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Effects of Chronic Alcohol Exposure on Motivation-Based Value Updating

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Abstract

Dysfunctional decision-making has been observed in alcohol dependence. However, the specific underlying processes disrupted have yet to be identified. Important to goal-directed decision-making is one's motivational state, which is used to update the value of actions. As ethanol dependence disrupts decision-making processes, we hypothesized that ethanol dependence could alter sensitivity to motivational state and/or value updating, thereby reducing the capability for adaptive behavior. Here we employed a sequential instrumental learning task to examine this hypothesis. In two experiments, mice underwent chronic intermittent ethanol (CIE) or air (Air) vapor exposure and repeated withdrawal procedures to induce ethanol dependence. Mice were then trained on a sequence of distal and proximal lever pressing for sucrose under either mild or more severe food restriction. Half of all Air and CIE mice then underwent a motivational shift to a less hungry state and effects of this motivational shift were evaluated across three days. First, mice were re-exposed to sucrose, and effects of food restriction state and CIE exposure on lick and consummatory behavior were examined in the absence of lever pressing. Over the next two days, mice underwent a brief non-rewarded test and then a rewarded test where the ability to retrieve and infer sucrose value to guide lever pressing was measured. In the sucrose re-exposure session, prior CIE exposure altered sucrose seeking in mice with a history of mild but not more severe food restriction, suggesting altered motivational sensitivity. During lever press testing, CIE mice were insensitive to decreases in motivational state and did not reduce proximal lever pressing regardless of food restriction state. Mildly restricted CIE mice, but not severely restricted CIE mice, also did not reduce distal pressing to the same degree as Air mice following a downshift in motivational state. Our findings suggest that ethanol dependence may disrupt motivational processes supporting value updating that are important for decision-making.

Keywords

alcohol dependence; decision-making; goal-directed; mice; incentive learning; motivational state

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Declaration of Interest

The authors have no conflicts of interest to disclose.

Introduction

Alcohol use disorder (AUD) is associated with long-lasting disruptions to decision-making processes. Decision-making often recruits our ability to use changes in our motivational state to appropriately adapt our behavior, often termed goal-directed control. In particular, deficits in goal-directed decision-making have been observed in human AUD (Gillan et al., 2016; Sjoerds et al., 2013) as well as in animal models of alcohol dependence (Corbit et al., 2012; Lopez et al., 2014; Renteria et al., 2018). As these decision-making processes and their control by motivational state support ongoing adaptive behavior as well as the continued use and misuse of alcohol, understanding their disruption may shed light on obstacles toward recovery faced by those with AUD.

Control over decision-making processes is often evaluated with outcome devaluation procedures. Normally, the subject is trained to work for either a food or alcohol outcome, and then that outcome is devalued using sensory-specific satiation or aversive pairing of the reward with illness. Goal-directed control over decision-making is observed as a reduction in the frequency at which subjects work to get the reward following outcome devaluation procedures (Adams and Dickinson, 1981; Dickinson, 1985). In the alcohol field, often put forth is the habit hypothesis, which suggests that individuals chronically exposed to alcohol are insensitive to the negative consequences associated with continued alcohol consumption, and hence continue alcohol seeking and show generally disrupted decision-making (Barker et al., 2015; Everitt and Robbins, 2005; Gremel and Lovinger, 2016). However, other bodies of work suggest alcohol seeking can still be goal-directed (e.g., Samson et al., 2004), and raise questions about the contribution of habit-related processes to continued ethanol dependence and misuse (Hogarth, 2020). Understanding the specific behavioral mechanisms that may be disrupted could shed light on the above discrepancies.

Rodent models of ethanol dependence have provided some evidence to support the hypothesis that people with AUD may be insensitive to negative consequences. Long-term ethanol self-administration renders lever pressing for alcohol insensitive to outcome devaluation (Corbit et al., 2012), an effect termed habitual alcohol seeking. This insensitivity to devaluation appeared to be driven by excessive alcohol exposure, as non-contingent ethanol exposure for the same extensive duration also left sucrose self-administration insensitive to outcome devaluation (Corbit et al., 2012). In addition, prior induction of ethanol dependence through vapor procedures rendered ethanol seeking behavior insensitive to outcome devaluation, an effect not observed when subjects were instead exposed to air vapor (Lopez et al., 2014; Renteria et al., 2020). Lastly, the effect of ethanol exposure seems largely due to direct actions on neural circuits that support goal-directed decision-making and not through other possibilities such as an effect on ethanol valuation. For instance, prior ethanol dependence produced food responding that was insensitive to outcome devaluation by disrupting output of a neural circuit shown to support goal-directed control (Gremel et al., 2016; Renteria et al., 2018). It thus appears that chronic exposure to ethanol itself can alter mechanisms supporting and/or underlying goal-directed decision-making.

However, as stated above, the particular disruptions to behavioral mechanisms that support or underlie goal-directed decision-making are unknown. Outcome devaluation procedures used to assess goal-directed control depend upon numerous components, including (but not limited to) general sensitivity to changes in motivational state, the ability to update the value of the reward (i.e., is the reward devalued), and the ability to infer and assign the updated value as a consequence of the associated action (i.e., using the devalued reward to update the action value; Balleine, 2011). In outcome devaluation procedures, the former two are confounded, as motivational state is altered by consumption of the reward itself. Procedures aimed at examining incentive learning, the process by which motivational states are used to assign value to the goals of our actions, have been used to examine how a change in motivational state (achieved through shifts in the degree of food restriction) can influence the updating and inference of reward value to control actions (Baltz et al., 2018; Wassum et al., 2009). Importantly, effects of shifts in motivational state on reward seeking and consumption can be examined in a re-exposure session. In this re-exposure session, the reward is delivered randomly in the absence of the normally-associated action. Differences in seeking and/or consummatory behaviors during the re-exposure session can reflect differences in motivational state (often hunger state) and/or palatability, respectively (Balleine and Dickinson, 1998; Wassum et al., 2009). In subsequent test sessions, the ability to infer and use the updated value to control actions is assessed. Differences between groups in the frequency of actions can indicate a deficit in updating and/or inferring the updated value associated with a particular action, as long as behavior during the re-exposure session was similar between groups in the same motivational state (Balleine and Dickinson, 1998; Wassum et al., 2009). Otherwise, motivational and/or palatability differences could contribute to the apparent alteration in the ability to update and infer a value change. Thus, use of the incentive learning task allows for the potential to examine whether there are alterations in 1) sensitivity to changes in motivational state, 2) sensitivity to changes in a palatability, and 3) ability to update and infer a value change.

One hypothesis for how ethanol dependence may disrupt goal-directed decision-making is by impeding these motivational and/or incentive learning processes. That is, the mechanisms underlying sensitivity to shifts in motivational state, assigning a less desirable value to the reward based on this motivational state, and/or inferring that new representation to control behavior may be dysfunctional in ethanol dependence. In the present studies, we employed the widely-used chronic intermittent ethanol exposure and repeated withdrawal (CIE) procedure (Becker, 1994; Lopez and Becker, 2005; Griffin et al., 2009) to induce ethanol dependence in mice, after which animals were trained in an incentive learning task. In a series of two experiments, we examined on separate days the capacity of mice to 1) use their current motivational state, 2) to update incentive value, and 3) guide instrumental actions. Furthermore, we examined these abilities within the context of differing magnitudes of motivational state shifts, using either mild (16-hr/day no access to food, 8-hr/day unlimited access) or more severe food restriction (gram restriction to 85% of baseline bodyweight).

