Brief Exposures to the Taste of Ethanol (EtOH) and Quinine Promote Subsequent Acceptance of EtOH in a Paradigm that Minimizes Postingestive Consequences

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Background: Aversion to the orosensory properties of concentrated ethanol (EtOH) solutions is often cited as a primary barrier to initiation of drinking and may contribute to abstention. These aversive properties include gustatory processes which encompass both bitter-like taste qualities and trigeminal-mediated irritation. Chronic intermittent EtOH access (CIA) results in substantial and persistent increases in EtOH consumption, but the degree to which this facilitation involves sensory responding to EtOH and other bitter stimuli is currently undetermined.

Methods: Long-Evans rats were given brief-access licking tests designed to examine the immediate, taste-guided assessment of the palatability of EtOH and quinine solutions. Rats were assessed once in a naïve state and again following previous brief-access exposure, or following 4 weeks of CIA. The relationship between the sensitivity to the aversive orosensory properties of EtOH and quinine following EtOH access and the impact of antecedent quinine exposure on the acceptance of EtOH were determined in 2 parallel studies.

Results: Both brief access to EtOH and 4-week CIA resulted in substantial rightward shifts in the concentration–response function of brief-access EtOH licking, indicating that EtOH exposure increased acceptance of the taste of EtOH. The initial sensitivity to the aversive orosensory properties of EtOH and quinine was positively correlated in naïve rats, such that rats that were initially more accepting of quinine were also more accepting of EtOH. Rats that sampled quinine immediately prior to tasting EtOH exhibited successive positive contrast in that they were more accepting of highly concentrated EtOH, relative to a water-control group.

Conclusions: Increased EtOH acceptance following exposure is, at least in part, facilitated by a decrease in its aversive sensory properties. Both long- and short-term access increase the palatability of the taste of EtOH in brief-access licking tests. Moreover, the sensitivity to the bitterness of quinine was predictive of acceptance of EtOH indicating some commonality in the sensory mechanisms that mediate the initial acceptance of the 2 stimuli. Accordingly, immediate prior exposure to quinine results in increased acceptance of EtOH, suggesting that successive positive contrast between oral stimuli may contribute to increased alcohol consumption.

Key Words: Alcohol, Taste, Bitter, Palatability, Contrast.

WHILE BOTH HUMANS and rodents are often hesitant to voluntarily consume ethanol (EtOH)-containing solutions, repeated experience with EtOH can facilitate voluntary EtOH consumption (e.g., Simms et al., 2008). This facilitation involves, at least in part, changes in the orosensory properties of EtOH (including taste and trigeminal irritation) as well as the development of conditioned responses to the taste of EtOH due to its reinforcing pharmacological and caloric effects (Ackroff and Sclafani, 2003). The taste of EtOH plays an integral role in its initial acceptance and likely serves to limit consumption (Bachmanov et al., 2003; Bice et al., 1992; Fischer et al., 2013; Lanier et al., 2005; Sherman et al., 1984; Thibodeau et al., 2017). Aversion to the taste of alcohol is often cited as one of the primary reasons for abstention from drinking behavior (Moore and Weiss, 1995). As such, the responsiveness to the taste of EtOH may play a large part in its consumption, particularly in the initiation of drinking behavior (Intranuovo and Powers, 1998; Lanier et al., 2005), and decreases in the negative sensory properties of EtOH that occur following repeated exposure may serve to facilitate intake (Kiefer and Dopp, 1989; Kiefer et al., 1994).

Human and rodent studies suggest that the taste profile of EtOH resembles that of bittersweet taste mixtures accompanied by trigeminal-mediated oral irritation (Bartoshuk et al., 1994; Dilorenzo et al., 1986; Green, 1988; Kiefer and Mahadevan, 1993; Kiefer et al., 1990; Lanier et al., 2005; Mattes and DiMeglio, 2001; Nolden and Hayes, 2015; Nolden et al., 2016; Scinska et al., 2000). For example, EtOH activates sucrose-responsive oral receptors and central

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gustatory pathways that, in turn, are integral for voluntary EtOH consumption (Blednov et al., 2008; Brasser et al., 2010; Coleman et al., 2011; Lemon et al., 2004), and adaptations in these pathways may be responsible for EtOH-induced increases in acceptance of sucrose–quinine hydrochloride (QHCl) mixtures (Lopez and Molina, 1999). While the physiological overlap between EtOH and other sweet-like stimuli has been demonstrated repeatedly, some studies have failed to find a relationship between the neural response to EtOH and either peripheral or central bitter-responsive neurons (Hellekant et al., 1997; Lemon and Smith, 2005; Lemon et al., 2011). Genetic and behavioral studies show that the taste of EtOH generalizes to bitter stimuli and that oral adaptation to the taste of EtOH decreases the perceived bitterness of subsequently presented QHCl concentrations (Mattes and DiMeglio, 2001). This suggests that EtOH activates physiological mechanisms involved in the perception of, and oral adaptation to, bitter-tasting stimuli. Furthermore, repeated intermittent EtOH access decreases the resistance to consume EtOH solutions adulterated with the bitter-tasting stimulus, quinine (QHCl; Hopf et al., 2010). Generally, this phenomenon is attributed to the development of compulsive-like drinking behavior rather than decreased sensitivity to bitter-tasting compounds. However, the behavioral assays used in these studies to assess whether intermittent EtOH access alters the response to QHCl solutions (Hopf et al., 2010) are limited in their ability to parse subtle differences in perceived intensity and are incapable of dissociating sensory phenomenon from postoral consequences (Loney et al., 2012; Ruiz et al., 2006; Spector and Glendinning, 2009). Therefore, the degree to which intermittent EtOH access induces changes in the response to the aversive components of EtOH taste, and whether these changes cause increased EtOH acceptance, has not been adequately assessed.

The present experiments were conducted to test the involvement of bitter-related taste phenomena in the acceptance of EtOH. Based on the equivocal nature of the available data on the similarity between the orosensory properties of EtOH and other bitter stimuli, we sought to systematically characterize the responsiveness to the aversive taste properties of EtOH and the prototypical bitter stimulus QHCl. First, in a large cohort of rats, we compared the EC_{50}, or the concentration at which half-maximal licking was observed, for both EtOH and QHCl in naïve rats in an attempt to determine whether the initial sensitivity to one stimulus predicted sensitivity to the other. Next, we determined whether chronic intermittent EtOH access (CIA) in adulthood impacted the taste of EtOH and QHCl in a manner similar to that seen following extensive prenatal exposure (Glendinning et al., 2017; Youngentob and Glendinning, 2009). We also tested whether previous exposure to the taste of EtOH facilitated free-access EtOH consumption. Finally, we examined whether an oral rinse with QHCl impacted the taste of EtOH in rodents, similar to a reciprocal relationship that has been previously demonstrated in humans (Mattes and DiMeglio, 2001) in an attempt to further implicate the involvement of orosensory responding to bitter stimuli in the acceptance of EtOH.

