S/12E, 0.01S/12E, and then 10E]. Mice were maintained on a FR1 schedule for 15-sec sipper presentations throughout the fading procedure. Sessions were conducted 5–6 days per week.

Over an ensuing 8-week maintenance phase, the schedule of reinforcement was incrementally increased to FR4. The appetitive and consummatory phases of self-administration were then procedurally separated (for reference, see [6]); the completion of 4 responses (response requirement 4; RR4) resulted in 30-min of continuous access to 10E. The RR was further increased to 8 during a subsequent 3-week period. A 20-min time limit to complete the RR was imposed.

2.6. Surgery

Mice were implanted with a unilateral guide shaft (26 ga. stainless steel, 11.0 mm long, imbedded in a threaded Teflon pedestal; Plastics One, Inc., Roanoke, VA) aimed at the lateral ventricle under anesthesia (ketamine/acepromazine/xylazine cocktail; i.p.) using a stereotaxic apparatus (Cartesian Research, Inc.) with the coordinates of anterior/posterior -0.46 mm, medial/lateral ± 1.20 mm, and dorsal/ventral 2.00 mm in relation to bregma [29]. Guide shaft placements were counterbalanced between the left and right lateral ventricles across subjects. Three stainless steel screws (size 000–120; Small Parts, Inc.; Miami Lakes, FL) were inserted into the skull to anchor the cranioplastic head mount that secured the cannula to the skull. Screw-down stylets (Plastics One, Inc.) were inserted into the guide shafts to maintain cannulae patency. A 0.3 mg/kg dose of the analgesic ketorolac (i.m.; Sigma-Aldrich Co.) was administered to each mouse immediately following surgery.

2.7. Tether habituation and allopregnanolone treatment

Stainless steel injectors (32 gauge; 11.5 mm long) were designed to extend 0.5 mm beyond the end of the guide shaft into the lateral ventricle. One end of the injectors was attached to PE-20 tubing leading from the drug syringe, and the other end was inserted into the guide shaft and stabilized to the threaded pedestal of the guide cannula by a screw-down cap. One week following surgery, mice were habituated to the experimenter handling associated with tether attachment. Following a 15-min time-out, tethered mice were given ICV infusions (1 μ l over 20-sec; computer-controlled) of 2% CDX in aCSF (vehicle; VEH), and then sessions were initiated after an additional 10-sec permitted for VEH diffusion. Once stable baseline responding (completion of RR8) and consumption ($< 15\%$ variability in g/kg consumed) were observed over three consecutive days, test sessions were conducted in which either VEH or ALLO (50, 100, 200, or 400 ng ICV) treatments were administered. Baseline responding and consumption were reestablished between ALLO treatments by conducting a minimum of 2 consecutive days of VEH infusions.

2.8. Blood ethanol concentration (BEC)

A 20 μ l tail vein blood sample was collected from each mouse immediately following 30-min access to 10E. The samples were assayed for BEC by gas chromatography as previously described [15]. Seven pairs of external standards with known ethanol concentrations (ranging from 0.25–4.00 mg/ml) were analyzed to construct a standard curve from which unknown ethanol concentrations were interpolated.

2.9. Locomotor activity

Locomotor chamber and automated activity monitoring (Accuscan; Columbus, OH) were employed as previously described in detail elsewhere [30]. Photocell beam breaks were tabulated by the Accuscan analyzer during testing and were converted to total horizontal distance traveled (cm). Activity sessions were conducted for 30-min to parallel the approximate time course of the self-administration sessions. Mice remained experimentally naïve for 4 weeks between the conclusion of the self-administration sessions and the assessment of locomotor activity. Mice were acclimated to the procedure room for 45-min prior to activity testing. Microinjections into the lateral ventricle were administered in a manner identical to that described above for the ethanol self-administration sessions. Each mouse was weighed, tethered, and returned to its home cage for 60-sec prior to infusion. Following infusion, the mice remained tethered for an additional 40-sec (to allow for diffusion), injectors were removed and replaced with stylets, and the mice were then immediately placed into the center of the activity chamber floor (40 × 40 cm). All mice received two ALLO doses (100 and 400 ng, ICV) over a 6 day period. Prior to each ALLO treatment day, locomotor activity levels were monitored for stability across 1–2 habituation sessions and 1 baseline session during which VEH was infused. Mice received the ALLO doses in a counterbalanced manner such that two groups were balanced for initial basal activity and received different initial doses of ALLO (i.e., either 100 or 400 ng the first week followed by the alternative dose the second week).