Materials and Methods

Animals

Male and female C57Bl/6J and B6.129S2-*Emx1*$^{tm1(cre)KrJ}$/J mice on a C57Bl/6J background (Emx1-Cre; bred in-house one generation from mice ordered from Jackson Lab, USA) were housed 2–4 per cage under a 14/10-hr light/dark cycle with access to food (Lab-diet 5015) and water ad libitum unless stated otherwise. C57Bl/6J mice were used as prior works have identified disrupted goal-directed decision-making processes following chronic ethanol exposure in this strain (Lopez et al., 2014; Renteria et al., 2018; Renteria et al., 2020). Emx1-Cre mice were employed for the potential of future neurobiological manipulations. Mice were at least 8 weeks of age prior to the start of CIE procedures. The Animal Care and Use Committee of the University of California San Diego approved all experiments, and experiments were conducted according to NIH guidelines.

Chronic Intermittent Ethanol Exposure

Multiple cohorts of mice were exposed to four rounds of ethanol vapor or air as previously described (hourly food restriction cohort $n = 3$, gram food restriction cohort $n = 2$; Renteria et al., 2018). Strain was kept consistent within each vapor cohort, with the first cohort of hourly food restriction including only Emx1-Cre mice and all other cohorts containing only C57 mice. Each round consisted of 16-hr of vapor (ethanol or air) exposure followed by an 8-hr withdrawal period. This was repeated for four consecutive days, with a three-day period in between rounds in which no vapor exposure occurred. Vapor exposure was done by placing mice in their home-cage into Plexiglass chambers (Plas Labs Inc., USA), and passing air or ethanol vapor through the chambers. Ethanol was volatilized by bubbling air through a flask containing 95% ethanol at a rate of 2.3 L/min, and was combined with a separate air stream to give a total flow of approximately 10 L/min. To avoid effects of stress on instrumental behaviors (Dias-Ferreira et al., 2009) and broad actions of pyrazole including actions at the NMDA receptor (Pereira et al., 1992), no pyrazole or loading dose of ethanol was administered prior to placement in the chamber. Animals were monitored for ill effects of vapor exposure. Blood ethanol concentrations (BECs) were collected at the end of each round from 11 total sentinel mice (mean BEC = 40.34 ± 2.81 mM; Analox, USA). Use of sentinels prevented the ability to correlate BEC measurements with the magnitude of behaviors observed.

Behavioral Training and Testing Procedures

Training was conducted as previously described (Baltz et al., 2018). In brief, mice were trained to press a distal “seeking” lever to gain access to a proximal “taking” lever that, when pressed, would produce delivery of 20% sucrose. In this paradigm, the distal lever has been shown to be sensitive to incentive learning processes while the proximal lever is directly sensitive to changes in motivational state and does not rely on incentive learning processes to control responding (Balleine et al., 1995; Corbit and Balleine, 2003). Following training, mice were either kept in the same motivational state or underwent a shift in motivational state. To provide an opportunity to update the value of sucrose, mice were then given a re-exposure session where sucrose was delivered but no levers were presented. Testing then occurred across two days. First, mice were given a brief non-rewarded test

where presses on the distal and proximal levers were recorded, but no sucrose was delivered. This test provided a measure of the ability to infer or retrieve and use the updated value gained on the re-exposure day to control responding. For the second test, mice were given a normal rewarded session and presses on the distal and proximal levers were recorded. This rewarded test provided an opportunity for mice to use the experienced updated value to control ongoing decision-making.

Experimental Design and Food Restriction

Two experiments were run using different types of food restriction. In Experiment 1, the total time with access to food was restricted (hourly restricted), whereas in Experiment 2, the amount of daily food available was restricted (gram restricted). Prior to the onset of experimental procedures, mice in each experiment were assigned to one of two vapor groups (Air or CIE) and one of two restriction groups (shift or no shift). In Experiment 1, all mice underwent lever press training with 16-hr of food restriction that began ~3–4 hours before lights out and ended immediately prior to daily training. Mice within each Air or CIE group in Experiment 1 were further assigned to one of two groups: 16–16 Group or 16–2 Group. Mice in the 16–16 Group were kept at 16-hr food restriction for the duration of the experiment. Mice in the 16–2 Group were shifted to a 2-hr food restriction the night prior to the re-exposure session, with the 2-hr food restriction beginning 1.5 to 3 hrs into their light cycle (Vollmers et al., 2009) and ending immediately prior to daily sessions. Mice in this group were kept at 2-hr food restriction for the remainder of experimental procedures. In Experiment 2, mice were food restricted to ~85% of their baseline bodyweight, and instrumental training was conducted under this gram restriction. Mice within each Air or CIE Group in Experiment 2 were further assigned to one of two groups: R-R Group or R-F Group. Mice in the R-R Group were kept gram restricted and maintained at ~85% bodyweight for the duration of the experiment. Mice in the R-F Group were switched from gram restriction to free-feed the night before the re-exposure session, and were maintained at free-feed for the remainder of the experiment. Thus, there was a total of four groups in each experiment; in Experiment 1 the groups consisted of Air 16–16, Air 16–2, CIE 16–16, and CIE 16–2, while in Experiment 2 the groups consisted of Air R-R, Air R-F, CIE R-R, and CIE R-F. Group assignment was counterbalanced across cohort, sex, squad, and operant box. Given limitations of vapor and food restriction procedures, groups were kept consistent within a cage.

Instrumental Training

Mice began instrumental training 3–5 days following the last CIE procedure. Mice were trained in standard operant chambers containing two levers situated around a food magazine containing a fluid well with contact lickometers and a house light on the opposite wall within sound-attenuating boxes (Med-Associates, Vermont, USA). On the first day, mice underwent magazine training on a random time (RT) schedule, with a 20% sucrose in water outcome (20–30 uL) delivered on average every 120-sec for 60-min. For the next 3–4 days, mice had access to the right (proximal) lever, and right lever presses were rewarded on a continuous reinforcement (CRF) schedule (one lever press produces a sucrose delivery; equivalent to a fixed ratio 1 or FR1 requirement). The session continued until mice earned 30 sucrose deliveries or until 60-min had passed. After CRF training on the right lever,

schedule training continued with introduction of the left (distal) lever into the chamber. During schedule training, the session began with the left lever out and right lever retracted. Mice had to press the left (distal) lever under a random ratio (RR) schedule requirement to get access to the right (proximal) lever. A right lever press under a FR1 schedule would then result in the delivery of sucrose and retraction of the right lever. As gram restriction can support higher levels of lever pressing than hourly restriction, we used higher RR requirements in Experiment 2 than Experiment 1. The RR schedule requirement for the left lever increased across six days. For Experiment 1, schedule progression occurred as follows: RR1 for one day, RR2 for one day, and RR4 for four days. In Experiment 2, schedule requirements progressed from RR2 for one day, to RR4 for one day, to RR8 for the final four days. Sessions ended when a mouse earned 30 sucrose deliveries or 60-min had passed.

Re-exposure and Testing

Prior to the re-exposure session, mice were or were not shifted in hunger state depending on group assignment, and were maintained at the assigned hunger state for re-exposure and testing sessions. In Experiment 1, mice in the 16–16 Group were kept at 16-hr food restriction, while mice in the 16–2 Group were food restricted for just 2-hr prior to the re-exposure session. In Experiment 2, R-R mice were kept at ~85% bodyweight by limiting food consumption. Mice in the R-F Group were allowed to free-feed starting ~16-hr prior to the re-exposure session. For the re-exposure session, mice were given re-exposure to sucrose during a RT session for 1 h, with sucrose delivered on average every 2-min. The next day mice were given a 5-min non-reinforced test session where responses on the left lever under RR schedule requirements (same RR schedule as the last four days of training) would produce the right lever; however, right lever presses were not reinforced. The following day, mice were given a 60-min rewarded session similar to the previous day, except that on this day right lever presses produced a sucrose delivery.