MATERIALS AND METHODS

Animals and Housing

Fifty-nine adult male Long-Evans rats (Envigo, St. Louis, MO) were individually housed in polycarbonate cages in a temperature- and humidity-controlled environment on a reverse 12:12 light cycle. Upon arrival to the animal colony, rats were handled daily for 3 days. All rats were maintained on ad libitum water and standard rodent chow (Harlan, East Millstone, NJ) unless otherwise stated. At the start of the experiment, rats were assigned to their respective groups in a weight-balanced fashion. All testing procedures occurred exclusively during the dark phase when rats tend to consume the most ad libitum EtOH (Gill et al., 1986). All experimental procedures were approved by the University at Buffalo Institutional Animal Care and Use Committee.

Taste Stimuli

Taste stimuli were prepared fresh daily in tap water and consisted of 6 concentrations of EtOH (Decon Labs, King of Prussia, PA; 1.25, 2.5, 5.0, 10, 20, and 40% v/v) diluted from 200 proof stock solutions and 6 concentrations of quinine dihydrochloride (Sigma-Aldrich, St. Louis, MO; 0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 mM).

Apparatus

Brief-access licking tests were conducted in a commercially purchased lickometer called a Davis rig (MS80; DiLog Instruments, Tallahassee, FL). The Davis rig consists of a polycarbonate cage (15 cm wide, 29 cm long, and 20 cm high) with a wire mesh floor and a centrally located opening that is occluded by a computer-controlled shutter (Smith, 2001). A fully automated sliding table, with 16 fluid reservoirs connected to sipper tubes coupled to a contact lickometer, is positioned on the other side of the shutter. This device allows each animal brief access to a single drinking spout through the centrally positioned slot in the front wall of the cage when the shutter is open. Lick contact is measured by a specially designed high-frequency AC electrical circuit that minimizes electrical stimulation of the tongue. Each individual lick contact is detected and recorded in the software control program for later analysis. In essence, these tests involve the immediate, unconditioned licking responses to randomized concentrations of a taste stimulus presented for a very brief period of time to minimize any potential contribution of postoral consequences (Brasser et al., 2012; Glendinning et al., 2017; Youngentob and Glendinning, 2009). Furthermore, the palatability-driven licking responses generated in the Davis rig closely mimic psychophysically derived taste quality assessments implicating their efficacy in measuring primarily taste-guided licking behavior (Loney et al., 2012). In an attempt to further minimize any potential cue effects due to the smell of the stimuli, particularly EtOH, fans were positioned such that a stream of air was continuously flowing through the gap between the shutter and the fluid reservoirs.

EXPERIMENT 1A: ARE THE IMMEDIATE, TASTE-GUIDED RESPONSES TO ETOH AND QUININE CORRELATED?

Here, a cohort of rats ($n = 40$) were exposed to a series of concentrations of both EtOH and QHCl in independent

brief-access licking tests. The EC_{50}s from each stimulus were plotted against each other to determine whether the initial taste-guided acceptance of EtOH and QHCl was predictive of each other.

Brief-Access Licking Tests

Training. All rats were water-deprived ~24 hours prior to the start of training and testing to promote stimulus sampling. On the first day of training, all rats were placed into the Davis rig and given access to 1 stationary reservoir of water and allowed to lick freely for 30 minutes. On the second day, rats were habituated to the opening and closing of the shutter and movement of the table holding the fluid reservoirs. During this 30-minute session, rats were given 60 seconds to initiate a lick; once a lick was counted, the rat was allowed 30 subsequent seconds to freely lick. This 30-second access period was followed by a 10-second intertrial interval before the presentation of a new trial. During this stage of training, a new trial was presented even if the rat failed to initiate a lick to the current trial. For the third day, rats were again given serial 30-second presentations, although now each trial was followed by a 1-second presentation that would serve as the water rinse during testing. On the final 2 days of training, the access period was shortened to 10 seconds. Following each trial, there was a 10-second intertrial interval followed by the 1-second presentation of a new water reservoir which, again, would serve as the water rinse trials during testing. This rinse was followed by another 10-second interval before the start of a new trial. A subsequent trial was not presented in the instance where the rat failed to initiate a lick during the current trial. This design ensured that the animals had to actively taste each concentration of each stimulus in order to advance the testing session. Doing so allowed us to analyze only the consummatory responding to each stimulus as a function of its concentration.

Testing. The testing sessions were identical to the final training conditions with the exception that all rats received their assigned taste stimulus presented in a randomized block consisting of each of the 6 concentrations of either EtOH (1.25, 2.5, 5.0, 10, 20, and 40%) or QHCl (0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 mM) and water. The licks elicited to each concentration of each stimulus on each trial were recorded, and the rats were free to initiate as many trials as possible during the 30-minute session.

Testing consisted of 3 testing days conducted on Monday, Wednesday, and Friday of a given week with each taste stimulus tested individually during said week allowing for at least 24 hours in between each test day and at least 72 hours before beginning testing with the other taste stimulus. Animals were allowed to rehydrate overnight following each testing day, and water was removed the following morning in preparation for the following test day. EtOH and QHCl testing order was counterbalanced across rats.

EXPERIMENT 1B: DOES CIA SHIFT THE IMMEDIATE, TASTE-GUIDED LICKING RESPONSE TO ETOH AND QUININE IN ADULT RATS?

This experiment was designed to determine whether, and to what degree, CIA in adulthood resulted in a palatability shift in the orosensory properties of either EtOH or QHCl. A subset of rats from Experiment 1a ($n = 20$) were subjected to continued testing within this experiment, which was comprised of 3 phases: an initial assessment of the brief-access licking response of naïve rats to both EtOH and QHCl (Pre tests; i.e., Experiment 1a); CIA in the animals' home cages (or an equivalent amount of time in the nonexposed, brief-access group); and finally, a reassessment of the brief-access licking response to both EtOH and QHCl (Post tests). The Brief-access group ($n = 10$) was only given brief access to EtOH in the Davis rig as described in Experiment 1a, while the CIA ($n = 10$) group was given 24-hour access 3 days a week for 4 weeks as described below. The testing procedures for the Pre and the Post brief-access licking tests were identical as described above.

