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Motivation for alcohol becomes resistant to quinine adulteration after 3-4 months of intermittent alcohol self-administration

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Abstract

Background—Continued consumption of alcohol despite deleterious consequences is a hallmark of alcoholism and represents a critical challenge to therapeutic intervention. Previous rat studies showed that enduring alcohol self-administration despite pairing alcohol with normally aversive stimuli was only observed after very long-term intake (> 8 months). Aversion-resistant alcohol intake has been previously interpreted to indicate pathological or compulsive motivation to consume alcohol. However, given the time required to model compulsive alcohol seeking in previous studies, there is considerable interest in developing more efficient and quantitative rodent models of aversion-resistant alcohol self-administration.

Methods—Outbred Wistar rats underwent 3-4 months or ~1.5 months of intermittent, home-cage, two-bottle access (IAA) to 20% alcohol (v/v) or water. Then, after brief operant training, the effect of the bitter-tasting quinine (0.1 g/L) on the motivation of to seek alcohol was quantified via progressive ratio (PR). Motivation for quinine-adulterated 2% sucrose under PR was assayed in a separate cohort of 3-4 months IAA rats. The effects of quinine on home-cage alcohol consumption in IAA rats and rats with continuous access to alcohol were also examined. Finally, a dose-response for quinine taste preference in IAA and continuous-access animals was determined.

Results—Motivation for alcohol after 3-4 months IAA, measured using an operant PR procedure, was not altered by adulteration of alcohol with 0.1 g/L quinine. In contrast, after 3-4 month of IAA, motivation for sucrose under PR was significantly reduced by adulteration of sucrose with 0.1 g/L quinine. In addition, motivation for alcohol after only ~1.5 months IAA was significantly reduced by adulteration of alcohol with 0.1 g/L quinine. Furthermore, home-cage alcohol intake by IAA rats was insensitive to quinine at concentrations (0.01, 0.03 g/L) that significantly reduced alcohol drinking in animals with continuous access to alcohol. Finally, no changes in quinine taste preference after 3-4 months IAA or continuous access to alcohol were observed.

Conclusions—We have developed a novel and technically simple hybrid operant/IAA model in which quinine-resistant motivation for alcohol is evident after an experimentally tractable period

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of time (3-4 months vs. 8 months). Quinine dramatically reduced sucrose and water intake by IAA rats, indicating that continued responding for alcohol in IAA rats despite adulteration with the normally aversive quinine might reflect maladaptive or compulsive motivation for alcohol. This model could facilitate identification of novel therapeutic interventions for pathological alcohol-seeking in humans.

Keywords

Motivation; alcohol; breakpoint; quinine; intermittent access

Introduction

One hallmark of human alcoholism is the continued seeking and consumption of alcohol despite deleterious health, economic, and/or societal consequences (Larimer et al., 1999; Sanchis-Segura and Spanagel, 2006). Since the compulsive drive to obtain alcohol represents a critical challenge to the therapeutic treatment of alcoholism, there is considerable interest in developing pre-clinical rodent models of continued alcohol consumption despite aversive consequences. Continued drug-seeking despite pairing with aversive stimuli has been interpreted as a model for the pathological, inflexible, and/or compulsive behavior of human alcoholics and addicts (Wolffgramm and Heyne, 1991; Spanagel et al., 1996; Spanagel and Holter, 1999; Wolffgramm et al., 2000; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2005; Vengeliene et al., 2009). However, previous studies have found that very long-term alcohol intake (>8 months) in rats is required for alcohol consumption to become resistant to adulteration with the aversive, bitter-tasting quinine (Wolffgramm and Heyne, 1991; Spanagel et al., 1996; Spanagel and Holter, 1999; Wolffgramm et al., 2000), making such methods impractical.

The long duration of alcohol consumption required in previous studies for the development of quinine-resistant intake may, in part, reflect the relatively low levels of drinking observed in many out-bred rat models of alcohol intake. Thus, we utilized an Intermittent Access to Alcohol paradigm (IAA), where rats are allowed access to 20% alcohol three of the seven days per week, with at least one day between the 24 hr alcohol-access sessions (Wise, 1973; Simms et al., 2008). IAA rats escalate intake across the first month of alcohol access, reaching plateau intake levels of ~6 g/kg/24 hr with blood alcohol concentrations greater than 50 mg% (Simms et al., 2008); importantly, these intake levels are significantly greater than those observed under continuous alcohol access (Wolffgramm and Heyne, 1991; Spanagel et al., 1996; Spanagel and Holter, 1999; Wolffgramm et al., 2000; Simms et al., 2008) and are more similar to those observed in alcohol-preferring rat strains (Bell et al., 2006). In addition, several compounds which may be efficacious at reducing alcohol intake by human alcoholics exhibit a greater effect on alcohol drinking under IAA compared to rats with continuous access to alcohol (Steensland et al., 2007; Simms et al., 2008; McKee et al., 2009). Thus, the IAA method appears to have some predictive validity.

Here, we utilized a novel hybrid of IAA and operant methods to demonstrate that quinine-resistant motivation for alcohol develops as early as 3 to 4 months after initiating IAA. This IAA/operant hybrid method may therefore model some aspects of compulsive alcohol-seeking in humans.

Materials and Methods

All experiments were performed in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the

Institutional Animal Care and Use Committee of the Ernest Gallo Clinic and Research Center and the University of California, San Francisco.

Alcohol self-administration under intermittent access two-bottle choice

Adult male, Wistar rats (250-275 g, Harlan, Livermore, CA) were individually housed and maintained on a 12-hr light/dark cycle (lights on at 7 am) in an AAALAC-accredited facility. Rats were allowed one week after arrival to habituate to the vivarium. Rats began alcohol self-administration at ~PN55. Self-administration was performed using a two-bottle, home-cage intermittent alcohol access model (IAA; Wise, 1973; Simms et al., 2008), where rats were given 24-hr concurrent access to two bottles, one with alcohol (20%, v/v) and the other with water, starting on the afternoon of Monday, Wednesday, and Friday. Thus, there was either 24 or 48 alcohol-free hr between each 24-hr period of alcohol access. The amount of alcohol or water consumed was determined by bottle weight before and after the 24 hr of alcohol access. Bottle position was randomly assigned for each session. Rats had ad libitum food and water access throughout experimentation.

