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Intermittent access to ethanol drinking facilitates the transition to excessive drinking after chronic intermittent ethanol vapor exposure

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Abstract

Background—Alcohol binge drinking in humans is thought to increase the risk for alcohol use disorder. Unclear is whether drinking patterns (e.g., binge-like or stable drinking) differentially affect the transition to compulsive-like drinking in dependent individuals. We examined whether chronic binge-like drinking facilitates the transition to compulsive-like drinking in rats.

Methods—Male Wistar rats were given 5 months of intermittent access to ethanol (IAE) or continuous access to ethanol (CAE) in a two-bottle choice paradigm. Then rats were given chronic intermittent ethanol (CIE) vapor exposure. Escalation of ethanol intake and compulsive-like responding for ethanol, using a progressive-ratio schedule of reinforcement and quinine-adulterated ethanol, were measured.

Results—IAE rats escalated ethanol drinking after 2 weeks of two-bottle choice, whereas CAE rats exhibited stable ethanol drinking for 5 months. After 8 weeks of CIE, both IAE+CIE and CAE+CIE rats escalated their ethanol intake. However, IAE rats escalated their ethanol intake weeks sooner than CAE rats and exhibited greater ethanol intake. No differences in compulsive-like responding were found between IAE+CIE and CAE+CIE rats. However, both IAE+CIE and CAE+CIE rats showed strong compulsive-like responding compared with rats without prior IAE or CAE.

Conclusions—Chronic ethanol drinking at stable or escalated levels for several months is associated with more compulsive-like responding for ethanol in rats that are exposed to CIE compared with rats without a prior history of ethanol drinking. Moreover, IAE facilitated the transition to compulsive-like responding for ethanol after CIE exposure, reflected by the escalation of ethanol intake. These results suggest that IAE may facilitate the transition to alcohol use disorder. The present study indicates that despite a moderate level of ethanol drinking, the IAE animal model is highly relevant to early stages of alcohol abuse and suggests that it may be associated with neuroadaptations that produce a faster transition to alcohol dependence.

Keywords

Alcohol; dependence; CIE; two-bottle choice; addiction

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Introduction

Alcohol use disorder (AUD) is a chronic relapsing disorder that is associated with the loss of control of ethanol drinking and compulsive drinking that is driven by negative affect (Koob et al., 2004; Koob and Volkow, 2010). In humans, evidence suggests that binge drinking may increase the risk for AUD, especially in adolescents and young adults (Delker et al., 2016; Llerena et al., 2015; Substance Abuse and Mental Health Services Administration, 2016). Currently unclear is whether the higher incidence of AUD is attributable to prior binge-like drinking *per se* or predisposing genetic factors. Additionally, the way in which prior ethanol use affects the transition to excessive ethanol drinking still needs to be elucidated. Prior chronic binge-like ethanol drinking might increase the rate of transition to excessive ethanol drinking, the level of ethanol drinking, or the compulsivity associated with ethanol drinking.

Two of the most prominent animal models in ethanol research are intermittent access to ethanol (IAE) using two-bottle choice (Lee et al., 2014; Seif et al., 2015; Millan et al., 2015; Hopf et al., 2010; Carnicella et al., 2014; Lim et al., 2012; Li et al., 2012; Carnicella et al., 2009; Barak et al., 2011b; Barak et al., 2011a; George et al., 2012; Wise, 1973; Momeni et al., 2014; Simms et al., 2014; Simms et al., 2008) and chronic intermittent ethanol (CIE) vapor exposure (Kissler et al., 2014; Gilpin et al., 2008c; Gilpin et al., 2008b; Staples et al., 2015; Leao et al., 2015; de Guglielmo et al., 2016). The IAE paradigm is commonly used to model binge-like drinking and compulsive-like responding for ethanol (Barak et al., 2011b; Nielsen et al., 2008; Simms et al., 2010; Feduccia et al., 2014) before dependence occurs, whereas CIE has been used to model postdependent states, as well as excessive drinking and compulsive-like responding for ethanol when combined with ethanol self-administration (Rogers et al., 1979; Roberts et al., 1996; Roberts et al., 2000; O'Dell et al., 2004; Schulteis et al., 1995). However, some groups but not all have reported the emergence of a dependent state using the IAE paradigm, characterized by somatic and emotional signs of withdrawal in mice and rats. This suggests that the IAE paradigm may model the dependence-induced escalation of ethanol drinking under specific conditions or in specific individuals. The combination of IAE and CIE models provides an opportunity to examine the effects of chronic binge-like drinking (IAE) on the transition to excessive drinking (CIE) and directly test the relevance of the IAE/CAE paradigm on vulnerability to the transition to dependence-induced escalation of ethanol drinking using the CIE model.