Chronic Intermittent EtOH Access

Three groups of rats were tested in the CIA paradigm: 2 groups of rats that were previously given access to EtOH in the Davis rig (Experiment 1a); one of these groups was given 24-hour access to EtOH for 4 weeks (CIA), as described below, while the other was only given water (Brief access) for an identical amount of time. The third group (Naïve; $n = 8$) was trained in the Davis rig as described above, but only received access to water, and thus were naïve to EtOH prior to consuming EtOH in their home cage for 4 weeks. This group allowed us to determine whether brief access to the taste of EtOH facilitated free-access EtOH consumption.

The Monday following the final testing day of the Pre tests, rats were given access to 2 bottles placed on the top of their home cage and allowed to consume from either bottle ad libitum. These glass bottles were equipped with straight, open, stainless steel sipper tubes and were placed on either side of a wire cage lid. In the CIA and Naïve groups, the rats were given access to 20% EtOH at dark onset on Monday, Wednesday, and Friday of a given week in one bottle, while the other bottle contained tap water. On the intervening days, both bottles contained tap water. In the Brief-access group, both bottles contained water at all times. Bottle positions were alternated on EtOH access days to control for any potential side preferences (Simms et al., 2008). This CIA testing lasted for 4 weeks for a total of 12 individual EtOH exposures in the rats with EtOH access, and they were followed with the brief-access licking Post tests.

EXPERIMENT 2: DOES ANTECEDENT QUININE EXPOSURE RESULT IN SUCCESSIVE POSITIVE CONTRAST IN A MANNER SUFFICIENT TO SHIFT THE PALATABILITY-DRIVEN LICKING RESPONSES TO ETOH IN ADULT RATS?

This experiment was designed to determine whether immediate preexposure to QHCl with an oral rinse impacted the palatability of the taste of EtOH. Two experimental groups of naïve rats (QHCl- and H_2O-adapted) were trained in the Davis rig exactly as described above with the exception that during the last 2 days of training, and throughout testing, the rinse trials were extended to 2 seconds, as opposed to 1 second as in Experiment 1. This was done to increase the number of licks taken during the rinse. Following training, rats were tested with brief access (10 seconds) to 6 concentrations of EtOH (1.25, 2.5, 5, 10, 20, and 40%, v/v) and water on Monday, Wednesday, and Friday of a given week. Prior to each test trial, the animals were tasked with licking to either QHCl or H_2O for 2 seconds before being presented with a random concentration of EtOH for 10 seconds. If at any point the animals failed to lick to the presented stimulus, including during the rinse, the trial stalled until licking was initiated. This ensured that all rats must have licked during the rinse trial before an EtOH test concentration was presented and, furthermore, that all rats sampled each concentration of EtOH. We used the data from Experiment 1 to guide our choice for QHCl rinse concentrations. Specifically, we chose 2 different concentrations of QHCl to be presented during the rinse: a relatively low concentration that impacted licking behavior (0.03 mM) and a log-step higher concentration (0.3 mM). Each QHCl rinse concentration was tested individually during a given week, and all were tested in ascending order with at least 72 hours in between testing with the higher concentration for a total of 2 weeks of testing.

Data Analyses

Licking data from the Davis rig were converted to lick ratios by dividing each animal's average licks to a given concentration of either EtOH or QHCl by that same animal's average licks to water. These data transformations control for individual differences in local lick rate and motivation to rehydrate. A lick ratio of 1.0 would indicate that the animal licked to the taste stimulus equal to H_2O; anything below 1.0 indicates that the animal licked more to H_2O than the taste stimulus. Curves were fit to the lick ratios, and the EC_{50} was calculated for both stimuli using a 3-parameter logistical function:

$$\frac{a}{(1+(10^{((\log \mathrm{conc}-c)\times b)}))}.$$

Here, a is equal to asymptotic licking, b is equal to the slope of the curve, and c is the EC_{50}, or the concentration at which one-half asymptotic licking was seen.

Mean lick ratios for EtOH and QHCl in Experiment 1a were analyzed with independent 1-way analyses of variance (ANOVAs). Further, the relationship between each animal's EC_{50} for EtOH and that same animal's EC_{50} for QHCl was compared with linear regression. Following completion of this testing, it was revealed that 2 rats were clear outliers (i.e., greater than 2 standard deviations beyond the mean) with regard to their EC_{50} for both EtOH and QHCl. As these rats were outliers for both stimuli, the data from these animals were excluded from the analysis.

Mean lick ratios to EtOH and QHCl from Experiment 1b were analyzed with independent 3-factor mixed ANOVAs with stimulus Concentration and Test (Pre vs. Post) as the within-group factors and EtOH Access as the between-group factor. The EC_{50}s for EtOH and QHCl from each Test were compared with independent 2-factor mixed ANOVAs with Test as the within-group factor and Access as the between-group factor. As in Experiment 1a, the EC_{50} for EtOH and the EC_{50} for QHCl generated during the Post tests were compared with linear regression.

Mean lick ratios to EtOH from Experiment 2 were analyzed with a 3-factor mixed ANOVA with EtOH Concentration and rinse Stimulus (QHCl or H_2O) as the between-subjects factors and Test as the within-subjects factor. The EC_{50} for EtOH from each test were compared with a 2-factor mixed ANOVA with Test as the within-group factor and rinse Stimulus as the between-group factor.

Tukey's post hoc analyses were conducted where appropriate.

RESULTS

Experiment 1a

Here, we tested the hypothesis that the sensitivity to the aversive orosensory qualities of EtOH was correlated with that of quinine. For both EtOH (Fig. 1*A*) and QHCl (Fig. 1*B*), water-deprived rats displayed a tendency to decrease licking as a function of increasing the stimulus concentration, indicating that responding was strongly influenced by the aversive properties of the stimuli. We found significant main effects of Concentration for both EtOH and QHCl, $F(5,185) = 638.42$, $p < 0.0001$ and $F(5,185) = 1{,}010.49$, $p < 0.0001$, respectively. Post hoc analyses revealed that rats began to significantly decrease their licking for EtOH and QHCl at the 5% and 0.03 mM concentrations, respectively, and licking remained significantly suppressed at each subsequently higher concentration ($ps < 0.05$).