2.10. Histological confirmation

Following all testing procedures, mice were infused with a 17 mg/ml methylene blue dye in VEH in a manner identical to that described above for locomotor activity assessment. Following euthanasia, intact whole brains were rapidly removed from the skull and flash frozen in isopentane and stored at –80°C until sectioned. Brain slices were prepared with a microtome (35 µm sections), mounted on glass slides, and photographed with an IM50 imaging system (Leica Microsystems Imaging Solutions Ltd.; Cambridge, UK) to confirm guide shaft placement. Correct guide shaft placement was determined by a dye-lined ventricular system and/or a visible cannula tract penetration through the corpus collosum into the lateral ventricle. Mice with incorrect or unverified injector placements were excluded from statistical analyses.

2.11. Data analysis, response criteria and statistics

Ethanol intake (grams of ethanol per kilogram of body weight) was determined from the 10E volumes depleted (to the nearest 1/20 ml) and pre-session body weights. Cumulative records of responding and licks were monitored via MED-PC software. Appetitive and consummatory measures were derived from these cumulative records. Based on previous experience [12,13, 14], an ethanol bout was defined as ≥ 20 licks separated by less than a 60-sec pause between successive licks. Mice were required to meet two performance-based criteria to be included in the analysis: 1) completion of the RR8 for at least three of the four ALLO doses examined (including the test session and preceding VEH treatment sessions), and 2) consistent demonstration of a 100-lick minimum (approximately 0.25 g/kg ethanol). Because neither the appetitive nor the consummatory response measures observed during baseline sessions (VEH infusion sessions the day prior to each ALLO test session) were significantly different, these data were collapsed and designated as the 0 ng ALLO dose.

For the locomotor activity sessions, there was no dose order effect detected between the two cohorts run (see above), so order was not included as a variable in further analyses. The total distance traveled was stable (less than 5% variability) across the baseline session and the habituation session conducted the day before. It also was found that within-group baseline activity levels established prior to each of the two neurosteroid doses were not significantly different across treatments, and these basal activity counts were collapsed and designated as the 0 ng ALLO dose.

All statistical analyses were performed using the SigmaStat version 2.0 software package (Jandel Scientific; San Rafael, CA). One-way repeated measures analysis of variance (ANOVA) was used to evaluate the within-subject effect of ALLO dose on appetitive and consummatory measures. Two-way ANOVA (factors: dose and interval/epoch) was similarly employed in the analysis of horizontal distance traveled. The Tukey's multiple comparisons procedure was conducted to delineate pair-wise differences in the event that a significant main effect or an interaction was determined. Correlations between variables were evaluated by the Pearson product moment test. In all cases, statistical significance was set at $P \leq 0.05$.

3. Results

The results represent two cohorts of mice that were trained and tested using identical procedures, and no statistical differences in appetitive or consummatory measures were found between cohorts. Of the 22 mice meeting the performance-based criteria described above, 17 were confirmed to have injector placements into the lateral ventricle, thereby resulting in a 77% hit rate. A representative photomicrograph is depicted in Figure 1. In cases where mice did not complete the RR-8, the corresponding appetitive and consummatory measures were not included in the analyses. One subject was unable to complete the RR-8 during the testing of the 50 and 400 ng doses of ALLO (not the same mouse for each), whereas two mice fell short of completing the RR-8 when the 200 ng dose was administered. The mean $\pm$ SEM body weight throughout the treatment time course was 29.0 ± 0.5 g, with average weights ranging between 28.6–29.4 g.

