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ALCOHOLISM CLINICAL & EXPERIMENTAL RESEARCH

ORIGINAL ARTICLE

The impact of intermittent exercise on mouse ethanol drinking and abstinence-associated affective behavior and physiology

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Abstract

Background: Negative emotional states are associated with the initiation and maintenance of alcohol use and drive relapse to drinking during withdrawal and protracted abstinence. Physical exercise is correlated with decreased negative affective symptoms, although a direct relationship between drinking patterns and exercise level has not been fully elucidated.

Methods: We incorporated intermittent running wheel access into a chronic continuous access, two-bottle choice alcohol drinking model in female C57BL/6J mice. Wheel access was granted intermittently once mice established a preference for alcohol over water. After 6 weeks, alcohol was removed (forced abstinence) and mice were given continuous access to unlocked or locked wheels. Negative affect-like behavior, home cage behavior, and metabolic activity were measured during protracted abstinence.

Results: Wheel access shifted drinking patterns in the mice, increasing drinking when the wheel was locked, and decreasing drinking when unlocked. Moreover, alcohol preference and consumption were strongly negatively correlated with the amount of running. An assessment of negative affect-like behavior in abstinence via the novelty suppressed feeding and saccharin preference tests (SPT) showed that unlimited wheel access mitigated abstinence-induced latency increases. Mice in abstinence also spent more time sleeping during the active dark cycle than control mice, providing additional evidence for abstinence-induced anhedonia- and depression-like behavior. Furthermore, running wheel access in abstinence decreased dark cycle sleep to comparable alcohol- and wheel-naïve mice. Given the positive impact of exercise and the negative impact of alcohol on metabolic health, we compared metabolic phenotypes of alcohol-abstinent mice with and without wheel access. Wheel access increased energy expenditure, carbon dioxide production, and oxygen consumption, providing a potential metabolic mechanism through which wheel access improves affective state.

Conclusions: This study suggests that including exercise in AUD treatment regimens has the potential to reduce drinking, improve affective state during abstinence and could serve as a non-pharmacological approach to prevent the development of an AUD in high-risk individuals.

KEYWORDS
abstinence, affect, EtOH, exercise, metabolism

INTRODUCTION

Alcohol use is commonly cited for its rewarding and social components, as well as its widespread use as a coping mechanism for alleviating stress and negative emotion (U.S. Department of Health and Human Services (HHS), 2016; NANOS Research, 2020). In a subset of individuals, casual alcohol use can shift to habitual use and negative reinforcement-driven dependence. While the focus of addiction and affective disorder treatment has been on discovering new pharmacotherapeutics, there are limited new treatments on the horizon. Preventing the emergence of an alcohol use disorder (AUD) or curbing the progression by promoting nonpharmacological interventional strategies for positively coping with stress could alleviate some of the dependence on pharmacological and clinical treatments.

Clinical and preclinical studies have demonstrated that exercise has a variety of positive actions on general health and on the brain (Cotman et al., 2007), including procognitive effects, mood elevation, and increased neurogenesis, in both healthy and psychopathological states (Bjornebekk et al., 2005; Cotman et al., 2007; West et al., 2019). Moreover, the potential for exercise in treating or preventing substance use disorders has been examined in several models (Lynch et al., 2013). To date, preclinical and clinical exercise studies have yielded somewhat mixed results in mitigating alcohol intake (Bardo & Compton, 2015; Costa et al., 2019; Jensen et al., 2019; Roessler et al., 2017; West et al., 2019), suggesting the need for a greater understanding of the intersection between exercise and brain centers involved in alcohol-related behaviors. A number of rodent studies have examined the relationship between alcohol drinking and voluntary exercise (for review see Leasure et al., 2015). Voluntary wheel running generally produces reductions in alcohol intake (Booher et al., 2020; Darlington et al., 2014; Gallego et al., 2015; McGonigle et al., 2016), although increases have also been reported (Lynch et al., 2019). In addition, withdrawal-associated seizures (Devaud et al., 2012; McCulley et al., 2012) and negative affective behavior (Pang et al., 2013a, 2013b, 2019) have been observed. Conflicting outcomes may in part be dependent on rodent strain, sex, EtOH exposure model, and wheel access schedule, outlining the need for more research in this area.

In this study, we begin to bridge the gap in knowledge between the benefits of exercise, drinking behavior, and the associated abstinence-induced negative affect. We tested the hypothesis that EtOH drinking behavior and exercise are negatively correlated, and that voluntary exercise can mitigate abstinence-induced negative affect and positively impact metabolic activity. We incorporated intermittent running wheel access into a continuous access chronic EtOH drinking model to determine the impact of wheel running on drinking behavior and motivation to drink in female C57BL/6J mice. This integrative model allows for repeated measurement, within-subject comparisons of individual drinking patterns with and without wheel access, facilitating precise correlative analyses. While physical exercise is known to have positive impacts on metabolic health and mood/affective state (Cotman et al., 2007; Dua & Hargreaves, 1992), and alcohol is known to negatively impact metabolic health (Manzo-Avalos & Saavedra-Molina, 2010), the direct combined impact of alcohol drinking and exercise on metabolic activity has not been well established. We characterized metabolic and behavioral activity in abstinence using indirect calorimetry. Incorporating metabolic activity markers into preclinical alcohol models and building a database of metabolic phenotypes has the potential to significantly advance our understanding of the relationship between exercise, EtOH drinking behavior, and abstinence-induced negative affect. To this end, we determined the effect of running wheel access throughout abstinence on negative affective behaviors associated with EtOH abstinence, and then subjected mice to home cage behavior and metabolic activity assessments in protracted abstinence. Establishing reliable preclinical models and basal metabolic and behavioral phenotypes for studying the relationship between exercise, drinking behavior, and abstinence-induced negative affect can widely impact treatment strategies and relapse potential in AUD patients.

MATERIALS AND METHODS

Animals

Female C57BL/6J mice (n = 55; The Jackson Laboratory) were delivered at 6 weeks of age, acclimated in standard group housing for 1 week, and then singly housed and acclimated for 1 week in modified cages with a full-sized running wheel. All mice were maintained on a 12 h light/dark cycle (lights on at 06:00 h) under controlled temperature (20°C to 25°C) and humidity (30% to 50%) levels. Mice were given access to food and water ad libitum. All experimental procedures were conducted with approval of the Institutional Animal Care and Use Committee at Vanderbilt and were within the guidelines set forth by the Care and Use of Mammals in Neuroscience and Behavioral Research (2003).

Chronic drinking with running wheel access

Chronic drinking followed by forced abstinence (CDFA) was conducted as previously described (Centanni et al., 2019; Holleran et al., 2016; Vranjkovic et al., 2018) with two modifications: (1) the addition of a running wheel factor and (2) an extension from 6 weeks drinking to 9 to 11 weeks (Figure 1). Mice were given two bottles, one with water and one with increasing concentrations of EtOH. The EtOH ramp involved increases from 3% to 7% to 10% over the course of the first 10 days. All mice were then maintained on a 10% EtOH solution in tap water for the remainder of the drinking period. The positions of the EtOH and water bottle were switched each week (Cohort 1 to 2) or every 12 days (Cohort 3) to account for a side preference. During the first 3 weeks of EtOH drinking, the running wheel was locked so as not to confound the acquisition of an EtOH preference. Following this acclimation phase, the intermittent wheel access phase began. Two intermittent access schedules