Behavioral and Statistical Analysis

The alpha level was set at 0.05 for all experiments. As all mice within an experiment experienced the same food restriction during training and there were no differences between final groups in acquisition, training data were collapsed across food restriction groups for ease of comparison. For behavior across training days, data were analyzed using two-way mixed ANOVAs (Vapor group x Day) performed on distal and proximal lever presses, lever press rates, head entries, and head entry rates. For re-exposure and test session data, the primary dependent variables were lick behavior and lever press rates during the test session (Baltz et al., 2018). Mice that experienced a reduction in hunger state were expected to reduce lick and response rates but mice that were maintained at the same hunger state as training were not. For re-exposure lick and head entry behaviors, data were analyzed using two-way ANOVAs (Vapor group x Hunger state). Test data were analyzed using two-way ANOVAs (Vapor group x Hunger state) performed on distal and proximal lever press rates. A priori pairwise Bonferroni-corrected comparisons between hunger states were used to examine effects of motivational state on sucrose seeking and consummatory behavior as well as to determine the presence of incentive learning in each Vapor group (Baltz et al., 2018). Data were analyzed using Prism 6 (GraphPad, USA). Data are presented as mean ± standard error of the mean (SEM).

Results

Attrition

As has been our prior experience (Baltz et al., 2018), the hourly food restriction used in Experiment 1 supported lower levels of behavior relative to more severe gram restriction (Experiment 2), with hourly food restriction not inducing weight loss (data not shown). In addition, in both experiments we shifted motivational state prior to testing by allowing mice increased food access, which also supported lower levels of behavior. Thus, not all mice had sufficient response rates (average rate of > 0.25 left lever presses/min to produce right lever access) during training or testing to be included in analyses (Experiment 1 $n = 12$ mice excluded; Experiment 2 $n = 10$ mice excluded). In Experiment 1 final Group ns are as follows: Air 16–16 = 9 (7F, 2M), Air 16–2 = 9 (9M), CIE 16–16 = 11 (5F, 6M), and CIE 16–2 = 10 (7F, 3M). In Experiment 2 final Group ns are as follows: Air R-R = 10 (8F, 2M), Air R-F = 6 (1F, 5M), CIE R-R = 10 (3F, 7M), and CIE R-F = 10 (6F, 4M). Lickometer technical malfunction resulted in excluding lick data from 8 animals in Experiment 2. Final ns for analysis of re-exposure lickometer data in Experiment 2 are as follows: Air R-R = 8 (6F, 2M), Air R-F = 6 (1F, 5M), CIE R-R = 7 (2F, 5M), and CIE R-F = 7 (3F, 4M).

Weights

Following CIE exposure but prior to the start of behavioral procedures, Air and CIE mice showed similar weights (unpaired t-tests, Experiment 1: $t(37) = 1.39$, $p = 0.17$; Experiment 2: $t(34) = 0.15$, $p = 0.88$). Average weights in Experiment 1 were 23.26 ± 1.09 g in the Air group and 21.41 ± 0.81 g in the CIE group. In Experiment 2, average weights were 21.13 ± 1.02 g in Air mice and 20.95 ± 0.73 g in CIE mice.

Experiment 1

Acquisition.—Five days post the last vapor exposure, all mice underwent magazine and CRF training (data not shown) prior to schedule training. Once schedule training began, Air and CIE mice similarly acquired distal lever pressing under a RR schedule (Figure 1A, D). Two-way mixed ANOVAs (Vapor group x Day) conducted on distal lever presses and distal lever press rate revealed main effects of Day (distal lever presses: $F(4, 148) = 25.83$, $p < 0.0001$; distal lever press rate $F(4, 148) = 29.37$, $p < 0.0001$), but no main effect of Vapor group (Fs < 0.17, ps > 0.05) or significant interactions (Fs < 1.12, ps > 0.05). Furthermore, presses on the proximal lever were acquired similarly between Air and CIE mice (Figure 1B, E), with two-way mixed ANOVAs (Vapor group x Day) showing main effects of Day for proximal lever presses ($F(4, 148) = 36.33$, $p < 0.0001$) and proximal lever press rate ($F(4, 148) = 31.79$, $p < 0.0001$), but no main effects of Vapor group (Fs < 0.03, ps > 0.05) and no significant interactions (Fs < 1.47, ps > 0.05). In addition to lever press behaviors, Air and CIE mice also showed similar levels of head entries (main effect of Day: $F(4, 148) = 9.19$, $p < 0.0001$; Figure 1C, F) and rate of head entries (main effect of Day: $F(4, 148) = 6.45$, $p < 0.0001$), with no other effects indicated (no main effects of Vapor group: Fs < 2.04, ps > 0.05; no interactions: Fs < 0.95, ps > 0.05).

Re-exposure.—The shift in hunger state was initiated prior to the re-exposure session, when Air 16–2 and CIE 16–2 mice were moved from a 16-hr restriction period to a 2-hr

restriction period. Mice in the Air 16–16 and CIE 16–16 groups were maintained at 16-hr food restriction prior to the start of the re-exposure session. During the re-exposure session sucrose was delivered on a RT schedule and licks and head entries were recorded. We found that a shift in hunger state altered the total number of head entries performed (main effect of Hunger state, $F(1, 35) = 18.63$, $p < 0.001$; no main effect of Vapor group or interaction: Fs < 0.83, ps > 0.05), with planned post-hocs revealing a decrease in head entries in the 16–2 group compared to the 16–16 group for both Air ($p < 0.01$) and CIE mice ($p < 0.05$; Figure 2A). The same pattern was observed for the rate of head entries (data not shown). We also found that a shift in hunger state altered the rate of licking (main effect of Hunger state, $F(1, 35) = 4.55$, $p < 0.05$; no main effect of Vapor group or interaction: Fs < 2.43, ps > 0.05). A prior planned post-hoc analysis showed a significant reduction in the 16–2 compared to 16–16 lick rate for the Air group (Bonferroni-corrected $p < 0.05$) and not the CIE group ($p > 0.05$; Figure 2B). The same pattern was observed for total licks (data not shown). This finding suggests that while a reduction in hunger state decreased head entry and licking behavior, it may have done so to a larger extent in the Air group.