When comparing the relationship between the EC_{50} of both EtOH and QHCl, we found that animals that found QHCl to be highly aversive were also likely to find EtOH to be more aversive and vice versa. Linear regression conducted on the EC_{50} of each animal for both stimuli revealed a modest, yet significant correlation coefficient, $r = 0.354$, $F(1, 36) = 5.17$, $p < 0.05$; Fig. 2, indicating that the evaluation of

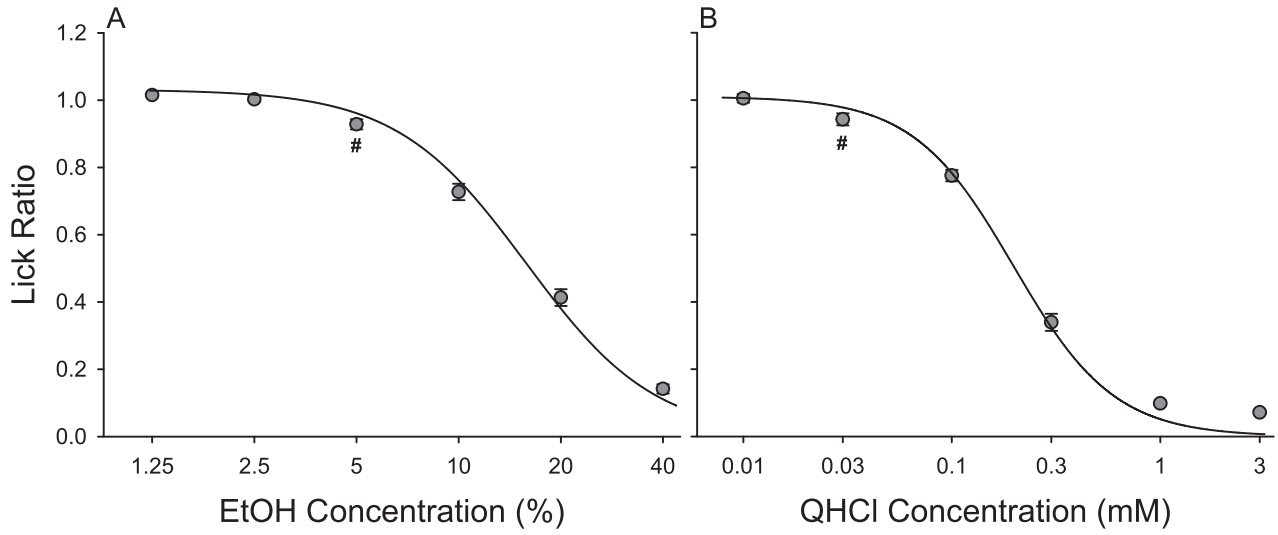

Fig. 1. Ethanol (EtOH) and quinine decrease licking rates in naïve rats as a function of increasing concentration. Mean (±SEM) brief-access lick ratios for randomly presented concentrations of EtOH (**A**) and quinine (QHCl; **B**). A ratio of 1.0 indicates that the rats licked an equal number of times to the stimulus as to H_2O; anything below 1.0 indicates that the rats licked more to H_2O relative to the stimulus. As a whole, rats displayed a tendency to decrease licking as a function of increasing concentration for both stimuli. Lick ratios were significantly suppressed beginning at the 5% concentration for EtOH and the 0.03 mM concentration for QHCl and remained suppressed at each subsequently higher concentration (#*p*s < 0.05).

the palatability of the concentration–response function for both EtOH and QHCl was positively correlated.

Experiment 1b

Brief-Access Licking Tests. Here, we examined the extent to which experience with EtOH resulted in a shift in the sensitivity to the aversive orosensory qualities of both EtOH and QHCl. Both brief access and extended access to EtOH through the CIA paradigm resulted in substantial lateral shifts in the concentration–response function to the taste of EtOH with no effect for QHCl. For EtOH (Fig. 3), regardless of the Test (Pre vs. Post) rats tended to decrease licking to EtOH as a function of increasing concentration resulting in a significant main effect of Concentration, $F(5, 90) = 330.62$, $p < 0.0001$. During the Post tests, rats were more accepting of the taste of EtOH as evidenced by an increase in licking to higher concentrations of EtOH resulting in a main effect of Test, $F(1, 18) = 47.67$, $p < 0.0001$. Rats that received substantially more exposure to EtOH in the CIA paradigm (Fig. 3*A*) showed an even larger lateral shift relative to rats that were only exposed to EtOH in the brief-access tests (Fig. 3*B*), thus resulting in a significant Test × Concentration × Access interaction, $F(5, 90) = 3.91$, $p < 0.01$. Within-group post hoc analyses revealed that upon their initial exposure to EtOH in the Davis rig during the Pre test, the first concentration at which all rats, regardless of group assignment, significantly reduced their licking was at the 10% concentration. During the Post tests, the rats, regardless of access condition, did not display a significant reduction in licking until the second highest (20%) concentration of EtOH that was tested. Within-group comparisons across tests revealed that both brief-access and CIA rats licked significantly more to the 10 and 20% concentrations (*p*s < 0.001) during the Post tests relative to their own initial baseline response during the Pre tests. Between-group post hoc comparisons revealed that the group differences in licking at each concentration failed to survive correction for multiple comparisons; thus, neither group significantly differed from the other at any concentration during both the Pre tests and Post tests.

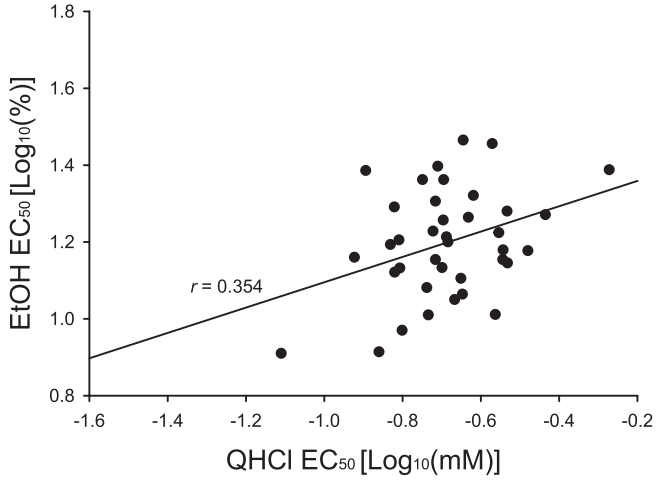

Fig. 2. The EC_{50}s of the concentration–response function for both ethanol (EtOH) and quinine (QHCl) are positively correlated. The EC_{50} for both EtOH and QHCl derived from the brief-access licking response of individual rats from a large cohort. There was a significant positive correlation between the EC_{50} for EtOH and QHCl, indicating that individual rats that were more sensitive to the aversive orosensory properties of QHCl were also likely to display a similar increased sensitivity to the aversive orosensory properties of EtOH.