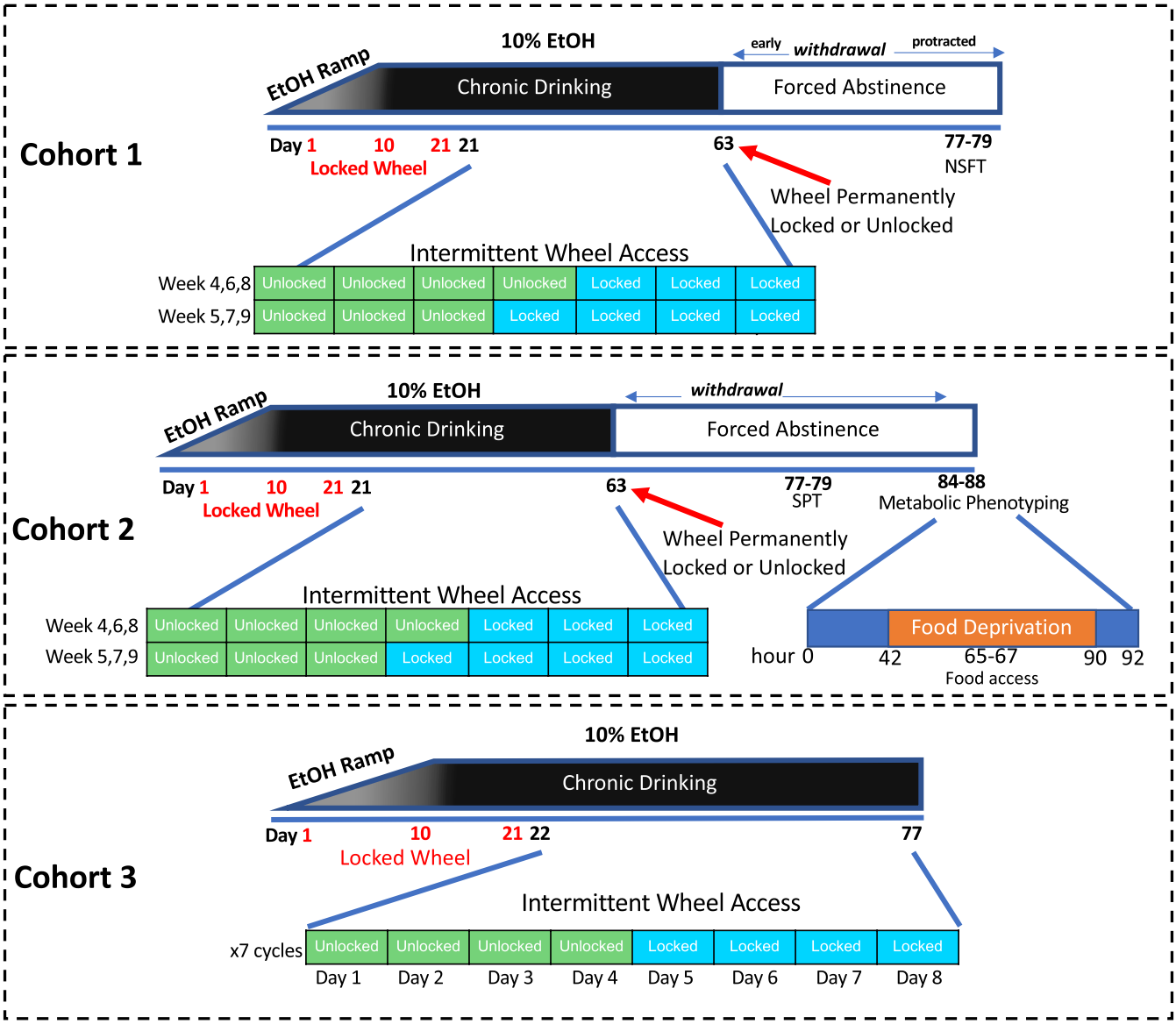


FIGURE 1 Experimental design. For all experiments, running wheels were locked for the first 21 days of two-bottle choice EtOH drinking. Cohort (1) on day 21, wheels were unlocked and remained unlocked for 4 days followed by three locked days, three unlocked days, and then four locked days. This pattern repeated for the remainder of the drinking period. On day 63 of drinking, EtOH was removed, and half of the mice were set in the wheel locked configuration and the other half in wheel unlocked configuration. Fifteen days into abstinence mice were subjected to the NSFT. Cohort (2) same as Cohort (1) except the SPT was performed 15 days into abstinence. Six days later mice were subjected to metabolic activity phenotyping. Cohort (3) mice were subjected to a 4-day unlocked wheel period followed by a 4-day unlocked wheel period. This cycle was repeated seven times

were used. The first ($n = 40$, Cohorts 1 and 2, Figure 1) involved a 2-week cycle; unlocked running wheel access for 4 days, followed by a 3-day locked wheel period, 3 days of unlocked wheel access, and 4 days of locked wheel. This cycle was the same for all mice and continued for 6 weeks for a total of 9 weeks of EtOH drinking. A separate cohort ($n = 20$, Cohort 3, Figure 1) underwent an 8-day cycle; 4 days of unlocked wheel access followed by 4 days of locked wheel. This cycle was repeated seven times for a total of 11 weeks of EtOH drinking. The amount of wheel running was quantified by determining rotations of the wheel using a magnetic counter attached to the cage and the wheel. Total rotations were recorded the same days as EtOH/water consumption measurements, and data were averaged as wheel rotations/day.

Abstinence behavioral tests

After 9 weeks of drinking in the 2-week intermittent wheel access cohorts (1 and 2), the EtOH bottle was removed, beginning the forced abstinence phase. Mice were split into two groups; half the mice received unlimited wheel access, while the other half maintained locked wheels for the remainder of the experiment.

Behavioral assessment of affective state as well as metabolic phenotyping was conducted as follows:

Novelty suppressed feeding test

The Novelty suppressed feeding test (NSFT) was conducted in Cohort 1 as previously described (Centanni et al., 2019; Holleran et al., 2016; Pang et al., 2013b; Vranjkovic et al., 2018). In brief, 13 days into abstinence, mice were food restricted for the 48 h leading up to the test. Food access was granted for a 2-h period, 23 to 25 h prior to NSFT. On day 15 of abstinence, mice were subjected to NSFT. Mice were placed into an open arena (50 × 50 cm^2) with a food pellet located in the center of the brightly lit apparatus (~300 lux). Latency to feed was measured as the amount of time elapsed prior to taking a bite of the food pellet. The latency to feed was assessed in real time by the experimenter and confirmed through video recording after the experiment. Mice were removed immediately after the first bite and placed back into their home cage with a preweighed food pellet. After 10 min, the food pellet was reweighed to determine home cage consumption. Mice that did not take a bite of food within the 20 min of experiment were excluded from the analysis ($n = 1$).

Saccharin preference test

The SPT was conducted over a 3-day period (Cohort 2). A 0.2% solution of saccharin (MilliporeSigma, Burlington, MA, USA) was added to a 50 ml conical tube with an attached sipper, weighed, and placed into the home cage opposite a preweighed 50 ml conical tube filled with water. After 24 h, weights were recorded, and the saccharin and water bottle sides were switched. This was repeated twice for a total of 72 h and three measurements. Preference was determined by comparing the total volume of the saccharin solution consumed in the 24-h period divided by the total volume of water consumed in that same period and multiplied by 100. Preferences were averaged for each mouse across the 3-day SPT.

Indirect calorimetry

Cohort 2 was subjected to metabolic phenotyping using indirect calorimetry (Promethion cages; Sable Systems International, Las Vegas, NV, USA) 5 days after the conclusion of the SPT (abstinence day 21). Mice were individually placed in metabolic cages (identical to home-cages with bedding) in a 12 h light/dark cycle, temperature/humidity-controlled dedicated room located in the Vanderbilt MMPC. The wheel conditions in abstinence (locked or unlocked) were maintained throughout the experiment. Energy expenditure measures were obtained by indirect calorimetry. The calorimetry system consists of cages equipped with water bottles and food hoppers connected to load cells for food and water intake monitoring. All animals had ad libitum access to water for the entire experiment and standard rodent chow for the first 42 h of testing. To determine the effects of the 48-h food restriction period used for NSFT on metabolic phenotype, food was removed from the cages 42 h into the experiment. Mice were given access to a single food pellet for 2 h (23 to 25 h into the 48-h food limitation period). Total experiment time was 92 h. A separate cohort of EtOH naïve female C57BL/6J mice ($n = 15$) were also run through metabolic cages; however, this cohort did not undergo a food restriction period. The air within the cages is sampled through microperforated stainless steel sampling tubes that ensure uniform cage air sampling, using a pull-mode, negative pressure system with an excurrent flow rate set at 2000 ml/min. Water vapor is continuously measured and its dilution effect on O_2 and CO_2 are mathematically compensated for in the analysis stream. O_2 consumption and CO_2 production are measured for each mouse every 5 min for 30 s. Incurrent air reference values are determined every four cages. Respiratory quotient is calculated as the ratio of CO_2 production over O_2 consumption. Energy expenditure is calculated using the Weir equation: EE (kcal/h) = 60 × (0.003941 × VO_2 (ml/min) + 0.001106 × VCO_2 (ml/min)) (Weir, 1949). Body composition was determined by NMR (Bruker Minispec, Billerica, MA, USA).