3.1. Appetitive response measures

Response frequencies on the ethanol-appropriate and inappropriate levers were unaltered by ALLO treatment (Table 1). Furthermore, ALLO exhibited no effect on any appetitive measure evaluated for ethanol-appropriate responding (Table 1). The response rate on the appropriate lever was significantly correlated with the size of the first bout ($r = 0.35$, $P < 0.001$, $n = 80$), whereby a higher response rate was associated with a greater number of licks in the first bout. A significant negative correlation between the latency to first lick and the ethanol dose (g/kg) consumed ($r = -0.23$, $P < 0.05$, $n = 80$) was also noted, with greater latencies being associated with reduced overall consumption.

3.2. Ethanol intake and bout patterns

A statistically significant correlation between ethanol dose (g/kg) and total licks was found ($r = 0.92$; $P < 0.001$, $n = 80$), indicating that sipper contacts accurately reflected actual ethanol consumption throughout the 30-min access period. A trend towards a significant effect of ALLO treatment for ethanol dose [$F(4,59) = 2.29$; $P = 0.07$] was noted. A main effect of ALLO dose on mean bout size [$F(4,59) = 4.12$; $P < 0.01$] was observed, with the 50 ng dose significantly augmenting the number of licks per bout ($P < 0.05$) when compared to baseline (Table 2). In addition, a statistically significant effect of ALLO dose on bout duration was detected [$F(4,59) = 3.09$; $P < 0.05$], which was due to the significant increase in bout duration ($P < 0.05$) following the 50 ng ALLO dose. ALLO dose was without influence on bout frequency and lick rate (Table 2).

3.3. Temporal distribution of lick patterns

Mean cumulative lick patterns following ALLO treatments were compiled from all mice, and are depicted as 10-min session intervals in Figure 2. A two-way repeated measure ANOVA revealed significant main effects of ALLO dose [$F(4,44) = 2.91$; $P < 0.05$] and session interval [$F(2,22) = 23.94$; $P < 0.001$], and a statistically significant dose X interval interaction [$F(8,88) = 5.03$; $P < 0.001$]. Subsequent simple main effect analyses for ALLO dose within each session interval detected a significant impact of ALLO dose only within the 0–10 min interval [$F(4,59)$]

= 6.46; $P < 0.001$]. The 50 ng dose significantly augmented licks during the initial 10-min of ethanol access ($P < 0.05$), whereas the 400 ng dose significantly reduced licks throughout this initial portion of the 30-min limited access ($P < 0.01$).

3.4. First bout analysis

From the temporal distribution analysis of licks (Fig. 2), it was apparent that the greatest proportion of total session licks occurred within the initial 10-min of ethanol access. Thus, analyses focused on parameters associated with the first consummatory bout (see Table 2), during which time the potential effects of ALLO on the regulation of consumption onset would most likely be observed. A significant influence of ALLO dose on the first bout size [$F(4,59) = 8.16$; $P < 0.001$] was detected. Consistent with the elevated number of licks during the 0–10 min session interval (Fig. 2), the 50 ng ALLO dose significantly enhanced first bout size ($P < 0.01$) by 62% when compared to baseline measures (Table 2). Although a significant main effect of ALLO on first bout duration [$F(4,59) = 3.20$; $P < 0.05$] also was observed, pair-wise comparisons were unable to detect significant differences between any single ALLO dose and vehicle treatment.

3.5. Blood ethanol concentrations (BECs)

Detectable BECs were observed immediately following 30-min ethanol access (mean: 29 ± 8 mg%; range: 0–107 mg%). A statistically significant correlation between BEC and g/kg intake was found ($r = 0.92$; $P < 0.001$; $n = 16$). The collection of a blood sample from one mouse could not be obtained.