Operant responding for alcohol or sucrose

After 3-4 months or ~1.5 months of IAA, rats were trained to operantly respond for alcohol in two overnight sessions, which were performed two days apart (typically over the Monday and Wednesday evenings). Rats were placed in standard operant chambers (10″ wide × 11″ long × 11″ high, Coulbourn Instruments, Allentown, PA) where two retractable levers located 5 cm above the cage floor flanked a 100 μL dipper cup. Two cue lights were located 6 cm above each lever (Bowers et al., 2008). Chambers were also outfitted with a house light, cue tone, and were enclosed in sound-attenuation cubicles that contained fans to provide white noise. Rats were randomly assigned the right or left lever as “active”. Depression of the active lever raised the dipper cup containing the reinforcer, illuminated the cue light above the lever and activated the tone for 4 sec. Reinforcers were delivered in a lick-contingent manner, where access to the reinforcer was terminated if licking did not commence within 2 sec (Bowers et al., 2008). Depression of the inactive lever had no programmed consequence. In the first 15 hr overnight session, the first ten reinforcers were delivered on an FR-1 schedule, with FR-3 thereafter. Also, session start was signified by raising of the dipper cup in concert with lever extension, tone activation and cue light illumination above the active lever. This occurred for up to 5 iterations if lever pressing or licking was not initiated within the first 3 min, and this cycle was repeated every 45 min until lever pressing behavior began. In the second overnight session, the first ten reinforcers were delivered on FR-3, with FR-5 thereafter. Table 1 shows the levels of lever pressing and licking during the second overnight session. Licks at the dipper were recorded with a lickometer to verify that lever presses were followed by consumption of the reinforcer. Reinforcer deliveries without greater than 3 licks were considered a null response and not used to advance the reinforcement schedule.

After the two overnight sessions, motivation to consume alcohol was determined once per week (either Wednesday or Friday) using an exponential progressive ratio (PR) schedule. PR sessions occurred late in the afternoon when access to the bottle containing alcohol would normally begin under IAA. Rats did not have access to alcohol from the bottle before each PR session. Thus, rats underwent one progressive ratio session for alcohol per week, with access to alcohol under the two-bottle, home-cage IAA paradigm for the other two days per week (for example, Monday = IAA-only, Wednesday = IAA-only, Friday = PR-only). An initial cohort of rats (Fig. 1) underwent one PR session a week for four weeks in order to determine the stability of PR responding for alcohol in the absence of quinine across multiple testing. A separate cohort of rats was then used to determine the impact of adulteration of alcohol with 0.1 g/L quinine on PR responding (Fig. 2). Adulteration of

alcohol with quinine occurred in one of the first two PR sessions; in the other PR session, rats were given access to alcohol without quinine. Presence of quinine in the first versus second PR session was counterbalanced across rats and subsequent data analysis did not reveal ordering effects (all $p > 0.1$).

PR methods were nearly identical to those previously described (Bowers et al., 2008), except that the PR session lasted 1.5 hr, and began in the late afternoon. Briefly, rats were placed in the operant chamber, and 2 min later responding was initiated by presentation of a compound cue (extension of the levers, illumination of the stimulus light over the active lever, tone sounding, and illumination of a raised dipper cup filled with alcohol or sucrose). After the compound cue, responding proceeded under a moderate PR schedule (Bowers et al., 2008): after the compound cue, rats could lick the dipper cup, press a lever, or do nothing. The progressive ratio schedule of reinforcement was determined from the following equation: $PR = 5e^{(0.1 \times \text{reinforcer(s) previously earned})} - 5$ (Bowers et al., 2008). Thus, if rats licked first (~50% of rats), a PR schedule of reinforcement of 1, 1, 2, 2, 3, 4, 5, 6, 7, 9, 10, 12, 13, 15, 17, 20, 22, 25, 28, 32, 36, etc. ensued. If rats pressed first (~50% of rats), a PR of 1, 2, 2, 3, 4, 5, 6, 7, 9, 10, 12, 13, 15, 17, 20, 22, 25, 28, 32, 36, etc. ensued, because rats had already pressed once and received a reward. Breakpoint was defined as the maximum number of presses performed in the last, successfully completed ratio in either a 1.5 hr session or after 15 min of non-responding, whichever came first. Greater than 90% of rats ended the session early by omitting response for 15 min. Null responses, where a rat completed the required number of lever presses but did not lick to consume the reinforcer, were not included in breakpoint determination.

A separate cohort of rats that self-administered alcohol for 3-4 months under IAA was trained to operantly respond for sucrose (2% w/v) so that the motivation to consume sucrose could be assayed under the PR schedule described above. Experiments were conducted as described above with the following modification: overnight operant training sessions and PR test sessions were performed on Tuesday and Thursday, and the bottle containing alcohol was removed before 8 a.m. on these days so that the majority of alcohol would be metabolized by the late afternoon when operant sessions occurred. This modification was employed to minimize interference with alcohol consumption under IAA. Addition of quinine to sucrose in the first versus second PR session was counterbalanced across rats and subsequent data analysis did not reveal ordering effects (all $p > 0.1$).

Approximately 25% of animals exhibited insufficient responding for alcohol or sucrose (<30 active lever presses during the second overnight session) and were not tested under PR. In addition, <5% of IAA rats drank less than 2 g/kg/24 hr after 1.5 months and were excluded from further analysis.