Ambulatory activity was determined simultaneously every one second with the collection of the calorimetry data. Activity and position are detected with XYZ beam arrays (BXYZ-R; Sable Systems) with a beam spacing of 1.0 cm interpolated to a centroid resolution of 0.25 cm. Consecutive adjacent infrared beam breaks are counted and converted to distance, with a minimum movement threshold set at 1 cm. A mouse was deemed asleep if no activity was detected on XYZ beams for >40 s. This can be interpreted as a proxy for what is likely time spent sleeping (Pack et al., 2007). A short lounge is defined as no Z beam breaks for 5 to 60 s and a long lounge is >60 s. Data acquisition and instrument control were coordinated by MetaScreen v2.2.18 and the raw data were processed using ExpeData v1.7.30 (Sable Systems). Sleep data are presented in Zeitgeber Time (ZT) with each animal's average percentage of time sleeping for that hour throughout the experiment.

Statistics

For the intermittent wheel access phase, within subject locked/unlocked wheel data were analyzed by taking the average consumption and preference values for each mouse during all locked/unlocked wheel phases and then using a parametric two-tailed paired Student's *t*-test comparing each mouse's average preference and consumption during the stated locked period versus the unlocked period. Correlation analysis was conducted using a Pearson's correlation coefficients reported as r with (95% confidence interval). To determine whether the correlation was statistically significant ($p \leq 0.05$), the slope of the test line was compared to a nonzero slope and the p value was derived from an *F*-test. In other words, the probability that randomly selected values would result in values obtained from the observed test slope. SPT and NSFT analyses was

conducted using a parametric two-tailed unpaired Student's *t*-test. Data were analyzed using GraphPad Prism 9 (Graphpad Software, San Diego, CA, USA).

Metabolic phenotyping analysis was conducted using the calorimetry analysis program CalR (Mina et al., 2018). In brief, CalR implements generalized linear models (including ANCOVA and ANOVA) to incorporate body mass as a covariate with the other variables; time (including photoperiod) and the specific metabolic parameters. For measurements not associated with mass (e.g., locomotion), CalR uses a one-way ANOVA to determine between group differences. For sleep behavior during metabolic phenotyping, data were collapsed into light and dark cycle and a cycle × wheel × treatment three-way ANOVA was run. This was followed by a post hoc two-way ANOVA removing the wheel condition. In all tests, a statistically significant difference was reported if $p \leq 0.05$.

RESULTS

Intermittent wheel access reduces EtOH drinking in female C57BL/6J mice

The first experiments sought to determine the impact of intermittent wheel access on drinking patterns in female C57BL/6J mice. A running wheel was present but locked for the first 3 weeks of drinking to establish a baseline EtOH preference independent of wheel running and to allow differentiation between any environmental enrichment produced by the presence of the wheel from actual wheel running. During the EtOH ramp mice established a preference for EtOH over water (Figure 2A) and consumed increasing concentrations of EtOH over time (Figure 2B) while water consumption was stable (Figure 2C). After 3 weeks, mice in Cohorts 1 and 2 were maintained on a 14-day intermittent wheel access cycle as described in the methods (four unclocked–three locked–three unlocked–four locked). This pattern resulted in notable fluctuations in both EtOH preference (Figure 2D) and consumption (Figure 2E). EtOH preference during the unlocked period was lower than the wheel locked period ($t_{(39)} = 4.78$, $p < 0.0001$; Figure 2F), while there was no significant effect on overall consumption ($t_{(39)} = 1.18$, $p = 0.245$; Figure 2I). Upon closer examination, the decreased preference effect was largely driven by the 4-day locked period that was followed by a 4-day unlocked period ($t_{(39)} = 9.22$, $p < 0.0001$; Figure 2G), and consumption was reduced in the 4-day unlocked wheel period relative to the 4-day locked wheel period ($t_{(39)} = 17.67$, $p < 0.0001$; Figure 2J). However, the preference and consumption during the 3-day unlocked period were increased relative to the preceding 3-day locked period (Preference: $t_{(39)} = 7.41$, $p < 0.0001$; Figure 2H, Consumption: $t_{(39)} = 9.40$, $p < 0.0001$; Figure 2K).

To follow up on this discrepancy, we altered the wheel access schedule in a separate cohort of mice (Cohort 3, Figure 1) to examine whether longer and/or consistent wheel access can reliably shift drinking patterns. After the EtOH ramp, mice were put on an 8-day intermittent wheel access schedule, 4 days with wheel access followed by 4 days with a locked wheel (Figure 3A,B). We observed a general preference for the bottle in closest proximity to the wheel, therefore bottle position was switched every three locked/unlocked cycles (as opposed to every week) to ensure bottle position was not unintentionally skewing the data as it may have in the first two cohorts. Similar to the 14-day schedule, mice with 4 days of wheel access had a significantly reduced preference for EtOH and consumed significantly less EtOH during the unlocked period (Preference: $t_{(19)} = 13.23$, $p < 0.0001$; Figure 3C, Consumption: $t_{(19)} = 7.74$, $p < 0.0001$; Figure 3D). Collectively, these data suggest consistent access to a running wheel reliably reduces EtOH drinking in a continuous access drinking model.

Voluntary wheel running is negatively correlated with EtOH drinking behavior

Since continuous access to a running wheel and EtOH drinking innately produces individual variability in both factors (wheel running and EtOH intake), we tested whether the amount of wheel running correlates with the magnitude of change in drinking behavior. Indeed, there was a significant negative correlation between wheel rotations and the subsequent change in preference and consumption (Figure 3E,F) between wheel locked and unlocked days (Preference: Pearson's correlation $r = -0.6388$, 95% confidence interval −0.7910 to −0.4122, $p < 0.0001$; Consumption: Pearson's correlation $r = -0.6096$, 95% confidence interval −0.7724 to −0.3716, $p < 0.0001$), suggesting exercise possesses a robust titrating effect to shift EtOH drinking behaviors.

Voluntary wheel running in abstinence reduces EtOH-induced negative affect-like behavior

For Cohorts 1 and 2, following the intermittent wheel access and EtOH drinking, EtOH was removed, and mice were split into two groups: continuous access to the running wheel throughout abstinence, and locked wheel throughout abstinence. Our lab and others have demonstrated a reliable increase in negative affect-like behavior in protracted abstinence (Centanni et al., 2019; Holleran et al., 2016; Pang et al., 2013b; Vranjkovic et al., 2018), and we tested whether access to a running wheel would curb this phenotype. In Cohort 1, mice were subjected to the NSFT on day 15 of abstinence. Mice with wheel access in abstinence had a significantly shorter latency to take the first bite of the food compared to mice with a locked wheel in abstinence ($t_{(16)} = 3.25$, $p = 0.005$; Figure 4A). Consumption 10′ after NSFT in the home cage was not different between groups ($t_{(16)} = 1.60$, $p = 0.130$; Figure 4C). However, mice with wheel access lost a significantly larger percentage of body weight than locked-wheel mice during the 48-h food restriction leading up to the NSFT ($t_{(16)} = 2.30$, $p = 0.035$; Figure 4B), suggesting increased energy expenditure in abstinence (and therefore increased hunger) in the wheel access