Analyses conducted comparing the EC_{50} for EtOH for each test (Fig. 3*A*, *B*, insets) revealed that both CIA and

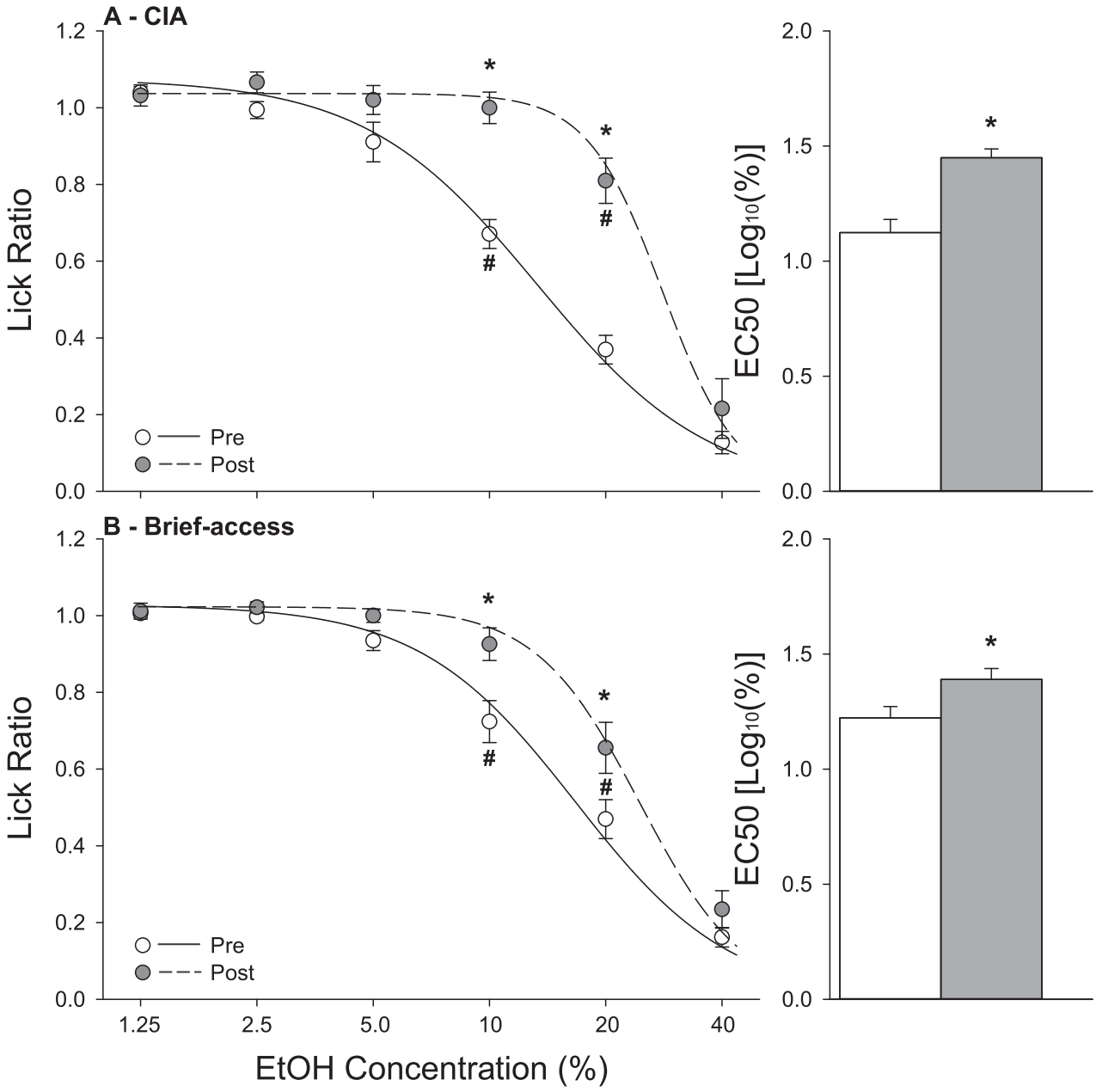

Fig. 3. Both brief access and chronic intermittent ethanol access (CIA) increase the acceptability of the taste of ethanol (EtOH). Initial brief-access licking responses to EtOH of naïve rats (Pre) were significantly lower than the subsequent (Post) brief-access licking response of these same rats following either brief-access exposure alone or 4 weeks of CIA. EtOH exposure resulted in a substantial decrease in the aversive orosensory responding to EtOH driving a lateral shift in the concentration–response function, indicating that, following extensive EtOH exposure, rats were more accepting of the taste of EtOH. During the initial test (Pre), all rats significantly suppressed their licking at the 5% concentration. Following either level of EtOH exposure (CIA: **A**; and brief access: **B**), rats did not significantly suppress their licking until the 20% concentration (#*p*s < 0.05). During the Post test, rats licked significantly more, relative to their initial response during the Pre test, at the 10 and 20% concentrations, thus driving a significant rightward shift in the EC_{50} (log_{10}[%]) of the lick curve following EtOH access (insets; **p*s < 0.05).

brief-access exposure resulted in a significant rightward shift in the EC_{50} for EtOH. The 2-factor ANOVA conducted on the EC_{50} generated for EtOH for each group on each test revealed a main effect of Test, $F(1, 18) = 55.55$, $p < 0.0001$, and a significant interaction between Test and Access group, $F(1, 18) = 5.75$, $p < 0.05$. Post hoc analyses indicated that each access group displayed significantly higher EC_{50}s upon the Post test (*p*s < 0.05) relative to their own baseline during the Pre tests.

Furthermore, to quantify the degree of shift across the 2 tests as a function of the differing level of EtOH access between the 2 groups, we subtracted the Pre test EC_{50} from the Post test EC_{50} for each rat in each group to generate a ΔEC_{50} (Fig. 4). Comparison of the ΔEC_{50} across groups

revealed a significantly greater shift in rats that received substantially more EtOH exposure in the CIA paradigm compared to rats that were only given brief-access exposure, $t(18) = 2.40$, $p < 0.05$, indicating that more EtOH exposure resulted in a greater increase in the acceptance of its taste.

Similar analyses conducted on the licking to QHCl (Fig. 5) revealed a main effect of Concentration, $F(5, 90) = 452.61$, $p < 0.0001$, and a significant Test × Concentration × Group interaction, $F(5, 90) = 2.54$, $p < 0.05$. However, post hoc analyses did not reveal any meaningful differences between Access group and Test, and thus, these effects were likely the result of stochastic differences in licking as a function of repeated testing. Within-group post hoc comparisons revealed that both groups on both tests significantly reduced their licking beginning at the 0.1 mM concentration, and thus, neither level of EtOH access resulted in a shift in the acceptance of QHCl. This indicates that rats tended to decrease their licking to QHCl as a function of increasing concentration, as expected, but that neither brief-access EtOH exposure nor CIA in adulthood had any effect on the acceptance of QHCl.