The transition to escalation of ethanol intake via CIE is thought to be attributable to negative reinforcement mechanisms during early withdrawal. The escalation of ethanol intake usually takes 4–8 weeks of CIE exposure (O'Dell et al., 2004; Gilpin et al., 2008c; Rimondini et al., 2003; Rimondini et al., 2005; Gilpin and Koob, 2010; Gilpin et al., 2009), although the rate of transition to the escalation of ethanol intake may be accelerated by other experimental factors, such as nicotine exposure (Leao et al., 2015). However, unclear are the effects of prior IAE on the transition to the escalation of ethanol drinking during CIE exposure.

In the present study, we hypothesized that IAE would facilitate the transition to the escalation of ethanol intake that is produced by CIE and increase compulsive-like responding for ethanol. Wistar rats were given 5 months of continuous access to ethanol (CAE) or IAE using a two-bottle choice paradigm (George et al., 2012; Wise, 1973; Momeni

et al., 2014; Simms et al., 2014; Simms et al., 2008) before being exposed to 8 weeks of CIE to produce escalation of ethanol intake. Ethanol drinking, fixed-ratio (FR) responding, progressive-ratio (PR) responding, and drinking despite adverse consequences (quinine adulteration) were measured to test the hypothesis that a history of IAE or CAE facilitates the transition to dependence-induced drinking in the CIE paradigm and increases compulsive-like responding for ethanol.

Materials and Methods

Animals

Young adult male Wistar rats (250–300 g) were used for all of the experiments. The experiments began when the rats were 60 days of age. The rats were maintained on a 12 h/12 h light/dark cycle with *ad libitum* access to food and water. All of the procedures were conducted in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by The Scripps Research Institute Institutional Animal Care and Use Committee.

Experimental design

Two groups of rats were first given 5 months of either IAE or CAE in two-bottle choice testing with ethanol and water. The same rats were trained to self-administer ethanol and then received CIE (IAE+CIE and CAE+CIE) for 8 weeks while being tested for ethanol self-administration. Following 8 weeks of self-administration testing, the rats were maintained on CIE and tested for ethanol responding on a PR schedule of reinforcement and with quinine adulteration of ethanol (Fig. 1)

A separate cohort of rats without prior two-bottle choice experience was trained to self-administer ethanol and then either given CIE or maintained on air. After 11 weeks of self-administration during CIE (8 weeks of escalation and 3 weeks of stabilization) or air exposure, the rats were tested for responding for quinine-adulterated ethanol (Fig. 1).

Intermittent access to 20% ethanol (two-bottle choice)

This procedure was identical to George et al. (2012) and based on the procedure that was developed by Wise (1973) and modified by Simms et al. (2008). The rats were given either continuous (CAE; $n = 10$; 24 h/day, 7 days/week) or intermittent (IAE; $n = 11$; 24 h/day, 3 days/week [Monday, Wednesday, Friday]) access to ethanol (20%, v/v) using a two-bottle choice procedure (ethanol vs. water) for 5 months.

The two-bottle choice procedure consisted of two 100 ml graduated plastic bottles with stainless-steel drinking spouts that were inserted through two grommets in front of each cage 15 min after the lights were turned off in the reverse light/dark cycle room. Both bottles were weighed 24 h after the fluids were presented, and measurements were taken to the nearest gram. The position of the bottles was switched every other day to avoid side preference. As a control, cages with no animal and only bottles were used to measure dripping. Dripping from these cages was subtracted from the 24 h rat intakes for both ethanol and water.

Operant ethanol self-administration

Four groups were trained to operantly self-administer ethanol: IAE and CAE rats with 5 months of prior two-bottle choice experience, CIE rats ($n = 7$), and control rats ($n = 7$). The latter two groups had no history with the two-bottle choice paradigm and instead only received operant self-administration training prior to CIE (CIE rats) or air (control rats) exposure. Self-administration sessions were conducted in standard operant conditioning chambers (Med Associates). The animals were trained to self-administer 10% (w/v) ethanol and water solutions until stable responding was maintained. The rats were first subjected to an overnight session in the operant chambers with access to one lever (right lever) that delivered water (FR1). Food was available *ad libitum* during this training. After 1 day off, the rats were subjected to a 3 h session (FR1) for 1 day, a 2 h session (FR1) the next day, and a 1 h session (FR1) the next day, with one lever delivering ethanol (right lever). All of the subsequent sessions lasted 30 min, and two levers were available (left lever: water; right lever: ethanol) until stable levels of intake were reached after 4 weeks of training. Upon completion of this procedure, the animals were allowed to self-administer a 10% (w/v) ethanol solution and water on an FR1 schedule of reinforcement (i.e., each operant response was reinforced with 0.1 ml of the solution).