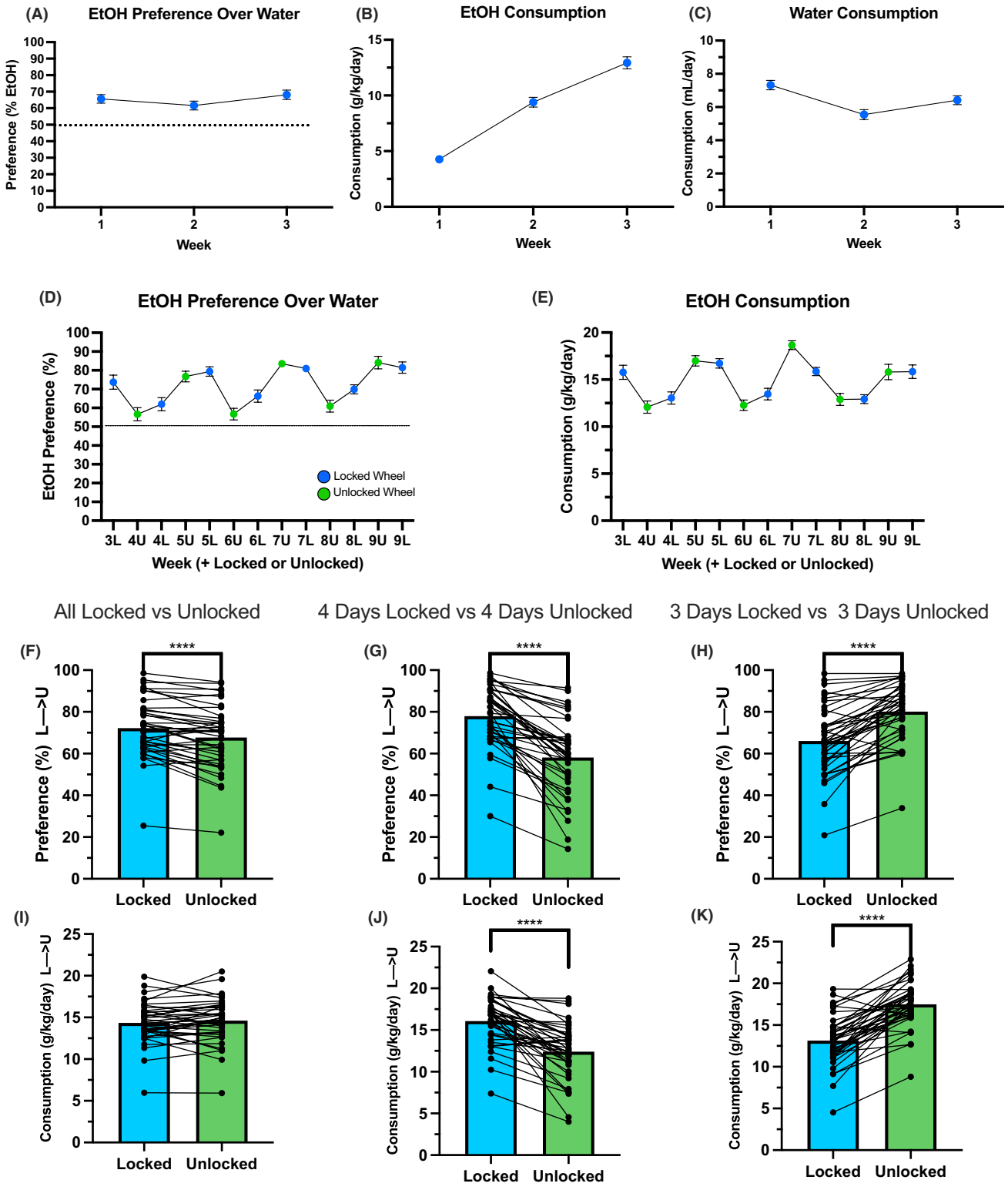


FIGURE 2 Intermittent wheel access during two-bottle choice EtOH drinking alters EtOH preference and consumption. (A–C) EtOH ramp phase. Mice established a significant preference for EtOH over water (A) and consumed increasing amounts of EtOH (B). (C) Water consumption during the EtOH ramp phase. (D, E) Intermittent running wheel access. EtOH preference (D) and consumption (E) throughout the drinking period. (F) Average EtOH preference during all locked days was significantly higher than all unlocked days. (G) Average of 4 days of locked wheel followed by 4 days of unlocked wheel significantly decreased individual preference for EtOH. (H) Three days of locked wheel resulted in an increase in the subsequent unlocked wheel preference. (I) Average EtOH consumption during all locked days was higher than all unlocked days. (J) Four Days of locked wheel followed by 4 days of unlocked wheel significantly decreased individual consumption for EtOH. (K) Three days of locked wheel resulted in an increase in the subsequent unlocked wheel preference. $N = 40$ mice. Data demonstrated as mean ± SEM (A–E) and individual averages per mouse with bars representing the group means (F–K). ****$p < 0.0001$