Two-bottle experiments with quinine

Preliminary experiments with continuous-access rats showed lower baseline operant responding compared to IAA. Specifically, responding for alcohol in the second overnight session as well as breakpoint obtained during the 1.5 hr PR test session were less than half of that observed for IAA animals (overnight presses: 167 ± 57, $n = 9$; breakpoint: 4.7 ± 0.6, $n = 7$). Since sucrose-responding animals still exhibited a breakpoint of ~5 in the presence of quinine, these low levels of responding under PR could represent a basal level of pressing in rats. Thus, in combination with the high level of variability in operant responding across continuous-access animals, the low breakpoint in the absence of quinine could preclude detection of any significant effect of quinine even with a large sample size. Therefore, operant experiments in continuous-access animals were not pursued further. Instead, we compared the impact of quinine adulteration on alcohol consumption in IAA rats versus continuous-access rats during home-cage, two-bottle choice drinking. Continuous access

animals had continuous access to 20% alcohol or water under a two-bottle choice paradigm, and testing of quinine effects on home-cage bottle drinking began after 3-4 months access to alcohol. Quinine was added to the alcohol bottle of IAA and continuous-access rats once per week, and the effect on alcohol consumption for the next 24 hr determined. These rats were run in two cohorts, one with 0, 0.003, 0.01, or 0.03 g/L quinine, and the other with 0, 0.003, 0.01, 0.03, or 0.1 g/L quinine, with concentrations counterbalanced across animals. Thus, each animal experienced 3 or 4 concentrations of quinine in addition to a no quinine condition.

For quinine taste preference tests, alcohol animals were exposed to 0.0003, 0.001, 0.003, 0.01, 0.03, or 0.1 g/L quinine in water in one bottle and water only in the other bottle, with one concentration of quinine versus water per week, with doses counterbalanced across animals and each animal experiencing 4 or 5 concentrations of quinine. For naive animals, some animals initially showed a very high preference during exposure to one of the three lowest quinine concentrations, perhaps because of the novelty of a slightly bitter substance (in contrast to alcohol drinkers who have been exposed to the slightly bitter alcohol for months). Thus, for the three lowest quinine concentrations, naive animals were given two sessions of access for the given quinine concentration before performing a session where preference was obtained (see also Tordoff et al., 2008).

Reagents

Quinine hydrochloride and sucrose were obtained from Sigma-Aldrich (St. Louis, MO). Saccharin sodium hydrate was obtained from Acros Organics (Morris Plains, NJ).

Statistical analysis

All data are shown as the mean plus or minus the standard error of the mean. Operant responding was evaluated with a one-way repeated measures ANOVA (with quinine vs without quinine) followed by a Bonferroni correction. Home-cage intake was analyzed with a one-way ANOVA over dose followed by Tukey post-hoc comparisons. Statistical analysis was performed with SigmaStat 3.1 (San Jose, CA). PR responding for sucrose or alcohol after 3-4 months or ~1.5 months IAA was performed in distinctly separate and non-overlapping cohorts. Thus, statistical differences in PR responding in the absence of quinine between groups were not determined, and instead we note that basal PR responding between groups was qualitatively similar. In addition, for the data in Figure 5, all animals did not experience every concentration of quinine, and therefore the effects of quinine between continuous-access and IAA animals were not compared.

Results

After 3-4 months consumption of alcohol (20% v/v) under IAA and 2 overnight operant training sessions, motivation for alcohol was quantified using an operant, exponential PR schedule of reinforcement (Fig. 1A). Rats underwent one, 1.5 hr PR session for alcohol per week, with access to alcohol under the two-bottle, home-cage IAA paradigm for the other two alcohol-access days per week (see Materials and Methods for details). The breakpoint, the maximum number of completed lever presses in a given ratio that resulted in reinforcement, was used as a quantitative measure of motivation (Richardson and Roberts, 1996;Sanchis-Segura and Spanagel, 2006;Bowers et al 2008). In an initial cohort of animals, the breakpoint for alcohol was stable across the four weeks of testing (Fig. 1B; $n = 18$; $F_{(3,68)} = 0.611$, $p = 0.611$). In addition, pressing on the inactive lever represented less than 20% of total lever presses (see below), suggesting that rats could discriminate the active and inactive lever even under a novel exponential PR response requirement.

We then examined, in a separate cohort of IAA rats, whether adulteration of alcohol with the normally aversive, bitter-tasting quinine (0.1 g/L) would alter motivation expressed to obtain alcohol during the PR session. Quinine was added to alcohol in either the first or the second PR session for a given rat, with alcohol alone in the other session, in a counterbalanced manner across animals (Fig. 2A). Quinine adulteration of alcohol following 3-4 months IAA did not reduce breakpoint (Fig. 2B; $n = 12$; $F_{(1,22)} = 0.633$, $p = 0.443$), the number of licks at the dipper cup containing alcohol (Fig. 2C; $F_{(1,22)} = 0.483$, $p = 0.501$), or active or inactive lever responding (Figs. 2D, E; active: $F_{(1,22)} = 0.239$, $p = 0.634$; inactive: $F_{(1,22)} = 0.190$, $p = 0.672$). Thus, operant responding for and intake of alcohol became resistant to the normally aversive quinine taste adulterant after only 3-4 months of IAA.

An additional cohort of rats that had consumed alcohol for 3-4 months under IAA was trained to operantly respond for sucrose (2%, w/v) using very similar operant conditions as utilized for alcohol (Fig. 3A). Breakpoint for 2% sucrose without quinine was qualitatively similar to breakpoint for 20% alcohol without quinine. However, unlike quinine-adulterated alcohol, quinine adulteration of sucrose significantly reduced the breakpoint for adulterated sucrose (Fig. 3B; $n = 10$; $F_{(1,18)} = 15.594$, $p = 0.003$). Quinine adulteration of the sucrose also significantly reduced the number of licks at the dipper cup (Fig. 3C; $F_{(1,18)} = 16.601$, $p = 0.003$) and active lever responding (Fig. 3D; $F_{(1,18)} = 6.563$, $p = 0.031$). No effect on inactive lever responding was observed (Fig. 3E; $F_{(1,18)} = 0.019$, $p = 0.894$), suggesting that quinine did not produce a general inhibition of motoric capacity. Thus, IAA rats can sense quinine and have it influence goal-directed behavior.