3.6. Locomotor activity

ALLO dose was without effect on the total horizontal distance traveled throughout a 30-min test (Fig. 3A). When locomotor activity was evaluated in 5-minute epochs, a significant influence of time [$F(5,30) = 244.64$; $P < 0.001$] was observed. Locomotor activity recorded during the first 5-min period (Fig. 3B) was significantly greater than all other epochs ($P < 0.001$ for all cases). However, no influence of ALLO dose was found.

4. Discussion

The current report is the first to examine the effects of intracranial ALLO on the responding for and self-administration of ethanol in mice, and to discern ALLO's influence on ethanol-reinforced responding versus consumption. ALLO did not influence the appetitive measures examined. In contrast, the lowest ALLO dose (50 ng) significantly enhanced mean bout size and duration, indicating that ALLO modulated the micro-architecture of consumption maintenance. The same ALLO dose promoted consumption onset by significantly augmenting the first bout size as well as the cumulative lick frequency during the initial 10-min of the session. The absence of ALLO effects on locomotor behavior and the frequency of total responses indicate that ALLO's impact on drinking patterns was not attributable to a non-selective effect on activity.

The effects of intracranial ALLO on drinking patterns closely resemble the previously reported influence of systemically-administered neurosteroid assessed within a 2-bottle choice procedure in mice (see [14]). The 50 ng (ICV) and 10 mg/kg (intraperitoneally; IP) doses similarly augmented both the mean and first bout sizes. Furthermore, a low systemic dose of ALLO (3.2 mg/kg, IP) significantly elevated the maintenance of ethanol licks, whereas higher doses (17 and 24 mg/kg, IP) suppressed licks [14]. This cumulative lick profile mirrored the current finding that 50 ng ALLO enhanced licks during the initial 10-min of the self-administration session whereas 400 ng led to a significant attenuation in licks. The acute application of the 5 α -reductase inhibitor finasteride, which blocks the synthesis of endogenous

ALLO, also affected mean bout size, first bout size, and bout duration components of bout micro-architecture (see [13]), but the effects were exactly the opposite to those observed following 50 ng ALLO in the current study. Thus, ALLO modulates ethanol intake patterns in a consistent manner regardless of the route of neurosteroid administration or the self-administration procedure utilized. The current observations, taken in concert with earlier findings following finasteride treatment, suggest that ALLO may be a physiologically-relevant endogenous modulator of regulatory processes underlying ethanol consumption.

Although the current work did not evaluate the specificity of ALLO's effect on ethanol self-administration, previous work from our laboratory and others have reported disparate findings on this issue. In a study evaluating concurrent responding for 10% ethanol and a 1% sucrose solution in male rats, a 5.6 mg/kg dose of ALLO selectively enhanced responding for ethanol while leaving sucrose responding unaltered [20]. Another study revealed that a 3-day regimen of 10 mg/kg ALLO administered to male mice augmented either 10% ethanol or a 0.033% saccharin solution provided under a limited access 2-bottle preference procedure in the home cage [42]. Consistent with the latter observation, ALLO (0.5 to 2 mg/kg) has been shown to elicit a hyperphagic feeding response in male mice [31]. Clearly, additional effort is needed to tease apart the specificity of ALLO's influence on ethanol reinforcement and consumption under both operant and non-operant conditions.