Progressive-ratio ethanol self-administration

To test the compulsivity of ethanol self-administration, IAE and CAE rats self-administered ethanol under a PR schedule of reinforcement. Operant self-administration on an FR1 schedule requires minimal effort by the animal to obtain the reinforcement and thus was considered a measure of intake during the data analysis. For five sessions, the rats were tested on a PR schedule, in which the number of lever presses that were necessary to obtain the next reinforcement progressively increased according to the following progression: 1, 1, 2, 2, 3, 3, 4, 4, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, etc. The PR session stopped after 90 min or when 15 min elapsed without the rat obtaining reinforcement. The rats were then maintained on an FR1 schedule until stable levels of ethanol self-administration were established before testing the effect of quinine on ethanol drinking.

Chronic intermittent ethanol vapor

The IAE (IAE+CIE), CAE (CAE+CIE), and CIE rats were exposed to CIE as described previously (O'Dell et al., 2004; Gilpin et al., 2008a). The rats underwent repeated daily cycles of 14 h vapor ON (blood ethanol levels during vapor exposure ranged between 150 and 250 mg%) and 10 h vapor OFF, during which behavioral testing occurred (i.e., 6–8 h after the vapor was turned OFF, the rats performed operant self-administration on a FR and PR schedule of reinforcement and responded for quinine-adulterated ethanol) when brain and blood ethanol levels are negligible (Gilpin et al., 2009). In this model, rats have been shown to exhibit somatic and motivational signs of withdrawal (Vendruscolo and Roberts, 2014). During cycles of CIE exposure, the rats were tested for the escalation of ethanol intake (6–8 h into withdrawal) using operant self-administration as described above.

Blood ethanol measurements

Tail blood was collected during CIE exposure in the last hour of vapor exposure (i.e. 13 h into vapor ON) and used to determine blood ethanol levels using an oxygen-rate ethanol analyzer (Analox Instruments).

Quinine adulteration of ethanol

To further test the compulsivity of ethanol intake, we used quinine adulteration. This test measures the persistence of rats to consume ethanol despite the aversive bitter taste of quinine that was added to the ethanol solution, which has been validated as a measure of compulsive intake (Vendruscolo et al., 2012; Seif et al., 2013).

All four groups of rats (IAE+CIE, CAE+CIE, CIE, and control) were maintained on an FR1 schedule until stable levels of ethanol self-administration were established before testing the effect of quinine on ethanol drinking. The ethanol solution was adulterated with increasing concentrations of quinine (0.005, 0.01, and 0.05 g/L; one concentration/session, one session/day). The concentrations of quinine that were used were similar to previous studies that measured the resistance to quinine in CIE rats without a history of IAE or CAE (Vendruscolo et al., 2012; Seif et al., 2013; Leao et al., 2015).

Statistical analysis

The results are expressed as mean \pm SEM. The data were analyzed using two-way repeated-measures analysis of variance (ANOVA), with group (IAE and CAE) as the between-subjects factor and day (days of sessions) as the within-subjects factor. The ANOVAs were followed by Fisher's Least Significant Difference (LSD) *post hoc* test as appropriate. The PR data were analyzed using *t*-tests. For quinine adulteration, a two-way repeated-measures ANOVA was used, with group (IAE+CIE, CAE+CIE, CIE, and control) as the between-subjects factor and concentration of quinine as the within-subjects factor. One-way repeated-measures ANOVA were also performed for each individual group to examine the reduction of ethanol responding relative to their own pre-quinine baseline.

Results

Blood ethanol levels

Blood ethanol levels were measured during the CIE transition to escalation of ethanol intake. Blood ethanol levels in IAE+CIE, CAE+CIE, and CIE rats did not differ and were maintained between 150–250 mg/100 ml during CIE exposure (data not shown).

Intermittent access to 20% ethanol (two-bottle choice)

Fluid leakage from the bottles in cages that contained no animals resulted in 0.5–1.5 ml of fluid loss during a 24 h period, which was 5–15 times lower than the level of drinking in IAE and CAE rats. For ethanol intake (expressed as ml/24 h), the repeated-measures ANOVA of days 10–70 of two-bottle choice testing revealed significant effects of group ($F_{1,19} = 5.57, p < 0.05$) and day ($F_{24,456} = 6.35, p < 0.0005$) and a significant group \times day interaction ($F_{24,456} = 2.56, p < 0.0005$). For ethanol intake (expressed as g/kg/24 h), the repeated-measures ANOVA of days 10–70 of two-bottle choice testing revealed significant

effects of group ($F_{1,19} = 4.46, p < 0.05$) and day ($F_{24,456} = 6.21, p < 0.0005$) and a significant group \times day interaction ($F_{24,456} = 2.49, p < 0.0005$).