To investigate this further, we performed additional analyses on variables related to the patterning of licking across the session. Mice often organize their licking into bursts (defined as 3 or more sequential licks with an interlick interval of less than 1 second) and emit many bursts of licking behavior independent of whether or not sucrose has been delivered. We examined whether Vapor and Hunger assignment would differentially alter lick bursts and related lick patterns. When we examined the number of lick bursts made by Air and CIE mice, we found a main effect of Hunger state ($F(1, 35) = 7.32$, $p < 0.05$; no main effect of Vapor or interaction, Fs < 2.76, ps > 0.05; Figure 2C). Planned comparisons within each group showed a significant reduction in the number of lick bursts for Air 16–2 compared to Air 16–16 mice ($p < 0.05$), but CIE mice showed similar numbers of lick bursts independent of hunger state ($p > 0.05$). Further, Hunger state and Vapor group status did differentially affect the time in between licking bursts. The interburst interval was significantly longer in Air 16–2 mice compared to Air 16–16, but CIE mice had similarly short interburst intervals regardless of hunger state. This was supported by a two-way ANOVA which found a significant interaction between Hunger state and Vapor group ($F(1, 35) = 6.96$, $p < 0.05$), as well as main effects of Hunger state ($F(1, 35) = 10.32$, $p < 0.01$) and Vapor group ($F(1, 35) = 6.53$, $p < 0.05$; Figure 2D). A priori Bonferroni-corrected comparisons confirmed that the difference between hunger state groups was significant in Air ($p < 0.05$), but not CIE ($p > 0.05$) mice. When we looked at lick behavior within a burst, we found similar average lick burst durations in all groups (no main effects or interactions, Fs < 2.9, ps > 0.5; Figure 2E) as well as similar interlick intervals during a burst (no main effects or interactions, Fs < 0.74, ps > 0.05; Figure 2F). Mice also emit bursts of licking following sucrose delivery, where licking behavior is more directly tied to consumption. When we examined the duration of the first burst following reinforcement delivery, we found that a reduction in hunger state on average tended to reduce lick burst duration (main effect of Hunger state, $F(1, 35) = 6.16$, $p < 0.05$), and there was no effect of Vapor group or interaction (Fs < 0.88, ps > 0.05; Figure 2G); however, prior planned comparisons found no significant differences between hunger state groups in either Air or CIE mice (ps > 0.05). These results suggest that though both Air and CIE mice showed similar lick behaviors when a reinforcer was likely present, CIE

mice spent more time seeking reinforcement as indexed by lick rate, increased number of lick bursts, and reduced time between bursts even in the reduced motivational state.

Non-rewarded test.—The following day, in a brief non-rewarded test session, we examined whether mice were able to retrieve and use the updated value of sucrose to control lever press behavior. Whereas Air mice lowered response rates on both distal and proximal levers following a decrease in hunger state, CIE-exposed mice did less so (Figure 3A, B). This magnitude effect was supported by a two-way ANOVA performed on distal lever press rate that showed a main effect of Hunger state ($F(1, 35) = 6.68$, $p < 0.05$), but no main effect of Vapor group or interactions (Fs < 1.5, ps > 0.05). A priori Bonferroni pairwise comparisons showed a significant reduction in distal lever press rate in Air 16–2 mice compared to Air 16–16 ($p < 0.05$) but not between CIE groups ($p > 0.05$). A two-way ANOVA performed on proximal lever press rate also showed a main effect of Hunger state ($F(1,35) = 5.15$, $p < 0.05$), with a priori Bonferroni pairwise comparisons also showing a significant reduction in Air 16–2 mice compared to Air 16–16 mice ($p < 0.05$), but no difference between CIE 16–16 and 16–2 mice ($p > 0.05$). Once again, there was no effect of Vapor group and no interaction (Fs < 1.38, ps > 0.05). Hence, these data suggest that prior CIE exposure disrupted sensitivity to motivational shifts as evaluated in the re-exposure state that may have contributed to deficits in updating and inferring value to control both distal and proximal lever pressing.

Rewarded test.—During the prior non-rewarded test, the sucrose value representation had to be inferred. In contrast, in the rewarded test mice could use the observable value of sucrose for decision-making control over distal and proximal lever pressing. Further, the rewarded test provided another opportunity for mice to update reward value and use the experienced reduced value of sucrose to concurrently control responding. When CIE 16–2 mice were able to use the observable value of sucrose to control decision-making, they subsequently reduced both distal and proximal lever pressing (Figure 3C, D). A two-way ANOVA performed on distal lever press rate found a main effect of Hunger state ($F(1, 35) = 6.95$, $p < 0.05$; no main effect of Vapor group and no interaction, Fs < 1.34, ps > 0.05), with a priori pairwise comparisons showing a significant difference between CIE 16–16 and CIE 16–2 groups ($p < 0.05$) and no difference between Air groups ($p > 0.05$), which had already retrieved and inferred the reduced value in the non-rewarded test session and reduced responding. There was also a main effect of Hunger state on proximal lever press rate ($F(1, 35) = 11.79$, $p < 0.01$), with only CIE 16–16 and CIE 16–2 groups differing as revealed by a priori pairwise comparisons ($p < 0.05$), and not Air groups ($p > 0.05$). There was again no main effect of Vapor group and no interaction (Fs < 1.76, ps > 0.05). These data suggest that CIE disrupted the ability to use motivational state to update and/or infer a relatively modest reduction in sucrose value to control lever press behavior. However, if able to experience the updated sucrose value during decision-making, CIE mice could use the experienced downshift in sucrose value to control decision-making.

Experiment 2

Acquisition.—Three days post the last vapor exposure, all mice were food restricted and dropped to 85% of their baseline bodyweights across two days. Instrumental procedures

began 5 days after the last vapor exposure. Again, Air and CIE mice similarly acquired lever press training. Following RT and CRF procedures, mice began RR training on the distal lever and maintained an FR1 schedule on the proximal lever. Air and CIE groups increased distal and proximal lever presses similarly across training (Figure 4A, B). This was supported by two-way mixed ANOVAs (Vapor group x Day) that showed main effects of Day for both distal ($F(5, 170) = 107.40$, $p < 0.0001$) and proximal ($F(5, 170) = 3.65$, $p < 0.01$) lever presses and no other main effects or significant interactions (Fs < 1.67, ps > 0.05). Furthermore, lever press rates were similar between Air and CIE mice, with a main effect of Day for both distal ($F(5, 170) = 92.08$, $p < 0.0001$) and proximal ($F(5, 170) = 18.05$, $p < 0.0001$) levers (no main effects of Vapor group or interactions: Fs < 1.08, ps > 0.05; Figure 4D, E). In addition, Air and CIE mice made similar numbers of head entries (main effect of Day: $F(5, 170) = 13.94$, $p < 0.0001$) at a similar rate (main effect of Day: $F(5, 170) = 4.57$, $p < 0.001$), with no other significant effects indicated (no main effects of Vapor group or interactions: Fs < 1.61, ps > 0.05; Figure 4C, F). All together, the data suggest that Air and CIE mice were able to similarly acquire instrumental chains of behavior.

Re-exposure.—In Experiment 2, the reduction in hunger state was achieved by shifting mice from a gram-based food restriction state to a free-feed state. Mice in Air R-F and CIE R-F groups were allowed to free-feed in their home-cage a minimum of 16-hr prior to the start of the re-exposure session and for the remaining duration of experimental procedures. During the first 16-hrs of free-feed, shifted mice gained 3.73 ± 0.40 g — significantly more than R-R groups, which gained on average 0.14 ± 0.06 g (unpaired t-test; $t(34) = 8.69$, $p < 0.05$). Mice in Air R-R and CIE R-R were kept in the gram restricted state for all experimental procedures.