To determine whether a shorter period of alcohol consumption under IAA might also lend resistance to quinine adulteration, we examined motivation expressed for quinine-adulterated alcohol during PR sessions after only ~1.5 months of IAA drinking (Fig. 4A). After the shorter IAA, quinine adulteration significantly reduced the breakpoint obtained for alcohol (Fig. 4B; $n = 17$; $F_{(1,32)} = 5.587$, $p = 0.031$), the number of licks at the dipper cup containing alcohol (Fig. 4C; $F_{(1,32)} = 16.220$, $p < 0.001$), and active lever presses (Fig. 4D; $F_{(1,32)} = 6.490$, $p = 0.022$), but did not alter inactive lever presses (Fig. 4E; $F_{(1,32)} = 0.646$, $p = 0.433$). Thus, ~1.5 months IAA drinking was insufficient to render responding for alcohol insensitive to adulteration with quinine.

In order to compare the quinine resistance after 3-4 months of IAA to the more commonly employed method of continuous, home-cage alcohol access, a dose-response relationship of quinine on home-cage alcohol intake after 3-4 months of IAA intake or 3-4 months of continuous access to alcohol was determined. Alcohol intake in continuous-access animals was significantly reduced by 0.01, 0.03, and 0.1 g/L quinine (Fig. 5A; $n = 11$-17; $F_{(4,69)} = 10.51$, $p < 0.001$ one-way ANOVA, Tukey post-hoc comparison: $p < 0.05$ for 0.01, 0.03, and 0.1 g/L quinine vs no quinine). In contrast, IAA alcohol intake was only reduced by 0.1 g/L quinine (Fig. 5B; $n = 15$-25; $F_{(4,92)} = 4.770$, $p = 0.002$ one-way ANOVA, Tukey post-hoc comparison: $p < 0.05$ for 0.1 g/L quinine vs no quinine). Thus, over a broad concentration range, quinine was less efficacious at reducing home cage alcohol intake in IAA versus continuous-access rats.

Since the continued alcohol consumption after 3-4 months of IAA despite addition of the bitter adulterant quinine could represent behavioral inflexibility, or instead could reflect an impaired capacity to detect quinine, we next examined the taste preference for quinine. Taste preference was assessed using a home-cage, two-bottle choice paradigm in which rats were given a choice between one bottle with water and a second bottle with water containing quinine (0.0003-0.1 g/L in half-log concentration increments). In order not to disrupt the alcohol-access schedule of IAA rats, water and water+quinine was presented in an overnight session on Tuesday or Thursday night with one session per week. Taste preference for

quinine was determined by the dose-dependent decrease in preference for water+quinine versus water alone. Importantly, no difference was detected between IAA rats, continuous-access rats, or alcohol-naive rats (Fig. 6A; n = 5-7 for 0.1 and 0.03 g/L quinine, n = 8-11 for other concentrations; group: $F_{(2,121)} = 0.223$, $p = 0.800$; concentration: $F_{(5,121)} = 18.544$, $p < 0.001$; interaction: $F_{(10,121)} = 0.310$, $p = 0.977$; two-way ANOVA with alcohol experience and quinine concentration as factors and data further analyzed via a Tukey post-hoc). There were also no differences in taste preference for saccharin (Fig. 6B; n = 6-10; group: $F_{(2,43)} = 0.273$, p = 0.762; concentration: $F_{(1,43)} = 13.547$, p < 0.001; interaction: $F_{(2,43)} = 0.502$, p = 0.609; two-way ANOVA with Tukey post-hoc comparisons). Thus, the quinine-resistant motivation to seek and consume alcohol expressed by rats after 3-4 months IAA did not result from altered quinine taste preference. These data suggest that behavioral inflexibility for continued alcohol consumption despite aversive consequences can be achieved in much shorter experimental paradigms than previously reported (Wolffgramm and Heyne, 1991;Spanagel et al., 1996;Spanagel and Holter, 1999;Wolffgramm et al., 2000).

Although quinine had a lesser effect on home-cage alcohol consumption in IAA relative to continuous-access rats, 0.1 g/L quinine did reduce alcohol intake in IAA rats under these purely consummatory conditions (Fig. 5B), In contrast, when the motivation for alcohol was more directly assessed, 0.1 g/L quinine had no effect on breakpoint obtained for alcohol by IAA animals after 3-4 months of home-cage intake (Fig. 2A). This did not reflect a difference in the time of access for alcohol, since 0.1 g/L quinine also reduced home-cage intake in IAA animals when measured after 1.5 hr access to alcohol (without quinine: 0.59 ± 0.07 g/kg; with quinine: 0.34 ± 0.05 g/kg; $F_{(1,28)} = 7.118$, $p = 0.018$; n = 15). Thus, quinine resistance in IAA rats could have a greater impact on IAA drinking under purely consummatory conditions. However, previous studies have shown greater quinine resistance after longer-term (> 8 mo) versus shorter-term (<6 mo) alcohol access, but some decrease in drinking with 0.1 g/L quinine was still observed in long-term access animals; this has been suggested to reflect differential populations with lesser or greater quinine resistance (Wolffgramm and Heyne, 1991;Spanagel et al., 1996;Spanagel and Holter, 1999;Wolffgramm et al., 2000;Turyakibahika-Thyen and Wolffgramm 2006). Thus, we re-examined the results shown in Figure 5 to compare home-cage alcohol intake with and without quinine across individual animals. There was variability in the effect of 0.1 g/L quinine on IAA drinking, with some animals showing similar intake with and without quinine while others showing a greater decrease in drinking with quinine (Fig. 7A), although the overall effect of 0.1 g/L quinine was much less pronounced than in continuous-access animals (Fig. 7B). In addition, home-cage alcohol intake in IAA animals was largely unaffected by 0.03 g/L quinine (Figs. 7C, 5B), a concentration which dramatically reduced water intake in IAA animals (Fig. 6A) and alcohol intake in continuous-access animals (Fig. 5A). In addition, baseline drinking in IAA animals did not correlate with the change in drinking with quinine for either 0.1 g/L quinine ($r^2 = 0.093$, $p = 0.270$ Pearson's regression) or 0.03 g/L quinine ($r^2 = 0.136$, $p = 0.120$, Pearson's regression), suggesting that quinine resistance was not related to basal intake levels. Taken together, our results suggest that 3-4 months of IAA alcohol consumption leads to development of resistance to the aversive stimulus quinine, perhaps indicating that motivation for alcohol in these animals has become pathological.