Consistent with previous findings (Wise, 1973; Simms et al., 2008; Carnicella et al., 2014; George et al., 2012), Fisher's LSD *post hoc* test revealed that rats that were given IAE presented significant escalation of ethanol drinking relative to their own baseline (4.3 ± 0.6 ml; 2.1 ± 0.3 g/kg on day 10 vs. 12.2 ± 2.3 ml; 5.8 ± 1.1 g/kg on day 70), whereas rats that were given CAE did not present escalation of intake (5.6 ± 1.3 ml; 2.7 ± 0.7 g/kg on day 10 vs. 6.3 ± 1.3 ml; 3.1 ± 0.7 g/kg on day 70). The escalation of ethanol intake in IAE rats was seen after day 15 and persisted throughout the 5 months of IAE (Fig. 2A, B).

Operant ethanol self-administration during CIE to measure the transition to the escalation of ethanol intake

The ANOVA revealed a significant effect of week ($F_{8,152} = 5.51, p < 0.0005$) and a significant week \times group interaction ($F_{8,152} = 2.09, p < 0.05$) in rats that underwent 8 weeks of CIE exposure after prior two-bottle choice experience, with no effect of group ($F_{1,19} = 3.53, p = 0.07$). Fisher's LSD *post hoc* test showed that both the IAE and CAE groups significantly escalated the number of ethanol rewards compared with their own baseline. However, rats that were given IAE+CIE presented escalation of ethanol intake that began in week 2 and lasted through week 8 of testing, whereas CAE+CIE rats presented escalation of ethanol intake in weeks 2 and 8 only. IAE rats presented a significant increase in ethanol intake compared with CAE rats in weeks 4–7 (Fig. 3). Both the IAE and CAE groups continued to show escalated ethanol intake that did not differ from each other for several weeks beyond week 8 of testing, during which time the groups were maintained on CIE exposure for PR and quinine testing.

Progressive-ratio schedule of reinforcement for ethanol self-administration

Once IAE and CAE rats escalated their ethanol intake after 8 weeks of CIE, they were tested for responding on a PR schedule of reinforcement. The *t*-test showed no significant difference in ethanol responding on a PR schedule between these two groups (Fig. 4A).

Quinine adulteration of ethanol

IAE+CIE, CAE+CIE, CIE, and control rats were presented with ethanol that was adulterated with quinine at increasing concentrations. The repeated-measures ANOVA for IAE+CIE, CAE+CIE, CIE, and control rats showed no effect of group or quinine concentration on ethanol responding and no group \times quinine concentration interaction. The one-way repeated-measures ANOVA for IAE+CIE rats showed no effect of quinine concentration. The one-way repeated-measures ANOVA for CAE+CIE rats showed no effect of quinine concentration. The one-way repeated-measures ANOVA for CIE rats revealed a significant effect of quinine concentration on ethanol responding ($F_{3,18} = 17.34, p < 0.0005$). Fisher's LSD *post hoc* test showed that CIE rats presented a significant decrease in ethanol responding relative to their own pre-quinine baseline when ethanol was adulterated with 0.05 g/L quinine. The one-way repeated-measures ANOVA for control rats revealed a significant effect of quinine concentration on ethanol responding ($F_{3,18} = 8.36, p < 0.005$). Fisher's LSD *post hoc* test showed that control rats presented a significant decrease in

ethanol responding relative to their own pre-quinine baseline when ethanol was adulterated with 0.005, 0.01, and 0.05 g/L quinine.

Fig. 4B shows that both groups of rats without a prior history of ethanol (control rats and CIE rats) exhibited a decrease in responding for ethanol relative to their own pre-quinine baseline responding when ethanol was adulterated with increasing concentrations of quinine. However, rats with prior ethanol experience (IAE+CIE and CAE+CIE groups) maintained ethanol responding that was similar to their pre-quinine baseline even at the highest quinine concentration tested.

Discussion

The present study tested the hypothesis that prior IAE facilitates the transition to the escalation of ethanol intake that is produced by CIE. Rats with IAE+CIE exhibited accelerated escalation of ethanol drinking compared with CAE+CIE rats (~5 weeks faster). However, IAE+CIE and CAE+CIE rats ultimately reached the same level of excessive ethanol drinking after 8 weeks of CIE. Both IAE and CAE rats exhibited a 2- to 10-fold decrease in the sensitivity to quinine adulteration compared with rats without a history of IAE.

In the IAE paradigm, IAE led to the escalation of ethanol intake, which was not observed in rats with CAE. These results are consistent with previous studies from our laboratory and others (Wise, 1973; Simms et al., 2008; Carnicella et al., 2014; George et al. 2012). A pattern of binge-like drinking in young adults has been hypothesized to be causally related to a higher risk for AUD later in adulthood, but unclear is whether this association is causal or only correlational (Delker et al., 2016; Llerena et al., 2015). The present results confirm the causal hypothesis, in which a history of IAE (i.e., a model of binge-like drinking) accelerated the transition to compulsive-like drinking in rats.