During the 60-min re-exposure session, head entry and licking patterns were similarly affected by hunger state in Air and CIE mice. We found that Vapor group and a shift in hunger state altered the number of head entries, as supported by a main effect of Hunger state ($F(1, 32) = 52.20$, $p < 0.0001$) and an interaction between Hunger state and Vapor group ($F(1, 32) = 6.32$, $p < 0.05$; no main effect of Vapor group: $F(1, 32) = 0.58$, $p > 0.05$; Figure 5A). However, post hocs for the interaction supported that head entries decreased in the R-F group compared to R-R group for both Air mice ($p < 0.0001$) and CIE mice ($p < 0.01$), albeit to a different degree. The same pattern was observed for head entry rates (data not shown). When we examined lick rate, a two-way ANOVA showed a main effect of Hunger state ($F(1, 24) = 114.20$, $p < 0.0001$; Figure 5B), with a priori pairwise comparisons showing a reduction in R-F compared to R-R in both Air and CIE groups (ps < 0.0001). There was no main effect of Vapor group and no significant interaction (Fs < 1.18, ps > 0.05). The same pattern was found for total licks (data not shown). The number of lick bursts made also followed a similar pattern. A two-way ANOVA revealed a main effect of Hunger state ($F(1, 24) = 111.00$, $p < 0.0001$) and Vapor group ($F(1, 24) = 4.34$, $p < 0.05$) but no interaction ($F(1, 24) = 3.00$, $p > 0.05$; Figure 5C). Planned comparisons once again showed reductions in the number of lick bursts made in the R-F compared to the R-R group for both Air and CIE (ps < 0.0001). Finally, time between bursts was also significantly affected by hunger state regardless of Vapor group. A two-way ANOVA found a main effect

of Hunger state ($F(1, 24) = 69.23$, $p < 0.0001$; Figure 5D) but no main effect of Vapor group or interaction (Fs < 1.02, ps > 0.05). Bonferroni comparisons supported that in Air and CIE groups, less hungry R-F mice exhibited significantly longer interburst intervals compared to hungrier R-R mice (ps < 0.0001).

Once again, when we examined patterns of licking, the average burst duration was not affected by Vapor group or Hunger state (no main effects or interaction; Fs < 4.01, ps > 0.05; Figure 5E). Within a burst, the average interlick interval was similar across all subgroups, as supported by a two-way ANOVA which found no main effects of Vapor or Hunger groups and no interaction (Fs < 1.46, ps > 0.05; Figure 5F). Regardless of Vapor group, less hungry mice decreased their lick burst duration following reinforcement compared to hungrier mice. This was supported by a two-way ANOVA which found a main effect of Hunger state ($F(1, 24) = 50.11$, $p < 0.0001$; Figure 5G) and no significant effect of Vapor group or interaction (Fs < 0.37, ps > 0.05). Bonferroni comparisons confirmed a significant reduction in lick burst duration in R-F mice compared to R-R mice for both Air ($p < 0.001$) and CIE ($p < 0.0001$) groups. These results suggest that following a severe shift in hunger state, CIE mice adjust seeking and consummatory lick behaviors similarly to Air mice.

Non-rewarded test.—In the subsequent 5-min non-rewarded test session the next day, the reduction in the more severe hunger state reduced distal responding for both Air and CIE mice (Figure 6A). A two-way ANOVA performed on distal lever press rate revealed a main effect of Hunger state ($F(1, 32) = 11.48$, $p < 0.01$), with a priori pairwise comparisons showing a reduction in R-F compared to R-R groups for both Air and CIE mice (ps < 0.05). In contrast, analysis of proximal lever press rates once again showed a main effect of Hunger state ($F(1, 32) = 8.04$, $p < 0.01$), and an a priori pairwise comparison showed a reduced press rate in the Air R-F group compared to Air R-R group ($p < 0.05$), but no difference between CIE groups ($p > 0.05$; Figure 6B). For both distal and proximal lever press rates, no main effects of Vapor group and no significant interactions were observed (Fs < 2.03, ps > 0.05).

Rewarded test.—During the subsequent 60-min rewarded test session, there was a significant reduction in lever press rate for both the distal and proximal lever in both Air and CIE groups (Figure 6C, D). A two-way ANOVA performed on distal lever press rate showed a main effect of Hunger state ($F(1, 32) = 37.63$, $p < 0.0001$), and a priori comparisons found significant reductions in distal press rate in R-F compared to R-R mice in both Air ($p < 0.01$) and CIE ($p < 0.0001$) groups. Unlike the non-rewarded test session, in the rewarded test session where mice were able to resample the sucrose delivered following completion of lever press requirements, CIE R-F mice reduced proximal lever press rate in a similar fashion to that of Air R-F mice. This was supported by a main effect of Hunger state ($F(1, 32) = 39.10$, $p < 0.0001$) and significant a priori pairwise comparisons between R-R and R-F mice in both Air ($p < 0.001$) and CIE groups ($p < 0.0001$). Once again, there were no main effects of Vapor group and no significant interactions for either distal or proximal lever press rates (Fs < 1.21, ps > 0.05). Together, this suggests that while CIE mice that had more severe food restriction still showed an insensitivity to devaluation on the proximal lever when value had to be inferred, goal-directed control over the more distal decision-making process remained intact. Furthermore, when allowed to use the current experienced action-outcome

relationship to guide behavior, CIE mice did show sensitivity to the reduced hunger state and decreased both the proximal as well as the distal action.

Discussion

Here we report that the prior induction of ethanol dependence can, but does not always, result in long-lasting deficits in motivational processes supporting goal-directed decision-making. Most notably, differences in the degree of food restriction determined whether CIE mice showed sensitivity to motivational shifts and whether their lever press performance was consistent with an updated value change. The present data suggest that long-lasting deficits in motivational processes and potentially aspects of value updating could in part explain deficits in outcome devaluation previously observed in ethanol dependent mice, and may in some cases support continued alcohol seeking even in the face of decreased motivational states. However, our data also suggest that the ability to recruit and infer outcome value for goal-directed decision-making is still possible within a more salient motivational state. Thus, we have shown that goal-directed processes can also remain largely intact following the induction of alcohol dependence in mice.

There has been interest in examining whether alcohol use, misuse, and dependence, as well as drug addiction in general, causes a shift from goal-directed to habitual control (Barker and Taylor, 2014; Everitt and Robbins, 2005; Gremel and Lovinger, 2016). By employing a decision-making task that separates processes supporting motivational versus inference components of goal-directed control, we find evidence that prior CIE exposure can reduce sensitivity to shifts in motivational state that could contribute to the ability to update and infer a value change for adaptive behavior. The insensitivity of sucrose seeking and related lick behaviors during re-exposure following a reduction in motivational state supports the hypothesis that CIE mice may be generally less sensitive to mild shifts in motivational state. During the re-exposure session, less restricted 16–2 CIE mice licked at a similarly high rate as more restricted 16–16 CIE mice, emitted a similar number of licks organized into bursts, and exhibited similarly low time between lick bursts. However, following a more severe shift in motivational state, CIE mice demonstrated patterns of licking that aligned with current motivational state and mirrored behavior seen in Air mice. That a more salient change in motivational state was able to support and recruit these same goal-directed processes for adaptive control lends support for this hypothesis.

Another possible explanation for changes in reward-seeking lick behaviors is that CIE produces a change in palatability, or the pleasantness of the outcome. Licking behavior has been proposed to reflect palatability (Berridge, 1991; Berridge, 2000), and can be separated from incentive processes at both the behavioral and neural level (Wassum et al., 2009). Altered palatability in CIE mice seems less likely, however, considering that across experiments, CIE and Air groups demonstrated similar patterns of consumption licking during periods that directly followed reinforcement. Of note, we cannot be confident that mice consumed all the sucrose in the first bout following its delivery. However, this interpretation is consistent with prior work showing no difference in sucrose consumption between ethanol dependent and control animals (Becker and Lopez, 2004). In light of these findings, we are prone to think a more likely explanation is that CIE induces changes

in motivational sensitivity. Further, we found that impairment was notably specific to seeking lick behaviors; it was not seen in head entries or when consuming sucrose itself. That impaired was not observed during a conditioned approach behavior (head entries) is somewhat surprising. It may be that mechanisms supporting conditioned approach behaviors and those supporting seeking licking are differentially affected by general motivational state shifts following alcohol exposure.