ALLO predominantly elicited its impact on consumption patterns at the lowest dose administered (i.e., 50 ng) whereas higher doses exhibited no difference from the vehicle control. The occurrence of a narrow effective dose range as well as a bimodal dose-response function in the current study is consistent with numerous earlier observations in rodent models and humans. A 3 mg/kg ALLO dose, but not 1 or 10 mg/kg, significantly enhanced ethanol-reinforced responding in rats [21]. Similarly, our laboratory previously reported a significant enhancement in ethanol intake following systemic pre-treatment with 3.2 mg/kg ALLO, no change with a 10 mg/kg dose, and a significant decrease with 24 mg/kg [14]. Intra-accumbal infusion of 0.55 and 1.0 ng ALLO (~1.5 and 3 pmol) partially substituted for a systemic ethanol injection in a drug discrimination procedure, whereas lower and higher doses were without effect [19]. Similarly, in a mouse model of aggression, 10 and 17 mg/kg ALLO elevated sideways threats and attack bites, whereas 3 mg/kg had no effect, and 30 mg/kg decreased these measures [11]. A recent clinical report also demonstrated that the ALLO concentrations stemming from 30 mg progesterone, but not 0, 60 or 200 mg were associated with enhanced negative mood scores in postmenopausal women [1]. Lastly, administration of 100 pmol ALLO into the lateral ventricle was found to elevate extracellular dopamine content in the NAc [32] whereas 45 nmol ALLO suppressed this measure [24]. Notably, ALLO's peak effect (50 ng; equivalent to 150 pmol) on bout micro-architecture in the present study closely resembled the concentration and route of ALLO administration (100 pmol; ICV) that led to enhanced dopamine content in the NAc. These observations collectively indicate that the behavioral and neurochemical manifestations of ALLO's CNS effects occur within a limited dose range. Multiple interpretations of ALLO's biphasic dose-response function have been offered. It is believed that higher ALLO doses reverse the influence of low to moderate doses by a mechanism that may involve disinhibition [1], shift from modulation to direct activation of chloride flux through GABA_A receptors [22], and loss of specificity for GABA_A receptors [8,34].

Because systemically-administered ALLO has been previously shown to produce locomotor stimulation in B6 mice [27], it was necessary to demonstrate a dissociation between ALLO's effects on ethanol self-administration versus general locomotor activity. The absence of locomotor effects with 100 and 400 ng ALLO is congruent with the unpublished observation that doses in excess of 1 µg were required to elicit locomotor stimulation when administered intra-VTA (Phillips & Finn, unpub). When taken in conjunction with the lack of ALLO-

induced changes in the number of total responses on both levers, it is unlikely that the altered drinking patterns following treatment with the 50 ng dose or higher doses were attributable to a general locomotor effect of ALLO.

As previously predicted [4], the procedural separation of appetitive and consummatory phases of ethanol self-administration in rodents has proven to be an effective means to dissociate the influence of pharmacological interventions on processes underlying ethanol seeking versus drinking. It has been demonstrated that systemic and brain site-specific pharmacological interventions can selectively impact appetitive versus consummatory processes [5,6,36,39] or influence both concomitantly [40]. In the present work, ALLO influenced consummatory processes. However, the absence of an effect of ALLO on appetitive processes associated with ethanol intake should be interpreted cautiously. The response requirement (RR) employed in the current work was modest (i.e., 8 lever presses) when compared to earlier work by Samson and colleagues in rats (i.e., RR30) using a similar operant procedure [6]. The lower RR in the present work was dictated by a general lower level of responding in mice when compared to rats. A more robust appetitive component (i.e., greater RR) may be necessary to reveal an effect of ALLO on ethanol-seeking behavior in mice.

In conclusion, the present findings provide evidence that centrally-active ALLO modulates ethanol intake patterns associated with the onset and maintenance of self-administration. When taken in conjunction with the recent observations that ethanol consumption increased endogenous ALLO concentrations in B6 mice [10] and human adolescents [43,44], and that the inhibition of ALLO biosynthesis attenuated ethanol intake [13], the current findings are consistent with the hypothesis that alterations in endogenous ALLO levels may influence the regulatory processes governing ethanol consumption and reinforcement. The development of pharmacological interventions that manipulate endogenous ALLO concentrations may prove to be a beneficial treatment strategy.

Acknowledgements

The authors would like to thank Sarah Eddy, Michelle Tanchuck, Cheryl Reed, and Carrie McKinnon for their technical assistance, and Christine Czachowski for her helpful insights.

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Fig 1.
Representative coronal brain section confirming guide cannulae placement into the lateral ventricle.

Fig 2.