IAE+CIE rats accelerated their escalation of ethanol drinking by ~5 weeks compared with CAE+CIE rats during CIE exposure. This suggests that both IAE+CIE and CAE+CIE rats were able to reach a state of escalated ethanol intake, but IAE+CIE rats transitioned to escalated ethanol intake earlier than CAE+CIE rats. This faster escalation of ethanol drinking in IAE+CIE rats cannot be explained by different exposures to ethanol vapors because IAE+CIE and CAE+CIE rats shared the same ethanol vapor chambers (in separate cages), and their blood ethanol levels did not differ and were maintained between 150–250 mg/100 ml.

When tested for ethanol responding on a PR schedule of reinforcement after 8 weeks of CIE, both IAE+CIE and CAE+CIE rats presented similar breakpoints. When ethanol was adulterated with quinine, both IAE+CIE and CAE+CIE rats presented equally high compulsive-like responding for ethanol. Both IAE+CIE and CAE+CIE rats maintained high ethanol responding at the highest quinine concentration tested (0.05 g/L). Control and CIE rats with no prior IAE or CAE experience reduced ethanol responding with concentrations of quinine that were 2- to 10-fold lower compared with IAE+CIE and CAE+CIE rats. These quinine concentrations were similar to previously reported studies with CIE and control rats

without a prior history of IAE or CAE (Vendruscolo et al., 2012; Seif et al., 2013; Leao et al., 2015). Altogether, the PR and quinine adulteration tests suggest that chronic access to ethanol drinking, regardless of whether it is in a stable manner or in a binge-like pattern, may enhance compulsive-like responding for ethanol in postdependent individuals. The lack of difference in compulsive-like responding between IAE+CIE and CAE+CIE rats may seem surprising when considering that IAE rats have been shown to be more compulsive than CAE rats (Hopf et al., 2010). However, the quinine adulteration test was performed after 8 weeks of CIE, a time point at which CAE+CIE rats were indistinguishable from IAE+CIE rats in terms of the escalation of drinking and PR responding. Our experimental design only tested the effect of quinine at the end of the escalation period to avoid interference with ethanol drinking during escalation. These results suggest that a ceiling effect might have been reached after prolonged exposure to CIE, and CIE will ultimately negate differences between IAE and CAE animals by producing animals with high levels of compulsive-like ethanol drinking.

The IAE model is usually not used as a model of ethanol dependence because of the relatively low blood ethanol levels that are achieved and low or lack of, somatic or emotional signs of withdrawal (Cippitelli et al., 2012; George et al., 2012). However, some research groups have observed significant withdrawal symptoms (Steenland et al., 2012; Fu et al., 2015) and evidence of compulsive-like drinking (Carnicella et al., 2014; Hopf and Lesscher, 2014; Seif et al., 2015; Darcq et al., 2016; DePoy et al., 2013) in the IAE model that may be relevant to alcohol use disorders. The present study confirmed the relevance of the IAE model to AUD by demonstrating that a history of moderate but prolonged (5-month) IAE in young adult rats accelerates the escalation of ethanol drinking and produces compulsive-like drinking when rats maintain blood ethanol levels that are greater than 150 mg/100 ml. We chose to use 5 months of access to replicate our previous work (George et al., 2012). This paradigm better resembles the human condition, in which years of moderate to heavy drinking often precede a diagnosis of AUDs. Most laboratories use shorter models, with 1–3 months of access. This shorter duration of access may not lead to an accelerated transition to dependence-induced drinking or higher compulsive-like responding for ethanol. Nevertheless, the present results with 5 months of access to two-bottle choice reinforce the relevance of the IAE model to study the transition from controlled ethanol drinking to a compulsive pattern of ethanol drinking that is observed in AUD. The present study also demonstrates the possibility of combining the IAE and CIE models to examine the neurobiological mechanisms that are involved in the transition from binge-like drinking to dependence. Finally, these results confirm human studies that reported a positive relationship between binge drinking and the development of AUD (Dawson et al., 2005; Hasin and Grant, 2015) and demonstrate a causal relationship between binge drinking and dependence, independent of genetic vulnerability, which may partially explain the high incidence of AUD in populations with a history of binge drinking.