We were unable to determine whether the observed decreased sensitivity to shifts in motivational state were solely responsible for the deficits in updating and inferring a change in value to control behavior. Oftentimes, the presence of incentive learning is determined by examining test lever pressing following a shift in motivational state in a group of animals that have been re-exposed to the reward compared to another group that underwent the same shift in motivational state but did not undergo a re-exposure session. What is typically observed is that subjects that have undergone the re-exposure use an updated value to control lever pressing, whereas subjects not given a re-exposure session do not (Balleine, 1992; Balleine & Dickenson, 1991; Baltz et al., 2018). The observation that mildly food-restricted CIE mice do not reduce lever pressing following a reduction in motivational state and re-exposure (akin to control animals that have not undergone re-exposure) could suggest deficits in updating or inferring an updated value to control lever pressing.

Of particular note, CIE mice showed impairments when incentive value had to be inferred to control lever pressing. This effect was consistently seen on the proximal lever across experiments. It has been proposed that the proximal response is influenced by immediate sensory and motivational aspects of the outcome to a greater extent than the distal response, which depends more on a diffuse representation of the outcome (Balleine, 2011). This hypothesis came in part from the finding that re-exposure to the outcome in the new motivational state was necessary for distal lever presses to be altered. However, proximal lever pressing in the incentive learning task often decreases following a downshift in motivation, independent of whether re-exposure occurred or not (Balleine, 1992; Balleine and Dickinson, 1991). Taking this hypothesis into account, one explanation for the present results in CIE mice is that the downshift in motivational state was not sufficient to reduce the value of immediate sensory or motivational aspects of reward used to control proximal lever pressing. However, CIE mice were able to use motivational state to guide lever pressing when the reward was directly available and consumable, such as in the rewarded test. This pattern of results notably reflects that observed in licking behavior during re-exposure, when mild shifted CIE mice displayed normal consumption but seeking behaviors that were inconsistent with motivational state.

Thus, we cannot rule out the hypothesis that CIE induced impairments in encoding or retrieving value representations to control lever pressing in addition to any long-lasting change in motivational sensitivity. Mice exposed to CIE exhibited behavior consistent with deficits in encoding or retrieving incentive value to control distal decision-making, as well as deficits in encoding or retrieving more immediate properties of outcome value to control proximal pressing. Importantly, these were magnitude effects, and the overall direction of effects did not differ between Vapor groups. There was a difference between experiments in the extent of possible incentive learning deficits; namely, behavioral patterns consistent

with incentive learning were intact in Experiment 2, as indexed by the decrease in distal lever response rate in both Vapor groups following the more severe reduction in motivational state. It is therefore possible that prior exposure to CIE produces deficits in encoding or retrieval processes—deficits that can be overcome with more salient shifts in motivational state.

As the habit hypothesis (or a lack of goal-directed control) is somewhat at odds with theories based on negative reinforcement, where continued drug use is supported by the outcome's ability to alleviate a negative state, and considering that there is a relative dearth of evidence for the habit hypothesis supporting substance misuse and abuse in humans (Hogarth, 2020), our data suggest more careful examination is warranted for parameters where goal-directed decision-making is used. The finding that a reduction in distal lever pressing following a shift in motivational state was observed in more severely restricted CIE mice in Experiment 2 confirms that there is not a complete absence of goal-directed decision-making in ethanol dependent mice. The more salient the animal's motivational state, the more likely it is to be able to recruit and use goal-directed processes to control reward seeking. It may be that a highly motivationally salient state (such as acute withdrawal) may be sufficient to drive goal-directed alcohol seeking, whereas in less motivationally salient states, subjects may appear habitual in their alcohol misuse. However, any interpretation should include caveats that stress and the potential for individual weight fluctuations across vapor exposure could be contributing to the observed effects, and the generalizability of our findings must be tempered as we only examined these processes in two strains of inbred mice.

Together, our data suggest that ethanol dependence does induce long-lasting alterations to motivational processes supporting goal-directed decision-making. Interestingly, our findings also suggest that future investigation of how ethanol dependence interacts with motivational states to influence decision-making processes is warranted. Key neural circuits underlying the ability of varied motivational states to engage decision-making processes should be identified and investigated. Restoring motivational sensitivity in individuals with AUD may promote successful treatment by allowing them to use motivational states to appropriately control decision-making processes.

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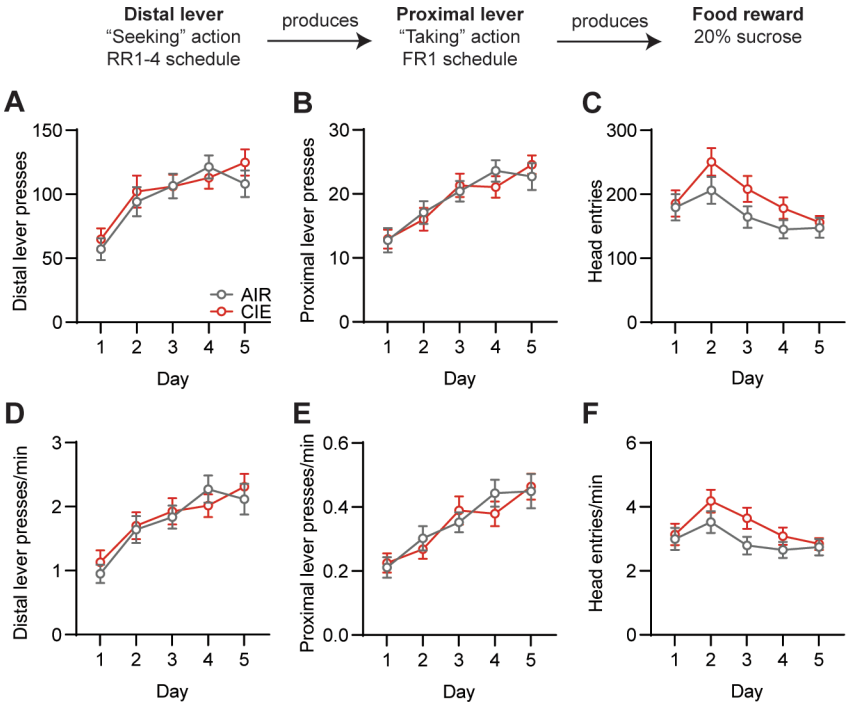

Fig. 1. Acquisition of instrumental task in Experiment 1 with mice under 16-hr food restriction.

Across 5 days of lever press training, chronic intermittent ethanol (CIE) and air control mice (**A**) increased the number of distal and (**B**) proximal lever presses. (**C**) Head entries mice made across days. Mice also increased the rate of (**D**) distal and (**E**) proximal presses. (**F**) Rate of head entries. RR = random ratio schedule; FR = fixed ratio schedule. Red circles (CIE mice) and grey circles (Air mice) are means ± SEM.