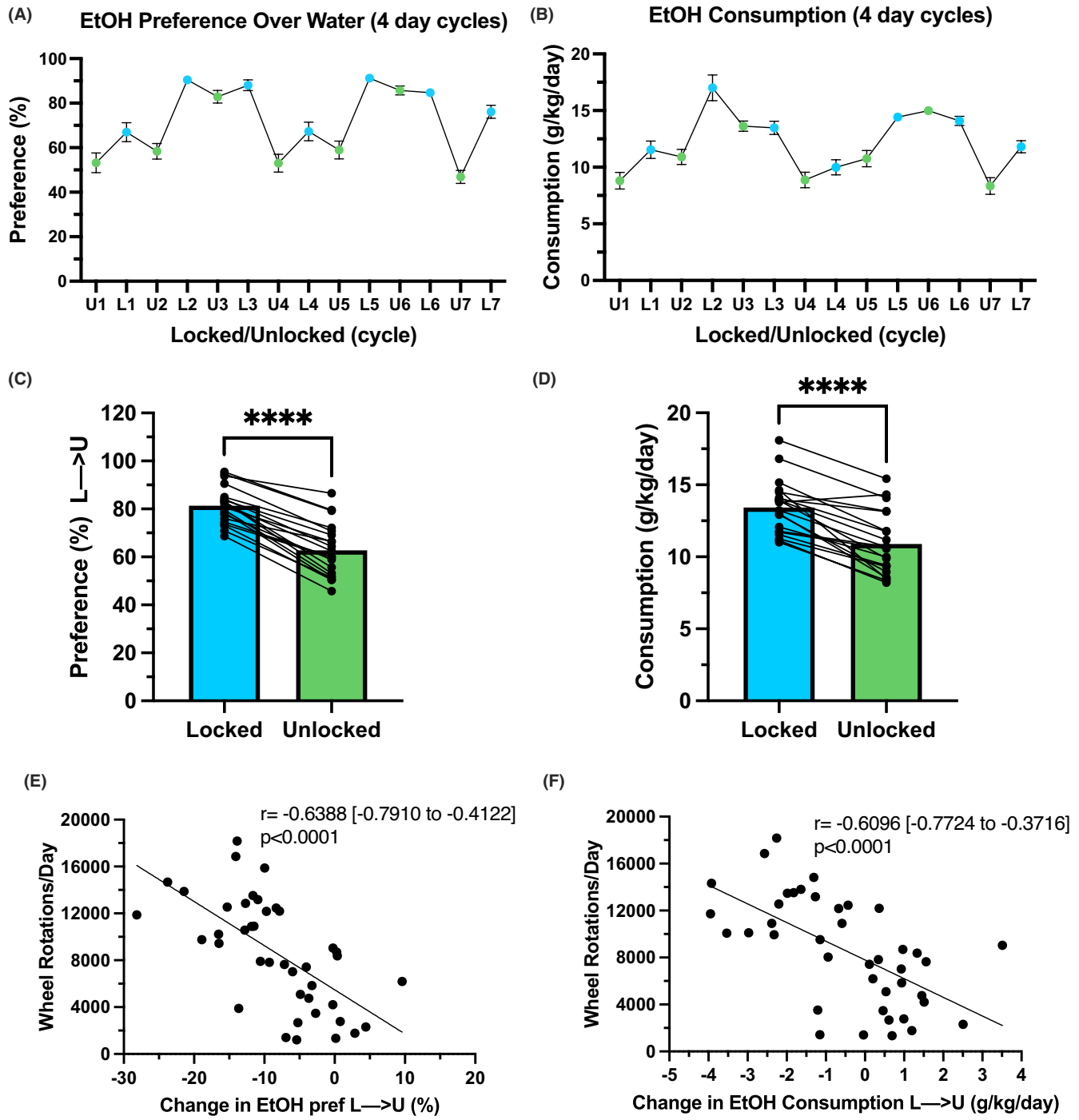


FIGURE 3 Alternative 4-day intermittent wheel access similarly alters EtOH drinking patterns and shows consistent negative correlation with amount of wheel running. (A) EtOH preference over water when the wheel is locked (cyan) and unlocked (green). (B) EtOH consumption (g/kg/day) when the wheel is locked (cyan) and unlocked (green). (C) Individual mouse data comparing overall average EtOH preference when the wheel was locked and unlocked. The preference for EtOH was significantly higher when the wheel was locked than when the wheel was unlocked (****$p < 0.0001$). (D) Individual mouse data comparing overall average EtOH consumption (g/kg/day) when the wheel was locked and unlocked. EtOH consumption was significantly higher when the wheel was locked than when the wheel was unlocked (****$p < 0.0001$). Data represented as mean ± SEM (A, B) and individual averages per mouse with bars representing the group mean (C, D). (E) Wheel running (wheel rotations) for all cohorts were negatively correlated with the magnitude of change in EtOH preference from the locked wheel to unlocked wheel. (F) Wheel running activity was negatively correlated with the magnitude of change in EtOH consumption from locked wheel to unlocked wheel (Pearson's correlation $n = 41$ mice)

group could contribute to the observed phenotype. To further assess affect in abstinence, we used a convergent behavioral assessment for affect, the calorie-independent SPT, in Cohort 2 to measure anhedonia-like behavior in abstinence. Indeed, saccharin preference over a 3-day period was significantly lower in mice with a locked wheel in abstinence compared to unlocked wheel

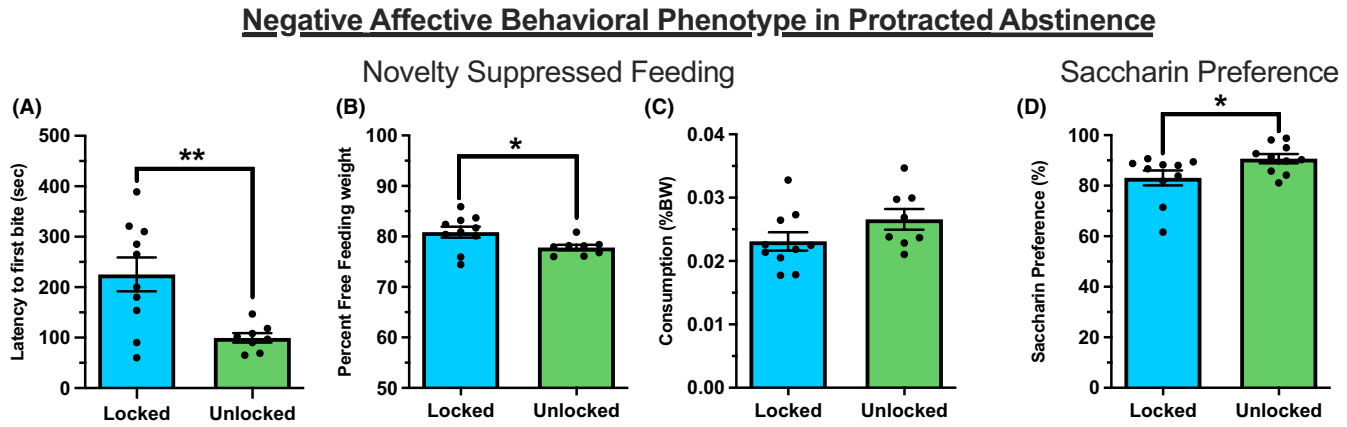


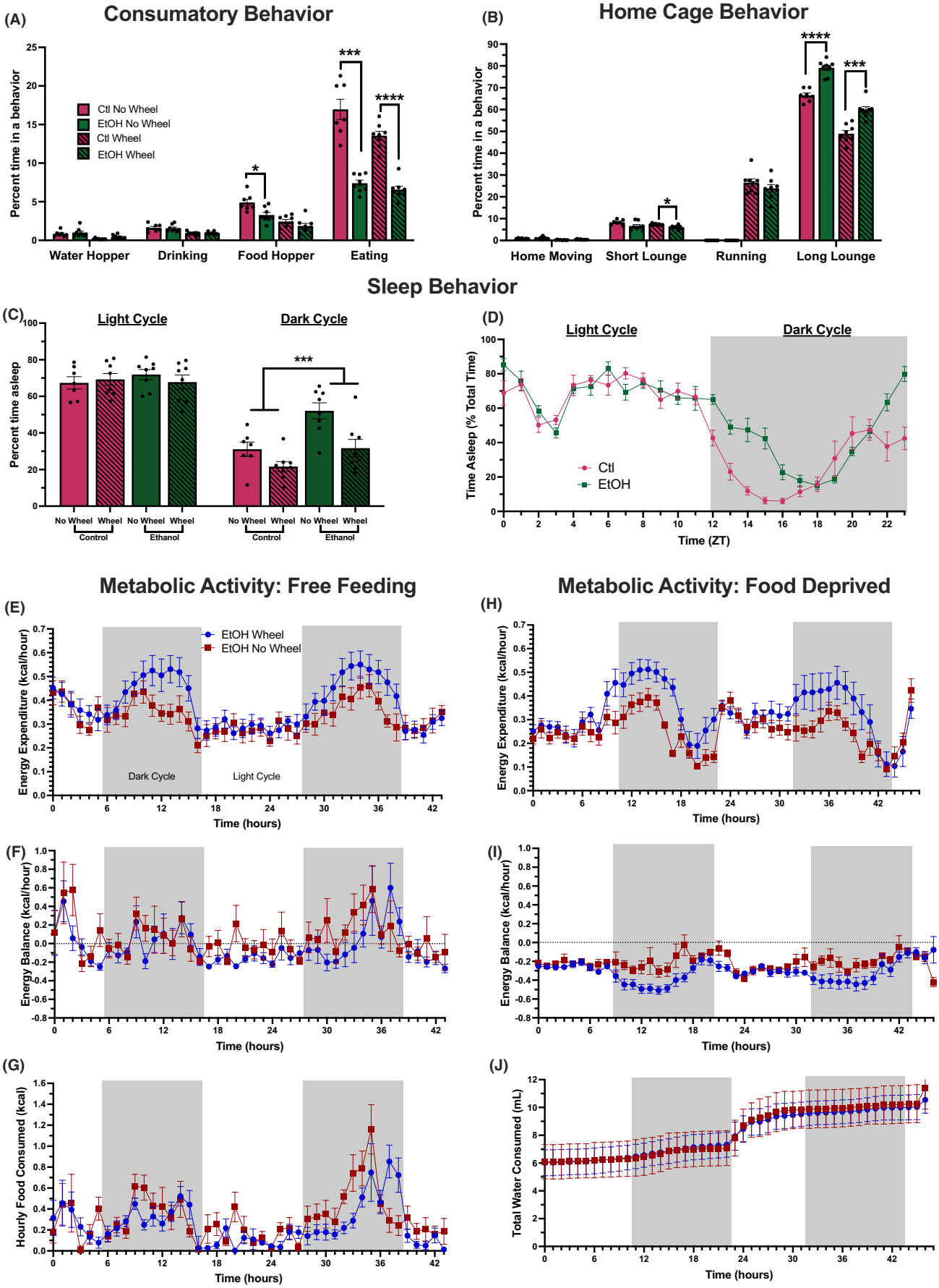
FIGURE 4 Wheel access in abstinence impacts affective state. (A) Latency to take the first bite on the NSFT was lower in EtOH abstinence mice with an unlocked wheel compared to abstinence mice with a locked wheel ($n = 8$ to 10 mice/group, $^{**}p < 0.01$). (B) Mice with an unlocked wheel had greater weight loss during the 48-h food restriction period leading up to the NSFT compared to mice with a locked wheel ($^{*}p < 0.05$). (C) Home cage consumption 10 min immediately following NSFT reveals no significant difference between wheel locked and unlocked mice. (D) Mice with an unlocked wheel in abstinence had a significantly higher preference for saccharin over water compared to mice with a locked wheel in abstinence ($n = 10$ mice/group, $^{*}p < 0.05$). Data demonstrated as individual mice with mean ± SEM