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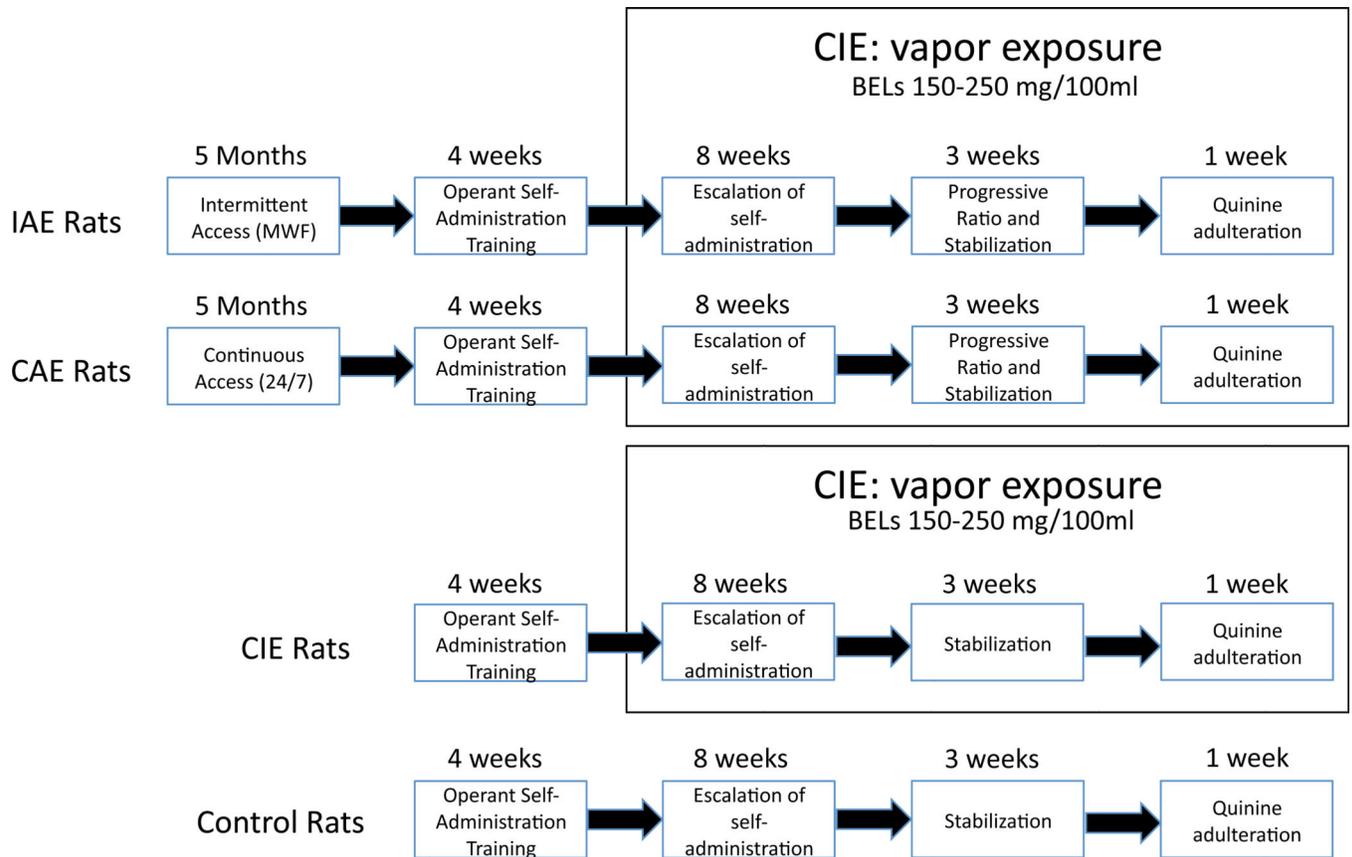


Figure 1.

Experimental timeline. IAE and CAE rats underwent two-bottle choice testing followed by operant self-administration and CIE. While maintained on CIE, IAE and CAE rats were tested for the escalation of ethanol intake followed by progressive-ratio responding and responding for quinine-adulterated ethanol. A separate cohort of rats (CIE and control) was trained to self-administer ethanol and then given CIE or air. CIE and air control rats were then tested for responding for quinine-adulterated ethanol after the stabilization of intake during CIE.