The temporal distribution of licks following ICV ALLO. Cumulative records were compiled and collapsed for all mice and depicted in 10-min session intervals. Each bar represents the mean $\pm$ SEM of 17 mice. * $p < 0.05$, ** $p < 0.01$ versus respective baseline (0 ng dose) values.

Fig 3. The effects of ALLO (ICV) on locomotor activity. The total horizontal distance traveled (centimeters) is depicted for the entire 30-min test (panel A) and in 5-min epochs (panel B). Each bar represents the mean $\pm$ SEM of 7 mice.

The effects of ICV ALLO on appetitive measures of operant self-administration. The values represent the mean $\pm$ SEM of 17 mice. The analyses of appropriate lever measures include only those mice completing the RR8. The latency to 1st lick depicts the time between completion of the RR8 and the first lick exhibited. ALLO did not significantly alter any appetitive measure evaluated.

Table 1

	Allopregnanolone Dose (ng)				
	0	50	100	200	400
Response Frequencies					
Ethanol-Appropriate Lever	8.00 $\pm$ 0.00	7.59 $\pm$ 0.41	8.00 $\pm$ 0.00	7.24 $\pm$ 0.54	7.53 $\pm$ 0.47
Inappropriate Lever	0.97 $\pm$ 0.28	1.18 $\pm$ 0.69	0.88 $\pm$ 0.45	2.47 $\pm$ 1.17	1.94 $\pm$ 0.84
Total Levers	8.97 $\pm$ 0.28	8.76 $\pm$ 0.84	8.88 $\pm$ 0.45	9.71 $\pm$ 1.32	9.47 $\pm$ 1.02
Appropriate Lever					
Response Latency (sec)	46 $\pm$ 8	76 $\pm$ 31	49 $\pm$ 14	67 $\pm$ 23	50 $\pm$ 12
Response Rate (presses/sec)	1.22 $\pm$ 0.34	0.98 $\pm$ 0.36	0.83 $\pm$ 0.30	0.86 $\pm$ 0.29	1.02 $\pm$ 0.35
Latency to 1 st lick (sec)	8.6 $\pm$ 2.5	14.4 $\pm$ 6.7	14.0 $\pm$ 7.6	29.2 $\pm$ 22.1	28.6 $\pm$ 23.0

The effects of ICV ALLO on consummatory measures of operant ethanol self-administration. The values represent the mean ± SEM of 17 mice. Lick rates refer to sipper contacts occurring only during the experimentally defined consummatory bouts. * $p < 0.05$, ** $p < 0.01$ versus respective baseline (0 ng dose) value.

Table 2

	Allopregnanolone Dose (ng)				
	0	50	100	200	400
Mean Consummatory Measures					
Ethanol dose (g/kg)	0.71 ± 0.07	0.89 ± 0.11	0.73 ± 0.09	0.74 ± 0.07	0.64 ± 0.10
Total licks	279 ± 29	334 ± 39	288 ± 37	302 ± 29	254 ± 39
Bout size (licks)	82 ± 4	149 ± 30*	78 ± 8	80 ± 12	76 ± 18
Bout frequency	3.5 ± 0.4	3.3 ± 0.6	3.6 ± 0.5	4.1 ± 0.4	3.7 ± 0.7
Bout duration (min)	0.8 ± 0.1	1.7 ± 0.4*	0.7 ± 0.2	0.9 ± 0.2	0.9 ± 0.3
Lick rate (licks/min)	292 ± 39	248 ± 47	308 ± 48	246 ± 43	279 ± 55
1st Bout Measures					
Bout size (licks)	129 ± 11	209 ± 25**	137 ± 22	101 ± 16	91 ± 20
Bout duration (min)	1.0 ± 0.1	1.9 ± 0.4	0.8 ± 0.2	1.0 ± 0.3	0.8 ± 0.3
Lick rate (licks/min)	250 ± 40	173 ± 35	335 ± 52	223 ± 52	292 ± 64