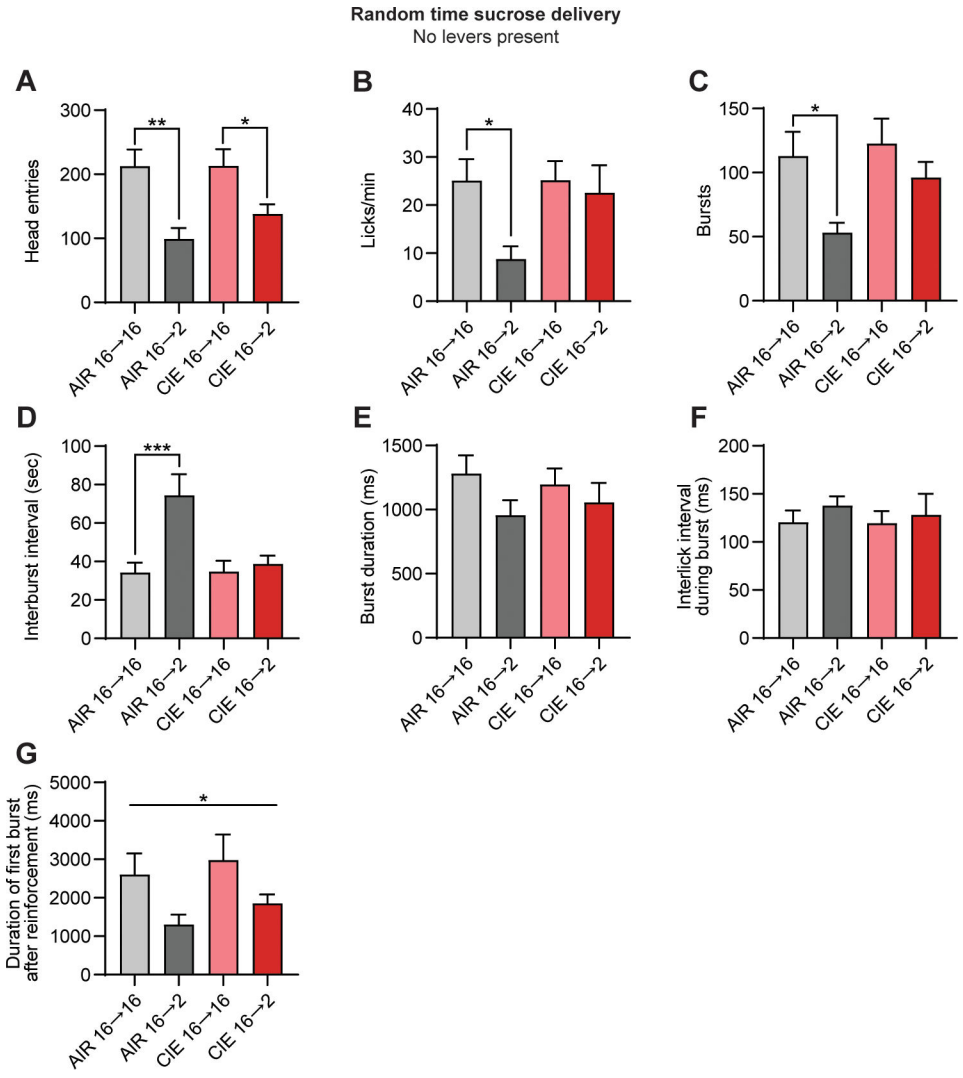

Fig 2. Re-exposure head entry and lick behavior for Experiment 1.

(**A**) Total head entries during the 60-min re-exposure session in chronic intermittent ethanol (CIE) and air control mice. (**B**) Rate of licking. (**C**) Total lick bursts, where a burst is defined as 3 or more sequential licks performed less than 1 second apart. (**D**) Time between bursts. (**E**) Duration of lick bursts. (**F**) Time between licks during a burst. (**G**) Duration of the first burst following delivery of a reinforcer. 16→16 = maintained at 16-hr restriction; 16→2 = shifted to 2-hr restriction. All data shown are means ± SEM. * = < 0.05, ** = < 0.01, and *** = < 0.001.

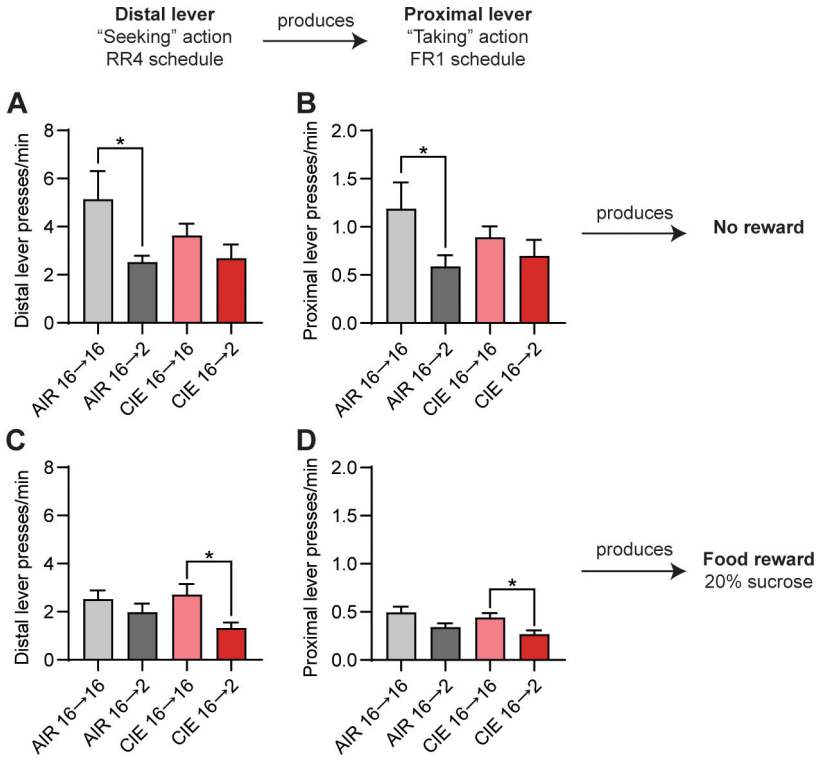

Fig. 3. Non-rewarded test and rewarded test for Experiment 1.

(**A**) Rate of distal and (**B**) proximal lever pressing in chronic intermittent ethanol (CIE) and air control mice during the 5-min non-rewarded test, where lever presses went unrewarded. (**C**) Rate of distal and (**D**) proximal lever pressing during the 60-min rewarded test session, where lever pressing did result in sucrose delivery. 16→16 = maintained at 16-hr restriction; 16→2 = shifted to 2-hr restriction. RR = random ratio schedule; FR = fixed ratio schedule. All data shown are means ± SEM. * = < 0.05.

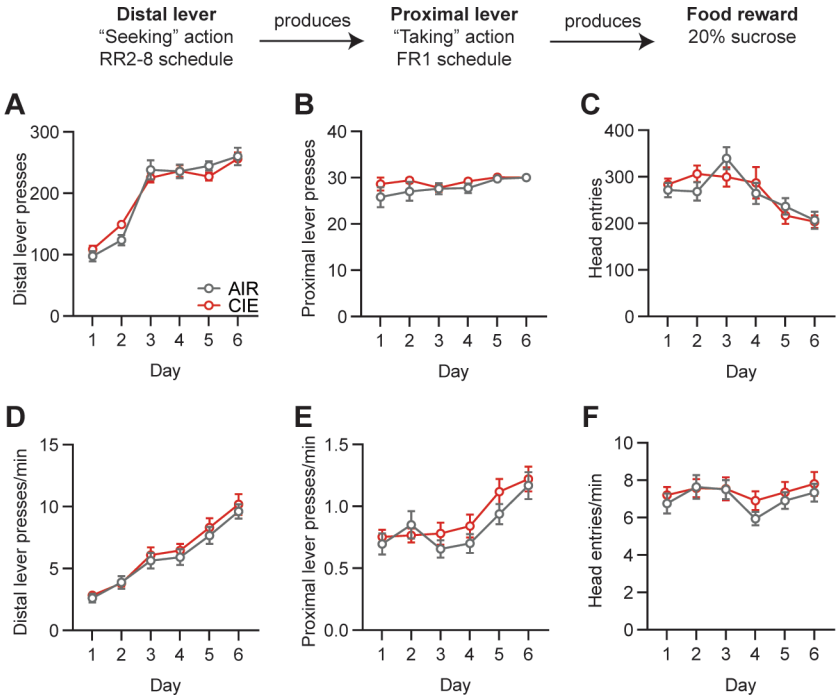

Fig. 4. Acquisition of instrumental task in Experiment 2 with mice under gram-based food restriction.