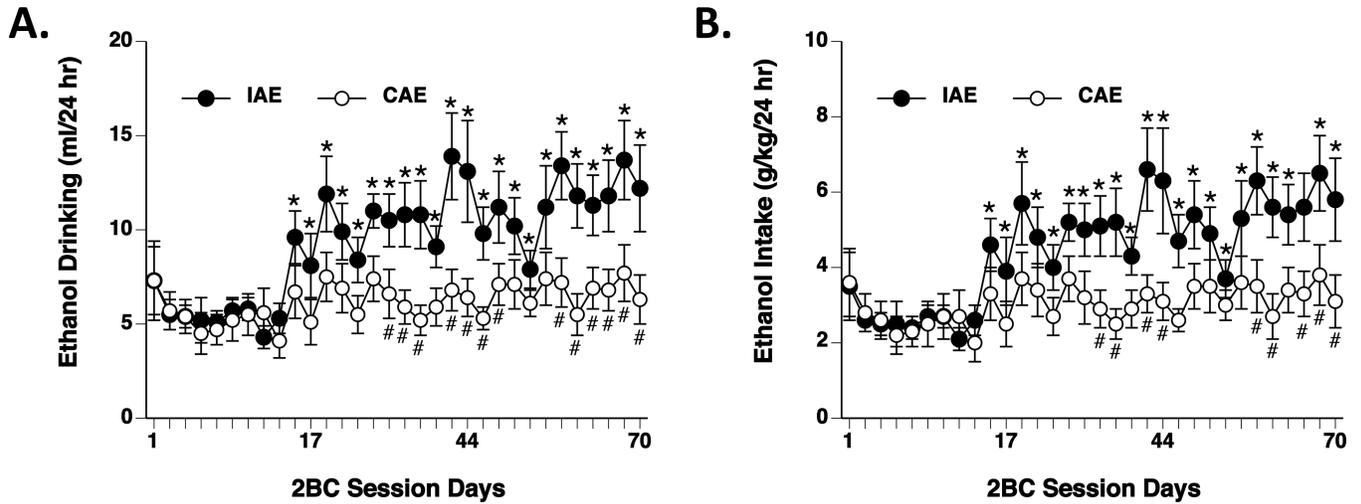


Figure 2.

Intermittent access to ethanol. Rats were given 24-h two-bottle choice (water and 20% ethanol, v/v) sessions either continuously (7 days/week) or intermittently (3 days/week) for 5 months. Data are shown through day 70. **(A)** Ethanol intake, expressed as ml/24 h. Rats that were given IAE (black circles) exhibited a significant increase in ethanol intake compared with rats that were given CAE (white circles). **(B)** Ethanol intake, expressed as g/kg/24 h. Rats that were given IAE (black circles) exhibited a significant increase in ethanol intake compared with rats that were given CAE (white circles). * $p < 0.05$, IAE intake on specific day compared with IAE intake on day 10; # $p < 0.05$, CAE vs. IAE on same day.