access ($t_{(16)} = 2.18$, $p = 0.043$; Figure 4D), collectively suggesting wheel access reduces negative affect-like behavior in protracted abstinence.

Wheel access in abstinence is associated with changes in home cage behavior

We next assessed home cage behavior in abstinence and compared mice with and without wheel access. EtOH abstinent mice underwent a 4-day metabolic (discussed below) and behavioral phenotyping. Wheel-locked abstinent mice were placed in chambers without wheels, while wheel-access mice continued to have wheel access. An EtOH naïve control group (with and without wheel access) was added as well. This group did not undergo food deprivation, therefore only free feeding time points in EtOH mice were used for analysis. Two-way ANOVA analysis revealed a significant effect of behavior type × treatment (F (21, 198) = 94.4, $p < 0.0001$). Post hoc analysis was conducted on each behavior (Tukey's Multiple Comparison Test). Because the wheel group had an additional behavior to track (wheel running), we compared all off-wheel activities to determine, which activities wheel mice were reducing to spend time on the running wheel (Figure 5A). In both control and EtOH abstinent mice, a majority of the time budget spent on the wheel was at the expense of time spent "long lounging" either in the home hut or elsewhere in the chamber (long lounge control no-wheel vs. control wheel, $p < 0.0001$; EtOH no-wheel vs. EtOH wheel, $p < 0.0001$; Figure 5A,B). Lounging data also revealed an EtOH effect specifically in the wheel groups, as EtOH mice with wheel access spent less time engaging in short lounges than control mice with a wheel ($p = 0.012$). In terms of consummatory behavior, mice in abstinence spent less time eating than control mice, regardless of wheel condition (no-wheel control vs. EtOH, $p = 0.001$; wheel control vs. EtOH, $p < 0.0001$). Interestingly, time spent at the food hopper (but not

FIGURE 5 Metabolic phenotype of mice with and without wheel access in abstinence. (A–D) Average time budget per group. A separate EtOH naïve group ($n = 15$ mice) was added to this study for comparison. For ease of interpretation, only the significant effects between EtOH and control mice in the wheel and no wheel conditions are displayed. (A) No wheel EtOH mice spent significantly less time at the food hopper than control no wheel mice. EtOH mice with and without a wheel spent less time eating compared to their control counterparts ($^{*}p < 0.05$, $^{***}p < 0.001$, $^{****}p < 0.0001$; $n = 7$ to 8 mice/group). (B) EtOH mice with wheel access spent significantly less time in the "short lounge" behavior, while EtOH abstinence resulted in more time spent in the "long lounge" behavior in both groups, independent of wheel condition. (C) EtOH mice spent significantly more time asleep during the dark cycle than EtOH naïve mice, regardless of wheel condition ($^{***}p < 0.001$; $n = 7$ to 8 mice/group). Wheel access did not alter sleep during the light cycle but did result in a significant reduction in sleep during the dark cycle. The presence of a wheel in EtOH abstinent mice reduced the percent time asleep to levels comparable to control mice without a wheel. (D) Sleep data presented in ZT with wheel condition removed. Observed differences in sleep are restricted to the dark cycle. (E–G) Free feeding period. Energy expenditure (E) was significantly higher during the active period (dark cycle) in wheel access mouse compared to no wheel access mice (see Table 1 for full statistics, $n = 8$/group). Energy balance (F) and food consumption (G) were not different between groups. (H–J) Food Deprivation Period. Energy expenditure (H) was significantly higher during the active period (dark cycle) in wheel access mice compared to no wheel access mice, which resulted in a lower energy balance (I) in wheel access mice during the food deprivation period. Water consumption (J) was not different between groups (see Table 1 for full statistics)


(A)
Consumatory Behavior
Percent time in a behavior
Ctl No Wheel
EtOH No Wheel
Ctl Wheel
EtOH Wheel
Water Hopper
Drinking
Food Hopper
Eating
(B)
Home Cage Behavior
Percent time in a behavior
Home Moving
Short Lounge
Running
Long Lounge
Sleep Behavior
(C)
Light Cycle
Dark Cycle
Percent time asleep
No Wheel
Wheel
Control
Ethanol
(D)
Light Cycle
Dark Cycle
Time Asleep (% Total Time)
Ctl
EtOH
Time (ZT)
Metabolic Activity: Free Feeding
(E)
Energy Expenditure (kcal/hour)
EtOH Wheel
EtOH No Wheel
Dark Cycle
Light Cycle
Time (hours)
(F)
Energy Balance (kcal/hour)
Time (hours)
(G)
Hourly Food Consumed (kcal)
Time (hours)
Metabolic Activity: Food Deprived
(H)
Energy Expenditure (kcal/hour)
Time (hours)
(I)
Energy Balance (kcal/hour)
Time (hours)
(J)
Total Water Consumed (mL)
Time (hours)

eating) was lower in no wheel EtOH mice compared to no wheel control mice ($p = 0.047$), however this effect was not present in the wheel groups ($p = 0.552$). Water drinking and time spent at the water hopper were not different between any of the groups.

Wheel access in abstinence is associated with changes in sleep behavior

The behavioral tracking also provides estimates for sleep (complete inactivity) time for each mouse (Figure 5C,D). Because mice were transferred to a novel environment during the light cycle, sleep data from the first day was removed from analysis. We first tested whether the food restriction period impacted sleep in the EtOH group. Two-way ANOVA analysis on the collapsed EtOH group data revealed a significant effect of the hour of day (F (6, 90) = 24.7, $p < 0.0001$), but no effect of day (F (1.5, 21.9) = 1.55, $p = 0.233$), therefore all data were included in the subsequent analysis ensuring an equal number of data points in the EtOH and control groups. Three-way ANOVA comparing treatment × wheel condition × light/dark cycle revealed significant effects of cycle (F (1, 27) = 299.7, $p < 0.0001$), wheel (F (1, 27) = 7.56, $p = 0.01$), cycle × treatment (F (1, 27) = 11.9, $p = 0.002$), and cycle × wheel (F (1, 27) = 11.7, $p = 0.002$), but no effect of treatment × wheel (F (1, 27) = 1.88, $p = 0.182$). We next performed post hoc two-way ANOVAs independently removing the cycle factor and the wheel factor. Removing the wheel factor and comparing EtOH treatment and percent time asleep revealed a significant cycle × treatment (F (1, 29) = 9.35, $p = 0.005$), cycle (F (1, 29) = 224.4, $p < 0.0001$), and treatment (F (1, 29) = 6.34, $p = 0.018$) effect driven by an EtOH-induced increase in sleep during the dark cycle (Sidak's multiple comparison, $p = 0.001$; Figure 5C,D). This effect is prominent in the beginning and end of the dark cycle (Figure 5D). Removing the cycle factor and examining treatment × wheel just in the dark cycle revealed significant effects of wheel (F (1, 29) = 13.6, $p = 0.001$) and treatment (F (1, 29) = 14.7, $p = 0.001$) driven primarily by wheel-EtOH versus no-wheel-EtOH ($p = 0.006$) and no-wheel-control versus no-wheel-EtOH ($p = 0.006$). Of note, there was no significant difference between no-wheel-control and wheel-EtOH groups ($p = 0.999$).