Discussion

Here, we describe a novel and technically simple pre-clinical method in which the motivation to seek and consume alcohol persists despite adulterating the alcohol with the bitter-tasting and normally aversive quinine. In particular, after 3-4 months of voluntary, intermittent 20% alcohol consumption and brief operant training, the motivation expressed for alcohol under an exponential progressive ratio was not altered by quinine adulteration of

the alcohol reinforcer. In strong contrast, motivation and responding for 2% sucrose after 3-4 months of alcohol intake under IAA was greatly reduced by adulteration with quinine. In addition, home-cage alcohol intake by IAA animals was insensitive to quinine at concentrations that significantly reduced alcohol drinking in continuous-access animals. Further, quinine taste preference of IAA animals was not altered relative to alcohol-naive and continuous-access animals. Additionally, water intake in IAA rats was nearly abolished at quinine concentrations that had no effect on alcohol intake. Taken together, these results suggest that IAA rats can sense the aversive tastant quinine and have it influence their motivation for and consumption of water and sucrose, but not alcohol. In contrast to previous studies, which have suggested that aversion-resistant alcohol intake develops after >8 months of alcohol consumption (Wolffgramm and Heyne, 1991; Spanagel et al., 1996; Spanagel and Holter, 1999; Wolffgramm et al., 2000; Sanchis-Segura and Spanagel, 2006; Vengeliene et al., 2009), 3-4 months of IAA intake leads to quinine-resistant and perhaps pathological motivation for alcohol. Thus, the IAA model may facilitate the identification of novel therapeutic interventions for compulsive alcohol-seeking in humans.

Although 0.1 g/L quinine did not alter operant responding for alcohol, it did reduce home-cage alcohol intake in IAA animals, although across a range of concentrations quinine was less efficacious in reducing home-cage intake in IAA versus continuous-access rats. Previous studies of quinine resistance after very long-term drinking have also observed some decrease in home-cage drinking by 0.1 g/L quinine that was suggested to reflect differential populations with lesser or greater quinine resistance (Wolffgramm and Heyne, 1991; Spanagel et al., 1996; Spanagel and Holter, 1999; Wolffgramm et al., 2000; Vanderschuren and Everitt, 2005; Turyakibahika-Thyen and Wolffgramm, 2006). Here, the effect of 0.1 g/L quinine on home-cage IAA drinking varied across animals, but home-cage IAA alcohol intake was essentially unaffected by 0.03 g/L quinine, a concentration that dramatically reduced alcohol and water intake in continuous-access animals as well as water intake in IAA animals and alcohol-naive animals (Tordoff et al., 2008; Turyakibahika-Thyen and Wolffgramm, 2006; Grobe and Spector, 2008). Thus, these data are in concordance with others that have examined quinine effects on alcohol intake after much longer drinking periods (~ 8 months). In addition, alcohol intake during operant PR sessions in 3-4 month IAA animals was 0.13 ± 0.01 g/kg, much lower than the ~0.6 g/kg intake in 1.5 hr of home-cage intake. These low intake levels under operant conditions could suggest that quinine-resistant responding for alcohol might be driven by alcohol-associated cues rather than pharmacological effect of alcohol, although this could also reflect the exponential response requirement for delivery of reinforcer. Regardless, our operant and two-bottle quinine data taken together both support the hypothesis that the motivation of rats to consume alcohol after 3-4 months IAA drinking was relatively insensitive to the aversive bitter tastant quinine.

Our results suggest that quinine adulteration reduced motivation for sucrose but not alcohol after 3-4 months IAA. However, sucrose and alcohol can exhibit distinct tastes on their own, with sucrose generally being perceived as sweet, and ethanol having sweet aspects and also bitter aspects that may subside somewhat after experience with alcohol (Fahlke et al., 1994; Kiefer et al., 2005). Thus, addition of the bitter quinine could lead to different effects on the taste of sucrose versus alcohol. Although the present results suggest that quinine adulteration reduces motivation for sucrose but not alcohol after 3-4 months IAA, a dose-response curve for the effects of quinine on PR responding would more clearly delineate the impact that quinine adulteration may have on responding for alcohol versus sucrose. In addition, we should note that, although PR responding for alcohol after 3-4 months IAA was stable across 4 weeks, we did not determine whether PR responding for sucrose under PR, or PR responding for alcohol after ~1.5 months IAA, was stable across 4 weeks of testing. Thus, less experience with the reinforcer in sucrose animals or after ~1.5 months IAA could result