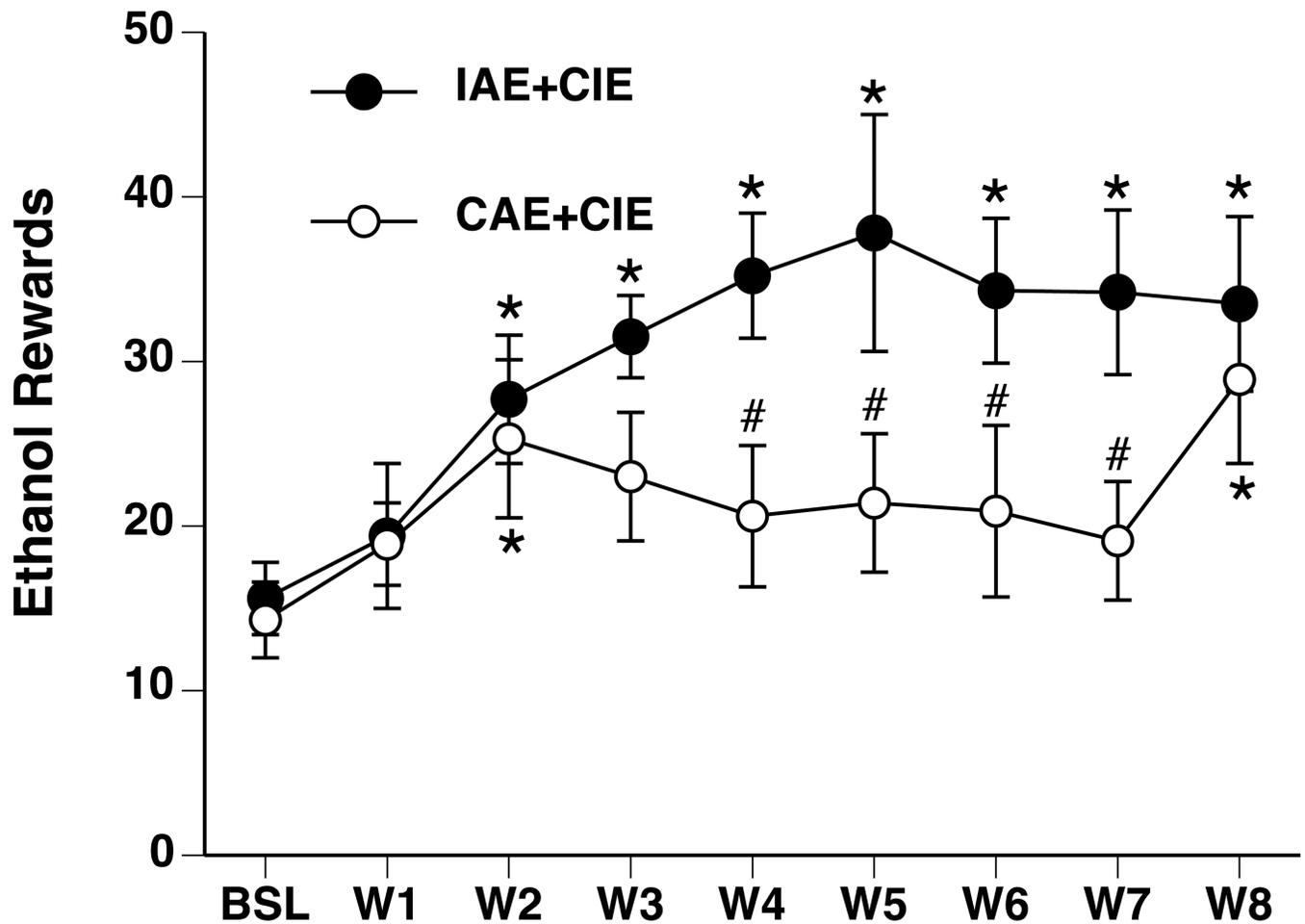


Figure 3. Escalation of operant responding for ethanol after CIE. Rats with prior continuous or intermittent access to ethanol were given chronic intermittent ethanol vapor exposure and then tested for operant ethanol responding in daily 30-min sessions. The data are expressed as an average of the 30-min sessions each week. CAE+CIE (white circles) and IAE+CIE (black circles) rats significantly escalated their responding for ethanol relative to baseline levels. However, IAE+CIE rats presented a significantly greater rate of escalation, reaching a dependent state faster, compared with CAE+CIE rats. W, week of testing. * $p < 0.05$, compared with baseline intake; # $p < 0.05$, CAE+CIE vs. IAE+CIE on same day.

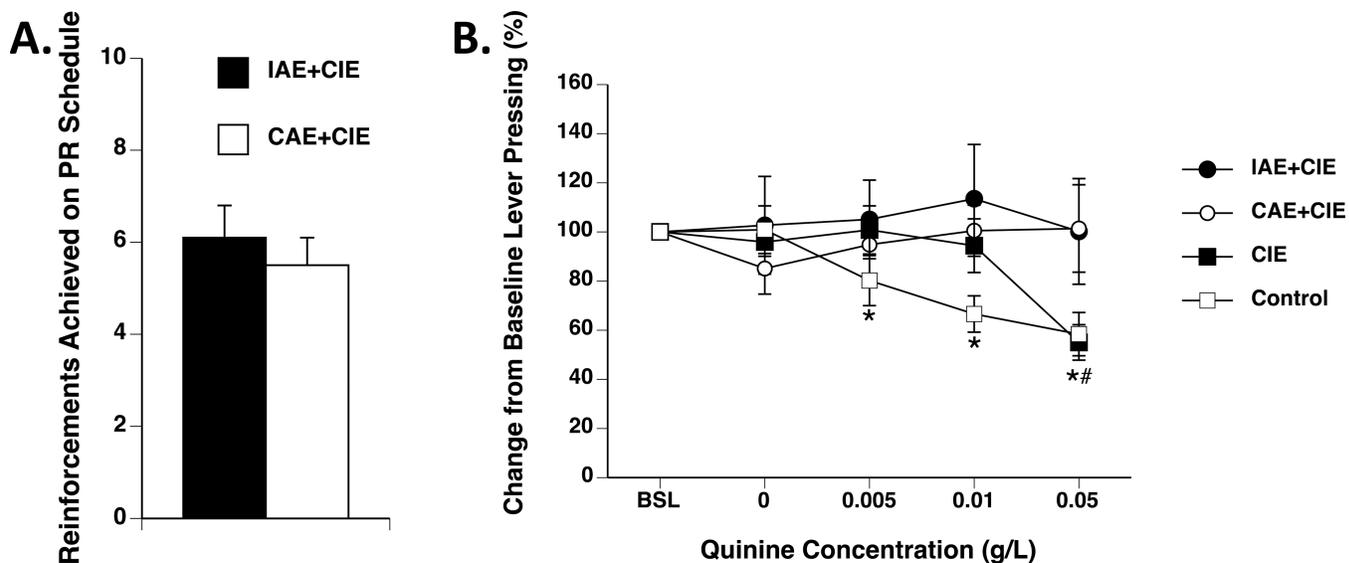


Figure 4. Compulsive-like responding for ethanol. **(A)** Mean responding for ethanol on a progressive-ratio schedule of reinforcement across 5 sessions of testing. No difference in ethanol responding on a progressive-ratio schedule of reinforcement was found between rats with prior continuous (white bar) and intermittent (black bar) access to ethanol. **(B)** Rats were tested for persistent ethanol drinking despite the aversive bitter taste of quinine that was added to the ethanol solution. Reductions of ethanol responding were measured relative to baseline levels. CAE+CIE (white circles) and IAE+CIE (black circles) rats did not reduce their responding for ethanol at the quinine concentrations tested. Control rats (white squares) presented a significant reduction of responding for ethanol at quinine concentrations of 0.005, 0.01, and .05 g/L. CIE rats (black squares) significantly reduced their responding for ethanol at 0.05 g/L quinine. * $p < 0.05$, reduction from control pre-quinine baseline in control rats at the specific quinine concentration; # $p < 0.05$, reduction from CIE pre-quinine baseline in CIE rats at the specific quinine concentration.