Additionally, there were no effects of EtOH access on any measure of change of the EC_{50} for QHCl (Fig. 5, insets), further establishing that EtOH exposure in adulthood had no measurable impact on the acceptance of the taste of QHCl.

As in Experiment 1a, when comparing the correlation between the EC_{50} for both EtOH and QHCl generated during the Post test, we found that even in the face of the increased acceptance of EtOH following repeated exposure, the animals that found QHCl to be more aversive were also likely to continue to find EtOH to be more aversive. Linear regression conducted on the EC_{50} of each animal for both stimuli again revealed a modest, yet significant correlation coefficient, $r = 0.467$, $F(1, 18) = 5.01$, $p < 0.05$; Fig. 6, indicating that the evaluation of the palatability of the concentration–response function for both EtOH and QHCl was positively correlated even though EtOH exposure had no impact on the acceptance of QHCl while substantially increasing the acceptance of EtOH.

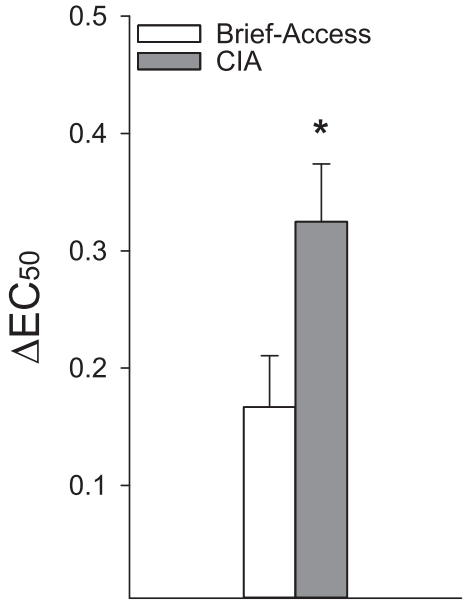

Fig. 4. More extensive ethanol (EtOH) exposure results in a significantly larger lateral shift in the concentration–response function of the taste of EtOH. The relative change in the EC_{50} (ΔEC_{50}) following chronic intermittent ethanol access (CIA) was greater than that seen following brief-access exposure alone. While both levels of EtOH access were sufficient to drive a significant rightward lateral shift in the concentration–response function of the taste of EtOH, more extensive exposure to EtOH resulted in an even greater shift (*ps < 0.05).

Chronic Intermittent EtOH Access. Previous brief access to the taste of EtOH in the Davis rig facilitated free-access EtOH consumption. In the rats that were given access to EtOH (Naïve and CIA), we found a significant main effect of previous exposure to the taste of EtOH, $F(1, 16) = 5.84$, $p < 0.05$, on the amount of EtOH consumed in the CIA paradigm (Fig. 7*A*). During the initial exposures to EtOH, Naïve rats drank less EtOH than the Brief-access rats (i.e., 1.78 ± 0.52 vs. 7.44 ± 1.1 g/kg/24 h on the first presentation, respectively). It was not until the ninth presentation of EtOH (i.e., third week of access) that the 2 groups consumed nearly identical amounts of EtOH. Likewise, we found a similar interactive effect on the preference (Fig. 7*B*) for EtOH over water, $F(11, 176) = 1.90$, $p < 0.05$, with Naïve rats initially showing a lower overall preference for EtOH, relative to the Brief-access group but eventually matching that of the Brief-access group following repeated presentations. These findings suggest that the brief-access Pre tests may have been sufficient to ameliorate the aversive nature of EtOH that putatively limits intake during its initial exposures, a hypothesis that is in line with the finding that just brief-access exposure to EtOH was sufficient to implement a significant lateral shift in the concentration–response function of the taste of EtOH (Fig. 3*B*).

Experiment 2

Here, we determined whether exposure to the taste of QHCl immediately before EtOH would result in an increase in the acceptance of the taste of EtOH. Replicating our findings from Experiment 1, brief-access exposure to the taste of EtOH was sufficient to result in a significant rightward shift in the concentration–response function to the taste of EtOH as seen in the H_2O-adapted rats. Furthermore, immediate preexposure to the taste of QHCl resulted in an immediate increase in the acceptance of the taste of EtOH. Analyses conducted comparing the lick ratios generated to concentrations of EtOH by rats rinsing with H_2O (as in Experiment 1) or 2 concentrations of QHCl (0.03 and 0.3 mM) revealed that rinsing with QHCl prior to EtOH presentation increased the immediate, taste-guided licking to EtOH as evidenced by a Test × Concentration × Stimulus interaction; Fig. 8*A*,*B*; $F(5, 45) = 5.91$, $p < 0.001$. There were also significant main effects of Test, $F(1, 9) = 45.39$, $p < 0.0001$, Concentration, $F(5, 45) = 280.47$, $p < 0.0001$, and rinse Stimulus, $F(1,$