Wheel access in abstinence is associated with changes in net energy balance

Both wheel running and EtOH drinking can impact metabolic activity in abstinence and lead to significant changes in affective state. We next examined the relationship between exercise, a history of EtOH drinking, and metabolic activity. Metabolic activity parameters were assessed for mice with versus without a wheel in abstinence. Abstinent mice with running wheel access exhibited greater energy expenditure, and a trend for lower energy balance, as food consumption was not different between groups during the free-feeding period (Figure 5E–G). The full list of comparisons for activity measures and metabolic phenotypes during the active period of the baseline free feeding period is listed in Table 1. This metabolic phenotyping approach can also be used to determine the effects of behavioral manipulation on metabolic activity. For instance, NSFT was accompanied by a higher percentage of weight loss during the food restriction period, which could be interpreted as an increase in hunger rather than a decrease in negative affective behavior. During the food deprivation period, energy expenditure was higher in the wheel access group compared to the no wheel group (Figure 5H,I). As expected during food deprivation, energy balance for both groups was negative, but tended to be even lower in the wheel access group. Water consumption was similar between groups (Figure 5J). The full list of comparisons for activity measures and metabolic phenotypes during the active phase of the food deprivation period is listed in Table 2. In all, these data outline unique metabolic phenotypes for mice in abstinence with or without wheel access.

TABLE 1 Free feeding metabolic phenotype of CDFA mice with versus without wheel access in abstinence

Condition	Wheel	No wheel	*p*-Value
Hourly food consumed (kcal)	0.466 ± 0.054	0.466 ± 0.047	0.7028
Hourly water consumed (ml)	0.187 ± 0.028	0.163 ± 0.023	0.1879
Total food consumed (kcal)	14.78 ± 2.03	16.40 ± 2.74	0.9964
Total water consumed (ml)	5.824 ± 0.926	5.634 ± 0.464	0.3269
Oxygen consumption (ml/h)	91.65 ± 3.47	72.65 ± 2.22	0.0338*
Carbon dioxide production (ml/h)	76.38 ± 3.15	62.66 ± 2.21	0.0473*
Energy expenditure (kcal/h)	0.446 ± 0.017	0.356 ± 0.011	0.0357*
Energy balance (kcal/h)	0.020 ± 0.040	0.110 ± 0.037	0.5351
Respiratory exchange ratio	0.831 ± 0.006	0.852 ± 0.006	0.2000
Locomotor activity (beam breaks)	44.038 ± 4330	55,274 ± 5509	0.0605
Pedestrian locomotion (m)	169.5 ± 15.78	275.4 ± 27.07	<0.001***
Total distance in cage (m)	209.6 ± 20.28	319.5 ± 32.31	<0.001***

Note: Statistical comparisons made during the dark cycle (active period). *$p < 0.05$, ***$p < 0.001$.

TABLE 2 Metabolic phenotype during a 48-h food restriction period in CDFA mice with versus without wheel access in abstinence

Condition	Wheel	No wheel	*p*-Value
Hourly water consumed (ml)	0.058 ± 0.006	0.046 ± 0.009	0.1107
Total water consumed (ml)	10.01 ± 0.90	10.24 ± 0.48	0.2835
Oxygen consumption (ml/h)	77.14 ± 5.37	53.25 ± 3.67	0.0063**
Carbon dioxide production (ml/h)	55.56 ± 4.14	38.09 ± 2.87	0.0064**
Energy expenditure (kcal/h)	0.366 ± 0.026	0.252 ± 0.018	0.0063**
Energy balance (kcal/h)	−0.348 ± 0.025	−0.196 ± 0.015	0.0184*
Respiratory exchange ratio	0.687 ± 0.011	0.693 ± 0.011	0.6828
Locomotor activity (beam breaks)	114,183 ± 3662	157,095 ± 4522	0.0616
Pedestrian locomotion (m)	454.2 ± 16.4	868.4 ± 33.9	0.0011**
Total distance in cage (m)	564.5 ± 19.8	993.3 ± 37.5	0.0012**

Note: Statistical comparisons made during the dark cycle (active period). *$p < 0.05$, **$p < 0.01$.

DISCUSSION

This study sought to determine the effect of intermittent running wheel access on drinking patterns during chronic continuous access two-bottle choice drinking. We found that EtOH consumption and preference were significantly altered by intermittent wheel access. Of particular note, the amount of wheel running was strongly correlated with the difference in preference and consumption during the locked-wheel phase compared to the unlocked wheel phase. We next determined that continuous access to a running wheel throughout abstinence was sufficient to reduce negative affect-like behavior in protracted abstinence. Given the independent impact of exercise and alcohol drinking on metabolic health and activity, we characterized metabolic activity in our model. Wheel access during abstinence resulted in differences in several measures of metabolic activity, although food and water consumption were not different between groups, suggesting the metabolic effects of wheel exercise may be beneficial to the improvement in negative affective state without impacting basal feeding characteristics. In addition, activity measured during metabolic phenotyping revealed a potential abstinence-induced shift in sleep patterns, as mice in abstinence were more active during their light cycle and less active during the dark cycle. Collectively, we outline a strong relationship between wheel running and EtOH drinking both in currently drinking and EtOH-abstinent mice. Future treatment strategies for abstinence that incorporate physical activity as an avenue to relieve stress before or at the onset of abstinence may have beneficial effects on negative affective symptoms mediated in part through increased metabolic activity.

Incorporating intermittent wheel access into continuous access EtOH drinking models

The previous literature investigating the relationship between wheel access and EtOH drinking is mixed. Intermittent wheel access switched from locked to unlocked on a daily or weekly basis has relatively little effect on EtOH preference or consumption (McGonigle et al., 2016; Ozburn et al., 2008). In contrast, EtOH consumption during continuous access two-bottle choice was lower in mice with continuous access to wheel compared to a locked wheel group (Ehringer et al., 2009). In a limited access drinking model, a similar decrease in EtOH drinking has been reported in mice with intermittent 24-h access to an unlocked wheel (Piza-Palma et al., 2014). Of note, all the referenced studies provided wheel access at the beginning of the drinking paradigm. A goal of the present study was to address these discrepancies by minimizing changes in housing conditions and limiting experimenter intervention. Mice were singly housed in a cage containing the locked wheel and acclimated for 1 week to remove any novelty associated with the wheel itself. Mice were also given time to establish a preference for EtOH prior to wheel access, avoiding any potential floor effect. While each model has advantages, the variability in paradigms prevents drawing overarching conclusions. Developing better standardized models would significantly benefit our understanding of the relationship between exercise and EtOH drinking.

Consumption and wheel rotations were initially measured on the same days each week to ensure experimenter intervention was consistent with weekly bedding changes, bottle side changes, veterinary wellness checks, and other external factors that could temporarily alter mouse behavior. However, in an attempt to control for factors that could influence drinking, the bottle positions were switched once a week on the same day the wheels were unlocked. This resulted in the bottle position being consistently on one side during the 3-day locked/unlocked phase and on the other side for the 4-day cycle. To follow up, we performed a modified version of this model that limited experimenter intervention even more and followed a consistent 4-day locked, 4-day unlocked pattern. Bottle position was switched every three cycles, ensuring the switch occurred during locked and unlocked periods. This model produced a consistent "sawtooth" pattern and confirmed a potential side preference for the bottle closest to the wheel. Moving forward we believe this model is superior to the 14-day cycle and will serve as a better model for future studies. While the caveats outlined above led us to

shift our wheel exposure timing, future studies further deconvolving wheel access time and drinking patterns could provide unique insight into this potential relationship.