in more variable PR responding across successive weeks. However, no order effects ($p > 0.1$) or significant differences between responding without quinine in the first versus second week of PR testing ($p > 0.1$) in either group were observed, suggesting that responding in the absence of quinine was not appreciably different in the two weeks of PR testing. Nonetheless, an important comparison that should be examined in future experiments is the impact of quinine adulteration on responding for sucrose in animals that intermittently consume sucrose for 3-4 months. While IAA rats showed behavioral flexibility when presented with quinine-adulterated sucrose, this additional experiment would provide insight into whether prolonged intermittent access specifically leads to behavioral inflexibility towards alcohol, or whether quinine resistance is a more general phenomenon that develops with prolonged intermittent access to any reinforcing substance. In addition, motivation for sucrose in IAA animals was examined on days where animals did not have access to alcohol, and thus the effect of quinine on sucrose intake could be influenced by being in a state of withdrawal from alcohol. This design was employed to minimize interference with alcohol availability under IAA. Despite these caveats, quinine adulteration of alcohol reduced home-cage intake in continuous-access rats at concentrations that had little effect on alcohol intake in IAA animals, with no differences in quinine taste preference between groups, suggesting that quinine-resistance in IAA animals was apparent when compared with control, continuous-access animals that could experience a similar taste effect of adding quinine to alcohol. Thus, the dramatic effect of quinine on responding for sucrose in IAA animals is more likely to reflect the aversive consequences of the tastant quinine rather than resulting from methodological or taste sensitivity differences. In addition, although self-administration conditions were very different during operant responding and home-cage two-bottle intake, our results taken together concur that IAA animals after 3-4 months of alcohol intake developed resistance to quinine adulteration relative to control conditions.

Previous studies using continuous-access models have shown that resistance to quinine adulteration develops after >8 months but not 6 months of alcohol intake (Wolffgramm and Heyne, 1991; Spanagel et al., 1996; Spanagel and Holter, 1999; Wolffgramm et al., 2000; Turyakibahika-Thyen and Wolffgramm, 2006; Vengeliene et al., 2009), and studies of other commonly abused substances also observe that very long-term intake can be required to develop resistance to aversive stimuli (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2005). Our results suggest that quinine-resistant motivation for alcohol develops after 3-4 months but not ~1.5 months of IAA intake, suggesting that ~1.5 months IAA is insufficient to produce quinine resistance. However, although breakpoint and active lever pressing were qualitatively similar between ~1.5 month IAA animals and 3-4 month IAA animals, ~1.5 month IAA animals showed greater licking under PR and a trend towards greater lever pressing during the second overnight training session (Table 1). Nevertheless, 3-4 months IAA intake was sufficient to develop resistance to quinine adulteration of alcohol, which is a relatively short, experimentally tractable period of time compared to that observed in previous studies. However, we also should note that previous studies of aversion-resistant alcohol intake which used a 4-bottle choice paradigm observed other aberrant, addiction-like behaviors, including a switch in preference for higher alcohol concentrations and changes in circadian patterns of alcohol intake (Wolffgramm and Heyne, 1991; Spanagel et al., 1996; Spanagel and Holter, 1999; Wolffgramm et al., 2000; Turyakibahika-Thyen and Wolffgramm, 2006; Vengeliene et al., 2009). Future studies will be required to determine whether these intriguing phenomena can also be observed using the present model.

PR studies usually include several weeks of operant self-administration to establish a baseline. Here, we undertook a different strategy, with the goal of quantitatively assaying the effects of taste adulteration on motivation with minimal operant training. PR responding for alcohol was stable across 4 weeks, and inactive lever pressing represented less than 20%

of total lever presses, suggesting discrimination of the active and inactive lever. Also, control experiments using the same brief operant training paradigm (motivation for sucrose after 3-4 months IAA, motivation for alcohol after ~1.5 months IAA) showed that this brief training method could detect quinine sensitivity. Our operant results are also supported by quinine-resistance in IAA animals compared to continuous-access animals during home-cage intake, with no differences in quinine taste preference. Thus, our data suggest that the brief operant training method was able to assess whether motivation for a reinforcer had become resistant to the aversive quinine.

In conclusion, we have demonstrated that rats consuming alcohol under the technically simple IAA paradigm developed resistance to the normally aversive quinine after 3-4 months of intermittent alcohol intake. In contrast, IAA animals exhibited a significant reduction in motivation for and intake of quinine-adulterated sucrose and a reduction in intake of quinine-adulterated water, suggesting that quinine can influence motivation in IAA rats. Although alcohol intake levels in IAA animals are less than those observed in human alcoholics (Sanchis-Segura and Spanagel, 2006; Simms et al., 2008), it is interesting that several compounds that can reduce alcohol intake by human alcoholics also significantly reduce alcohol intake in IAA rats but have relatively little effect in continuous-access rats (Steensland et al., 2007; Simms et al., 2008; McKee et al., 2009). In this regard, we observed that quinine reduced home-cage alcohol intake in continuous-access animals at concentrations that had little effect on IAA intake. Our studies thus validate and extend the IAA paradigm as a simple and useful pre-clinical method with which to explore the development and expression of aversion-resistant alcohol consumption, as well as the efficacy of novel therapeutic interventions for compulsive alcohol-seeking in humans.

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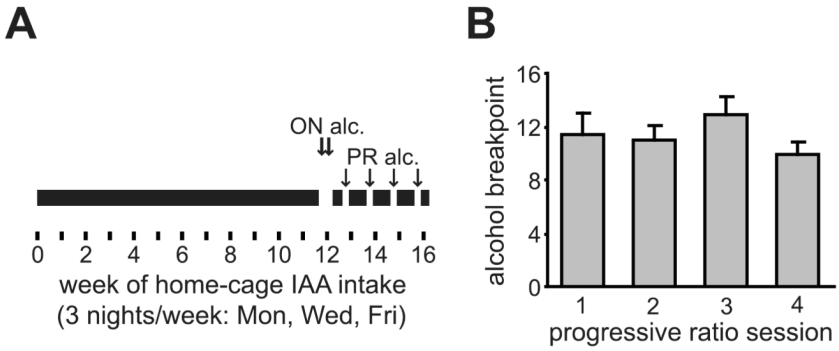

Fig. 1.