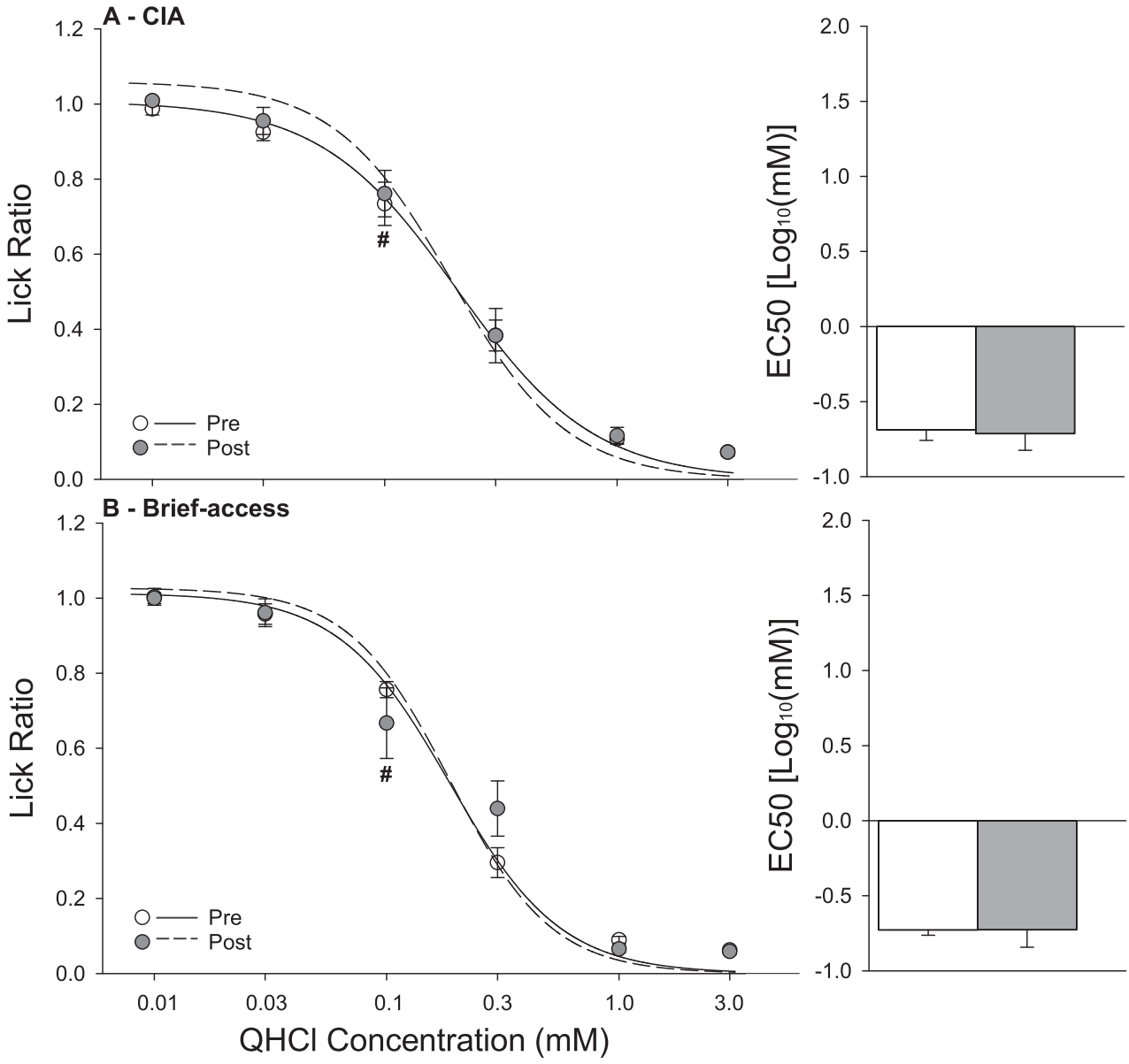

Fig. 5. Neither brief access nor chronic intermittent ethanol (EtOH) access (CIA) alters the taste-guided responding to quinine (QHCl). Initial brief-access licking responses to QHCl of naïve rats (Pre) and the subsequent (Post) brief-access licking response of these same rats following either 4 weeks of CIA exposure (**A**) or brief-access exposure (**B**). In contrast to the robust rightward shift in the licking response to EtOH following exposure, we found no effect of EtOH exposure on the licking response to QHCl or the EC_{50} (log_{10}[mM]), indicating that this level of EtOH exposure was not sufficient to affect the responding to the aversive orosensory properties of QHCl.

9) = 9.42, $p < 0.05$. Post hoc analyses revealed that on the initial Test (Fig. 8*A*; Test 1), both groups of rats significantly decreased their licking beginning at the 10% concentration (*p*s < 0.001). Rats that were rinsing with 0.03 mM QHCl licked more to EtOH, relative to rats rinsing with H_2O, at the 20% concentration ($p < 0.05$). On the subsequent Test (Fig. 8*B*; Test 2), rats rinsing with H_2O now significantly decreased their licking starting at the 20% concentration, while rats rinsing with 0.3 mM did not decrease their licking until the highest concentration tested (40%; *p*s < 0.001). Comparisons across groups indicated that the QHCl group, relative to the H_2O group, licked significantly more to the 40% concentration ($p < 0.001$) with a trend to lick more to the 20% concentration ($p = 0.08$).

Comparing the EC_{50} for each test (Fig. 9) revealed that the EC_{50} for each group was significantly shifted rightward in both groups upon the second Davis rig test, main effect of Test, $F(1, 9) = 46.13$, $p < 0.0001$, again demonstrating that repeated brief-access exposures to an array of EtOH concentrations were sufficient to increase acceptance of the taste of EtOH as was seen in Experiment 1. Moreover, rinsing with QHCl prior to sampling EtOH resulted in an increase in acceptance of the taste of EtOH, relative to the group rinsing with H_2O, main effect of rinse Stimulus, $F(1, 9) = 37.95$,

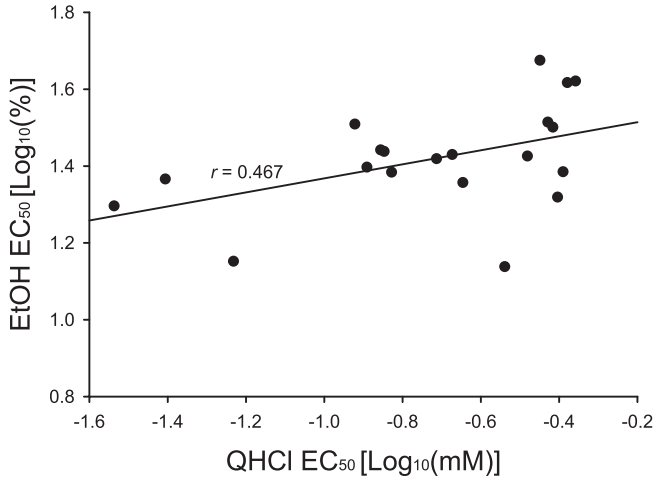

Fig. 6. The EC_{50}s of the concentration–response function for both ethanol (EtOH) and quinine (QHCl) remain positively correlated following exposure to, and thus an increase in acceptance of, EtOH. The EC_{50} for both EtOH and QHCl derived from the brief-access licking response generated during the Post test. There was a significant positive correlation between the EC_{50} for EtOH and QHCl, indicating that individual rats that were more sensitive to the aversive orosensory properties of QHCl were also likely to be more sensitive to EtOH, despite the large increase in acceptance of EtOH seen following repeated exposure.

$p < 0.01$. Planned comparisons revealed that both groups had a significantly higher EC_{50} on the second test relative to their own baseline EC_{50} from the first test ($ps < 0.01$) and that on each test, the group rinsing with QHCl had a higher EC_{50} relative to the H_2O group ($ps < 0.05$), further indicating that rinsing with QHCl prior to tasting EtOH resulted in an immediate increase in the acceptance of the taste of EtOH.