The relationship between wheel access and drinking patterns

A number of studies have observed changes in drinking behavior in the presence of a running wheel (Darlington et al., 2014; Hammer et al., 2010; McMillan et al., 1995; Piza-Palma et al., 2014). Our within subject design provided multiple data points for each subject and revealed a collective sawtooth drinking pattern. However, one caveat to this design is the inability to determine the motivation for the changes in drinking behavior. We propose two possible motivating forces behind the changes in drinking pattern. First, the hedonic properties of running may outweigh the hedonic properties of drinking, thus drinking behavior may be displaced by access to the unlocked wheel. This idea is supported by the correlation data in Figure 3E,F. This is consistent with several other pivotal studies that have established a "reward hierarchy" for other substances (Banks & Negus, 2017; Lenoir et al., 2007). However, the opposite has also been observed, where drug abuse can shift behavior away from non-drug rewards to drug reward (Negus, 2006). More work is needed to fully test this idea in our model. Alternatively, the "frustration" of not being able to run when the wheel is locked could lead to an increase in drinking. This theory fits with work by the Grisel group that suggests blocking access to a running wheel induces frustration stress or the loss of something desired or pleasurable (McGonigle et al., 2016; Piza-Palma et al., 2014). In support of this, mice deficient in β-endorphin, a neuropeptide thought to decrease the stress response, exhibited increased EtOH consumption in response to a locked wheel compared to wild-type mice (McGonigle et al., 2016). While the approach used in this study does not directly test these possibilities, this model lays the groundwork for future studies to explore the motivation behind this reliable effect.

Wheel access in abstinence and negative affect-like behavior

There were no differences in basal food or water consumption between wheel and no-wheel access mice in abstinence (Table 1), suggesting differences in consumptive behaviors are not likely driving the lower latency to feed on NSFT (Figure 4A) or increased saccharin preference on the SPT (Figure 4D). These findings are in agreement with previous work by Pang et al. (2013b) showing EtOH abstinence-induced deficits on NSFT and SPT that were mitigated by wheel access in abstinence. While we did observe differences in energy expenditure and energy balance during the food deprivation period, this was only evident during the active period for mice (dark phase). There were no differences between the groups in the light cycle, when NSFT was conducted, suggesting at the time of testing, both groups were in a similar metabolic state, decreasing the probability that hunger was a driving factor behind the decreased latency to first bite. There was also no difference in food consumption in the 10′ home cage feeding period after NSFT (Figure 4C), further suggesting the EtOH abstinence effect on negative affect is not attributable to changes in metabolic activity.

Mouse metabolic phenotyping in EtOH abstinence

Our approach using home cage monitoring and metabolic phenotyping with indirect calorimetry provided a wealth of information that has yet to be studied in a chronic EtOH drinking paradigm. The basal metabolic analysis revealed significant wheel versus no wheel group differences in energy expenditure, oxygen consumption, and carbon dioxide production during the active period for the mice (Figure 5E–G, Table 1), suggesting general increased metabolic activity in abstinent mice with wheel access. Collectively, the metabolic phenotyping study provides unique high temporal resolution insight into the effects of exercise on metabolism during EtOH abstinence, and these metabolic factors should be strongly considered in all future studies examining the effects of exercise on the many facets of AUD. Understanding the relationship between the basal metabolic phenotype and mechanistic changes in brain circuitry involved in exercise and AUD could be important for determining the effectiveness of exercise on relapse potential and/or the risk of an individual to relapse. The strong literature characterizing binge EtOH-induced reduction in neurogenesis (Crews et al., 2004; Nixon, 2006; Nixon & Crews, 2002) and increased neurodegeneration (Leasure & Nixon, 2010) that is prevented by exercise serves as an excellent blueprint for this strategy.

EtOH abstinence, activity, and disrupted sleep

The metabolic phenotyping chambers also provide precise high temporal resolution activity measurements, thus this approach can glean information on the time budget for each group. Observed differences in pedestrian locomotion and total distance in cage can likely be attributed to the presence of, and therefore time spent on, the running wheel. This is supported by the time budget for mice with and without wheels while in the indirect calorimetry chambers (Figure 5A,B). Wheel mice spent roughly a quarter of their time (average 25.1%, calculated from Figure 5B) on the wheel, and a majority of this time was taken away from time spent in the home hut engaging in "long lounges" (average 73.3% without wheel, 54.4% with wheel, calculated from Figure 5B). This suggests the lower overall activity of the no-wheel group may contribute to the changes in metabolic activity. Importantly, food and water consumption during the basal assessment period were not different between groups, suggesting wheel running is not driving consumptive behavior, but does influence affective state (Figure 4). This is in line with earlier work in EtOH-preferring and nonpreferring rats suggesting

decreased drinking in EtOH-preferring rats in the presence of an unlocked wheel, but no changes in food or water consumption in either strain (McMillan et al., 1995).

Interestingly, removing the wheel condition from our analysis identified unique EtOH abstinence-induced shifts in sleep patterns consistent with a hypersomnia phenotype. EtOH mice spent more time sleeping during the active dark cycle relative to EtOH naïve mice. This is in agreement with previous work demonstrating sleep disturbances in early withdrawal after repeated cycles of chronic intermittent EtOH vapor exposure (Veatch, 2006). However, this study observed decreased non-REM sleep in both the dark and light cycles, whereas our data suggest increased sleep during the active dark cycle. Both hypersomnia and insomnia can be comorbid with AUD (Hasler et al., 2014), therefore both results fit with clinical observations. Importantly, the stage of AUD and abstinence can be important in sleep disturbances. Early withdrawal disturbances may be driven by the homeostatic imbalance and physical withdrawal symptoms whereas increased sleep during protracted abstinence may reflect an anhedonia and depressive-like phenotype. In agreement with preclinical studies, the vast clinical literature suggests sleep is impacted in nearly every phase of addiction (for review see Koob & Colrain, 2020). Acute and chronic alcohol exposure can impact the duration and quality of sleep, and many of these deficits persist at least 30 days into abstinence. Sleep deficits may relate to increased negative emotional states that are often attributed to relapse (Colrain et al., 2009; Drummond et al., 1998; Rundell et al., 1977). In this study, EtOH-abstinent mice with wheel access slept less during the dark cycle than mice with no wheel access. Interestingly, EtOH-treated mice slept a similar amount of time as the no-wheel EtOH naïve mice, suggesting exercise may prevent or reverse EtOH-induced sleep deficits. Further, this supports the idea that exercise can promote wakefulness and in turn decrease abstinence-induced negative affective behaviors like depression and anhedonia. It is worth noting that to fully support this hypothesis, more accurate sleep studies using for example, EEG are needed to confirm sleep patterns in these mice.

CONCLUSION

Integrating an intermittent wheel access element into the established CDFA model produced robust changes in EtOH preference and consumption, and this effect was strongly correlated with the amount of wheel running. Interestingly, activity monitoring in protracted abstinence yielded a potentially unique disrupted sleep pattern phenotype that was independent of wheel access. The presence of a running wheel in abstinence reduced negative affect-like behavior compared to mice with a locked wheel in abstinence. Given the impact of exercise on metabolic state, we characterized the metabolic phenotype of mice in abstinence with a locked or unlocked wheel and observed differences in net energy balance between the groups. While compromised metabolic health associated with chronic drinking is well documented in clinical literature (Fan et al., 2006; Vieira et al., 2016), this integral factor has been neglected in many preclinical studies and should be considered in future preclinical studies examining the relationship between exercise and EtOH drinking. Building a greater database of metabolic phenotypes in relation to EtOH drinking behavior, and home cage behavior in general, in mice will greatly enhance our understanding of the interplay between alcohol drinking, abstinence, and exercise. In addition, assessing metabolic activity during an acute food deprivation period used in several behaviors including NSFT (Figure 4A–C) could increase confidence in behavioral outcomes. Future studies using this approach could ultimately provide insight into how the general human population varies in response to exercise based on individual drinking habits. Furthermore, incorporating exercise into AUD treatments has the potential to prevent or reduce negative affect associated with alcohol abstinence.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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