Motivation for alcohol was stable over time. (**A**) Schematic illustrating that, after 3-4 months voluntary access to alcohol (20%, v/v) under IAA, animals were allowed 2 overnight sessions to acquire operant responding for alcohol. The motivation to obtain alcohol was quantified as the breakpoint on an exponential progressive ratio schedule of reinforcement (1.5 hr/day, once/wk, performed on a day where rats would normally have access to home-cage alcohol). Rats were returned to the home-cage IAA schedule on days where operant testing was not performed. (**B**) Breakpoint was stable across the 4 weeks of PR testing. Mon, Wed, Fri = Monday, Wednesday, Friday; ON = overnight; PR = progressive ratio; alc. = alcohol.

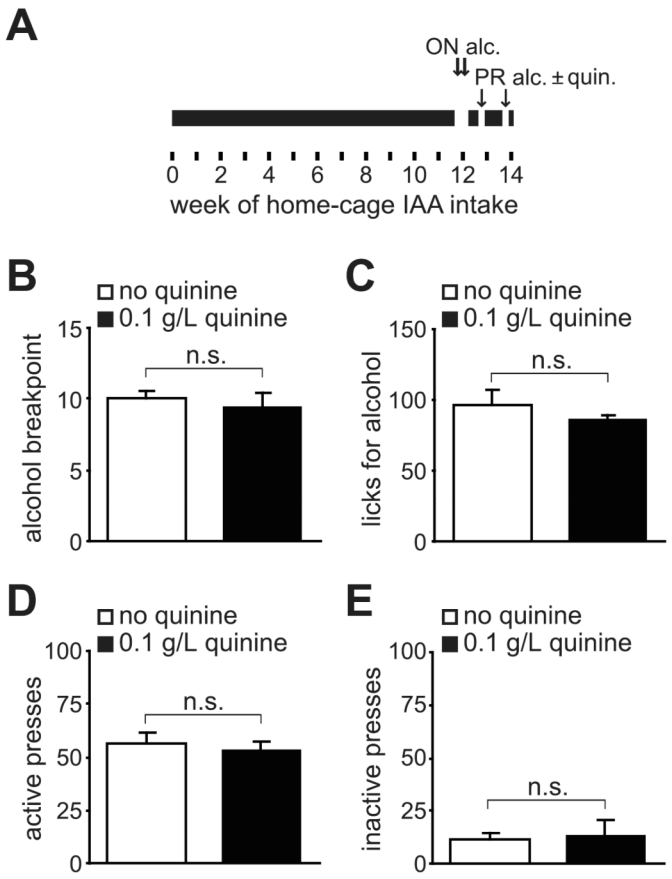

Fig. 2.

IAA rats exhibit behavioral inflexibility during operant responding for alcohol. (**A**) Schematic illustrating that, after 3-4 months of voluntary IAA drinking, the effect of taste adulteration of alcohol with 0.1 g/L quinine on motivation and responding for alcohol was examined under a progressive ratio schedule. (**B-E**) No effect of quinine on (**B**) breakpoint for alcohol, (**C**) licks at the dipper cup containing alcohol, (**D**) active lever responding, or (**E**) inactive lever responding. ON = overnight; PR = progressive ratio; alc. = alcohol; quin. = quinine; n.s. = not significant.

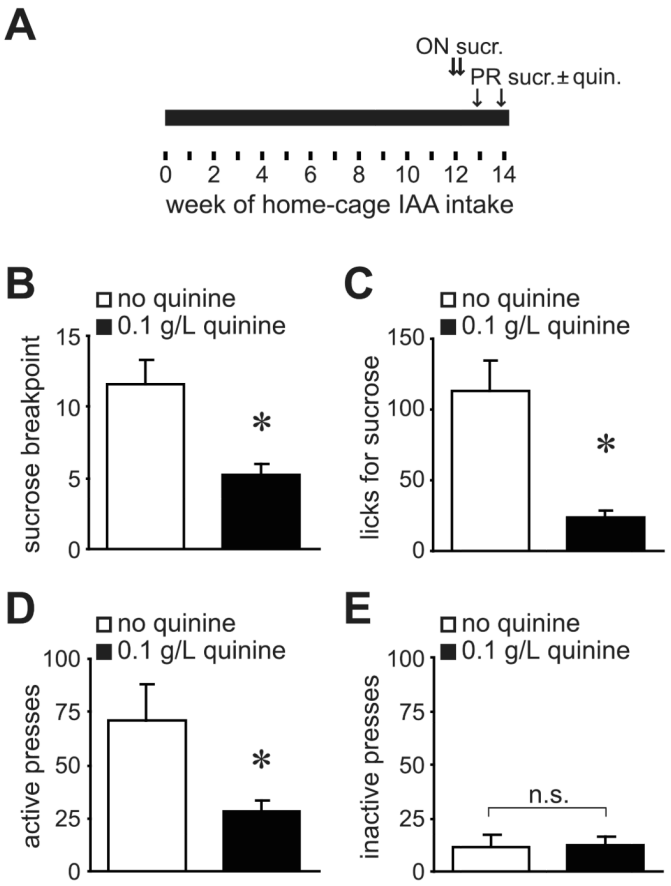

Fig. 3.

Quinine (0.1 g/L) reduced the motivation of IAA rats to consume 2% (w/v) sucrose. (**A**) Schematic illustrating that, following 3-4 months alcohol intake under IAA, rats were briefly trained to operantly respond for sucrose, and motivation for sucrose was then assayed under a progressive ratio schedule. Sucrose training occurred on days that rats did not have access to alcohol. (**A-C**) Quinine significantly reduced (**A**) the breakpoint obtained for sucrose, (**B**) licks at the dipper cup containing sucrose, and (**C**) active lever responding. (**D**) Quinine did not reduce inactive lever responding. ON = overnight; PR = progressive ratio; sucr. = sucrose; quin. = quinine; n.s. = not significant. * $p < 0.05$.