Across 6 days of lever press training, chronic intermittent ethanol (CIE) and air control mice (**A**) increased the number of distal and (**B**) proximal lever presses. (**C**) Head entries mice made across days. Mice also increased the rate of (**D**) distal and (**E**) proximal presses. (**F**) Rate of head entries. RR = random ratio schedule; FR = fixed ratio schedule. Red circles (CIE mice) and grey circles (Air mice) are means ± SEM.

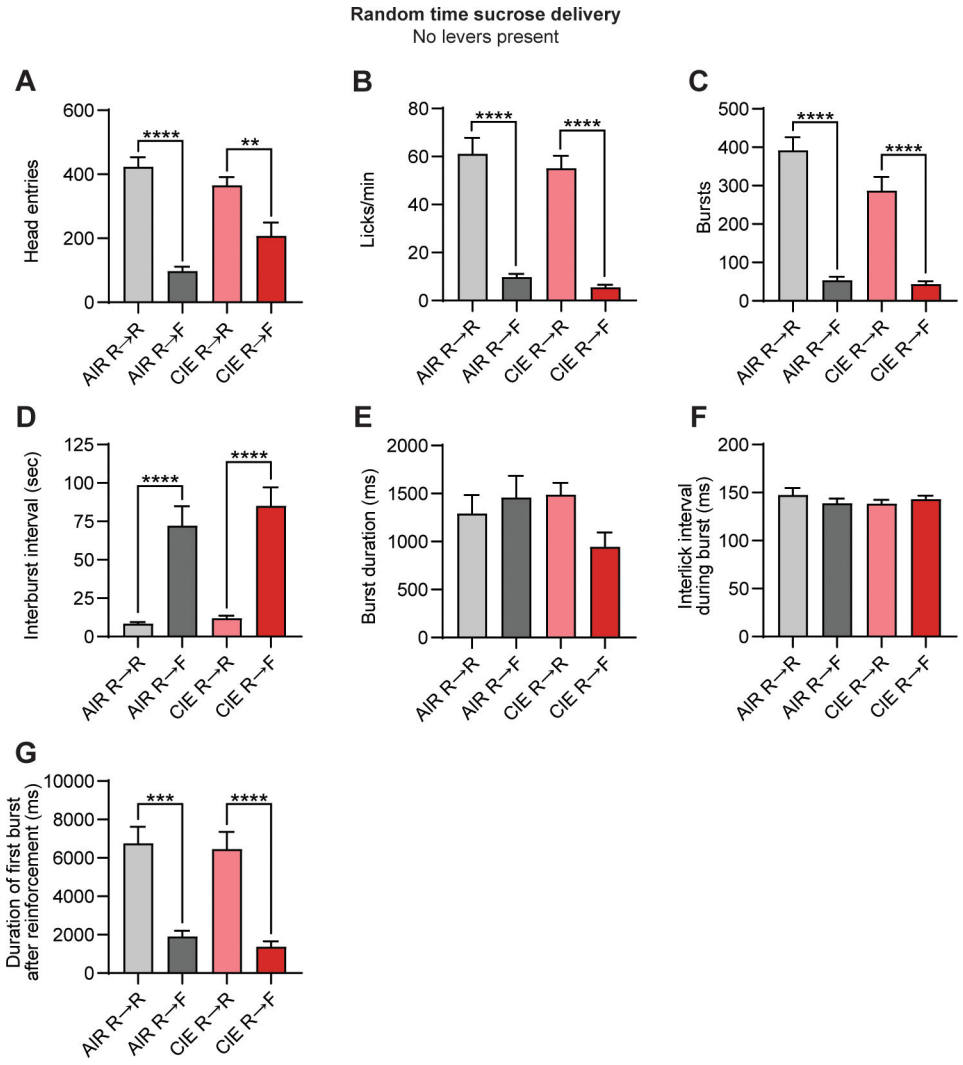

Fig 5. Re-exposure head entry and lick behavior for Experiment 2.

(**A**) Total head entries during the 60-min re-exposure session in chronic intermittent ethanol (CIE) and air control mice. (**B**) Rate of licking. (**C**) Total lick bursts, where a burst is defined as 3 or more sequential licks performed less than 1 second apart. (**D**) Time between bursts. (**E**) Duration of bursts. (**F**) Time between licks during a burst. (**G**) Duration of the first burst following delivery of a reinforcer. R→R = maintained at gram restriction; R→F = shifted to free-feed. All data shown are means ± SEM.** = < 0.01, *** = < 0.001, and **** = < 0.0001.

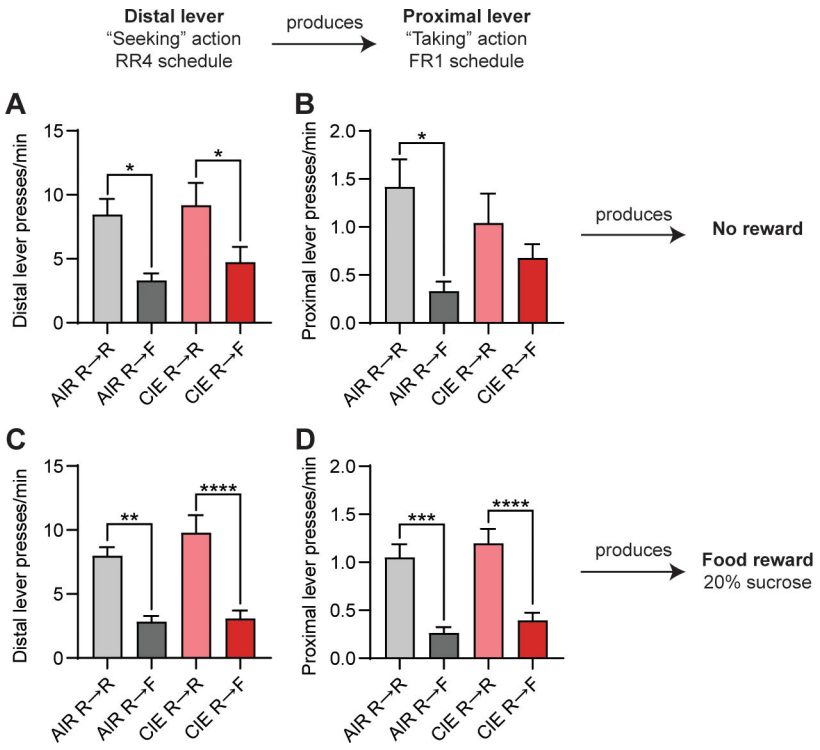

Fig. 6. Non-rewarded test and rewarded test for Experiment 2.

(**A**) Rate of distal and (**B**) proximal lever pressing in chronic intermittent ethanol (CIE) and air control mice during the 5-min non-rewarded test, where lever presses went unrewarded. (**C**) Rate of distal and (**D**) proximal lever pressing during the 60-min rewarded test session, where lever pressing did result in sucrose delivery. R→R = maintained at gram restriction; R→F = shifted to free-feed. RR = random ratio schedule; FR = fixed ratio schedule. All data shown are means ± SEM. * = < 0.05, ** = < 0.01, *** = < 0.001, and **** = < 0.0001.