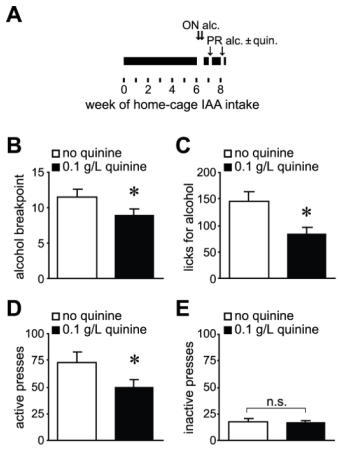

Fig. 4.

Motivation for alcohol was sensitive to quinine after shorter (~1.5 months) IAA experience. (**A**) Schematic illustrating that motivation for alcohol in the presence and absence of quinine was examined under PR after ~1.5 months of home-cage IAA. (**B**-**D**) Quinine significantly reduced (**B**) the breakpoint obtained for alcohol, (**C**) licks at the dipper cup containing alcohol, and (**D**) active lever responding. (**E**) Quinine did not reduce inactive lever responding. n.s. = not significant. * $p < 0.05$.

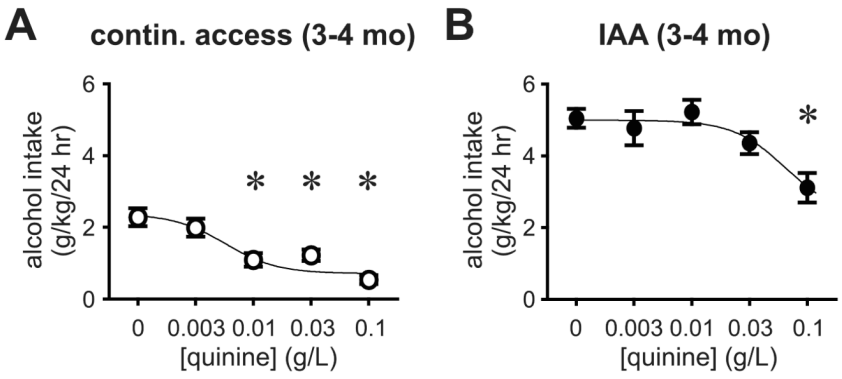

Fig. 5.

Quinine was less efficacious in reducing home-cage alcohol intake after IAA. A dose-response curve of the effect of quinine on alcohol intake was generated in IAA rats and in rats with continuous access to alcohol. (**A**) As little as 0.01 g/L quinine significantly reduced alcohol intake in animals with continuous access to alcohol. (**B**) In contrast, only 0.1 g/L quinine significantly reduced alcohol intake in IAA animals. Lines indicate a best-fit curve. * $p < 0.05$.

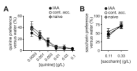

Fig. 6.

Quinine taste preference, determined by home-cage two-bottle choice between water and water+quinine, was not altered in IAA drinkers. (**A**) Quinine adulteration dose-dependently reduced water intake, with a similar dose-response relationship in IAA drinkers, animals with continuous access to alcohol, and age-matched, alcohol-naive controls. (**B**) No differences in preference for saccharin among groups. Cont. acc. = continuous access.

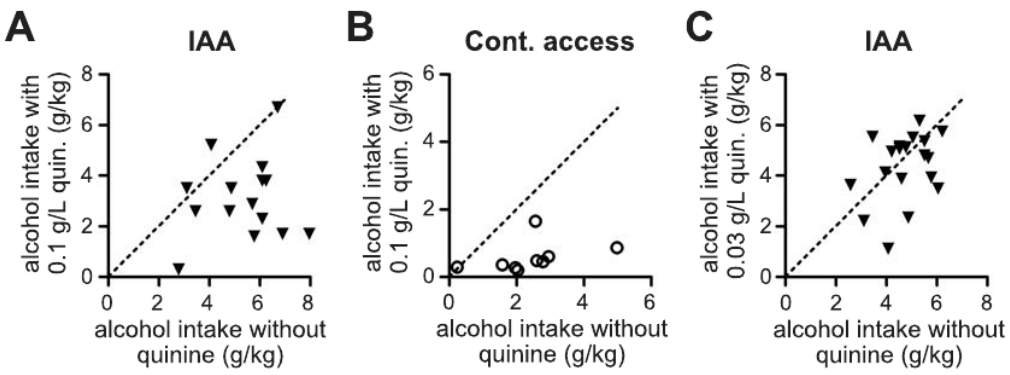

Fig. 7.

Scatter plots of results in Fig. 5 showing home-cage alcohol intake across individual animals with and without quinine. (**A**) IAA animals show variability in the effect of 0.1 g/L quinine, with some animals showing similar intake with and without quinine and others showing a greater decrease in drinking with quinine. (**B**) 0.1 g/L quinine dramatically reduced alcohol intake in all continuous-access animals. (**C**) Home-cage alcohol intake in IAA animals was largely unaffected by 0.03 g/L quinine. Dotted lines indicate where alcohol intake levels would be identical with and without quinine. Cont. = continuous, quin. = quinine.

Table 1
Levels of responding during the second overnight operant session

There were no significant differences across groups for any measure. However, when only ~1.5 month alcohol animals and 3-4 month alcohol animals were compared, there was a trend for greater active lever presses (p = 0.059) and licks (p = 0.092) in the ~1.5 month access group.

	Active lever presses	Inactive lever presses	Licks at dipper cup
3-4 mo IAA rat responding for 20% alcohol	336 ± 53	36 ± 7	1189 ± 179
3-4 mo IAA rat responding for 2% sucrose	413 ± 127	42 ± 8	1383 ± 455
~1.5 month IAA rat responding for 20% alcohol	520 ± 61	44 ± 5	1732 ± 202
$F_{(2,43)}$ =	1.587	0.487	1.221
p =	0.216	0.618	0.305