DISCUSSION

The present experiments determined the relationship between the response to the orosensory properties of EtOH and that of the bitter stimulus quinine dihydrochloride in an attempt to elucidate putative mechanisms involved in the evaluation of the palatability of the taste of EtOH. We found that the initial sensitivity to the taste of EtOH and QHCl is positively correlated, as indicated by the relationship between the EC_{50} for both stimuli in naïve rats (Fig. 2). Furthermore, brief access to EtOH, as well as intermittent 24-hour EtOH access, shifted the acceptance of the taste of EtOH but not QHCl (Figs 3 and 5, respectively) and facilitated initiation of free-access EtOH consumption (Fig. 7). Finally, an oral rinse with QHCl increased the acceptance of the taste of EtOH (Figs 8 and 9) in that rats that rinsed with QHCl were more accepting of higher concentrations of EtOH than rats that rinsed with H_2O. Overall, these results indicate that the initial sensitivity to the aversive orosensory properties of at least 1 bitter stimulus (QHCl) is predictive of the sensitivity to EtOH. Moreover, these results indicate that even brief exposure to the taste of EtOH is sufficient to increase its acceptance and ultimately, its consumption.

The positive relationship reported here between the sensitivity to the aversive tastes of QHCl and EtOH upon the animals' initial exposures to the stimuli is in line with reports in humans demonstrating that increased sensitivity to bitterness

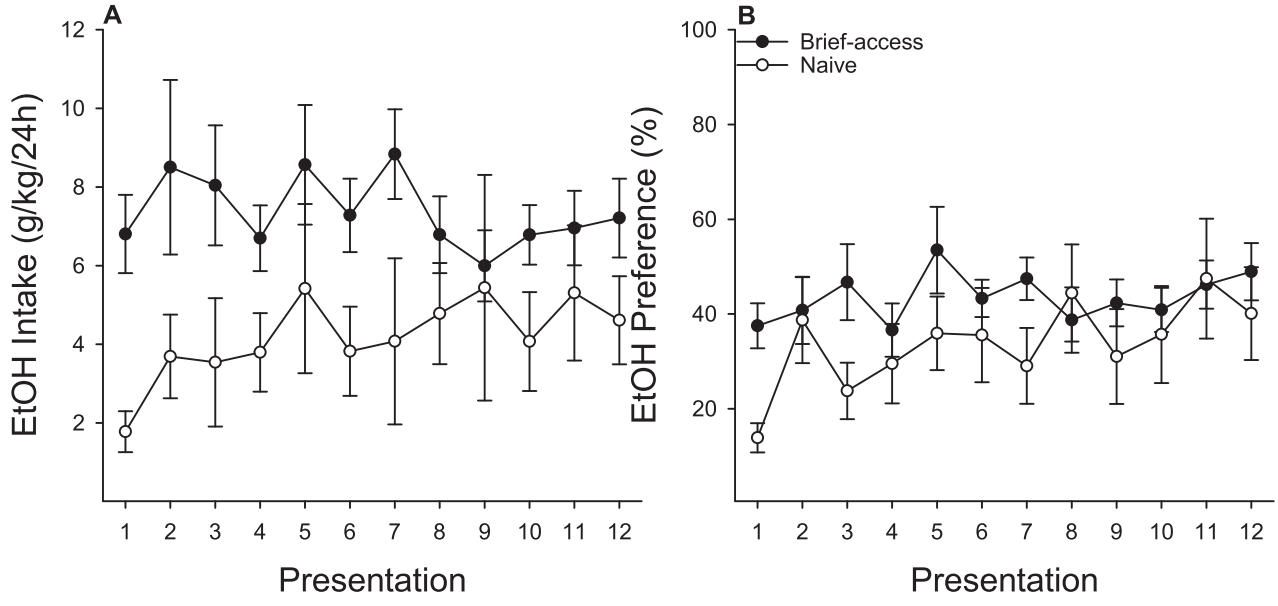

Fig. 7. Free-access ethanol (EtOH) intake remained consistently high in rats previously given brief access to EtOH. Mean EtOH intake (**A**; g/kg/24 h) and preference (**B**; %) across the 12 EtOH presentations during the 4-week CIA procedure. Previous exposure to the taste of EtOH in the Davis rig facilitated free-access EtOH intake, particularly during the initial presentations. Specifically, brief-access-exposed rats consumed a rather large amount of EtOH upon its first presentation and continued, on average, to consume this amount throughout the 4-week period, while the naïve rats escalated intake across the presentations until matching the intake of the previously exposed rats. This suggests that the limited brief-access exposures in the Davis rig were sufficient to decrease the aversive-like responding to EtOH thus increasing its intake.

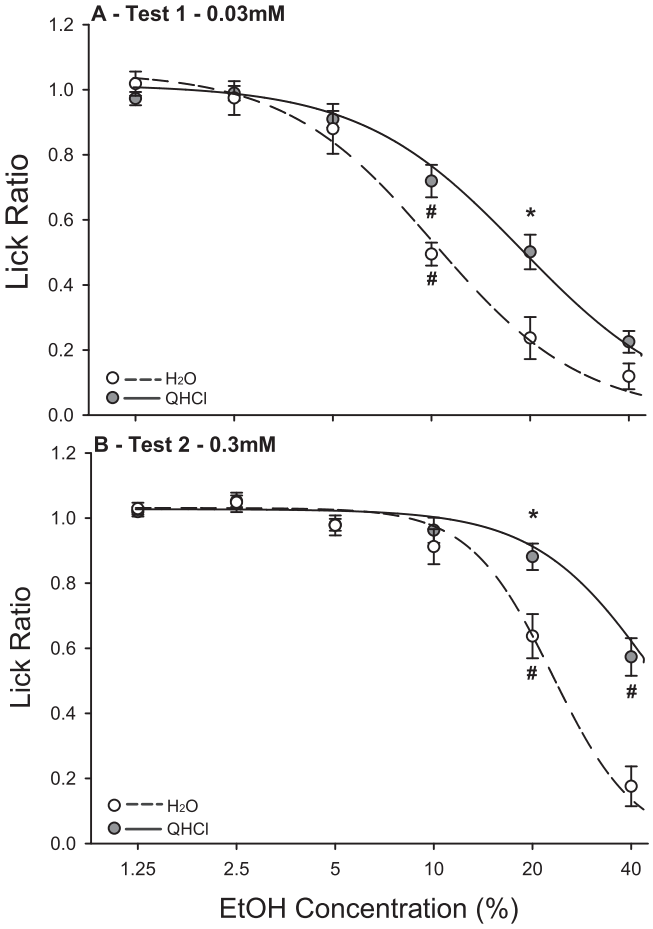

Fig. 8. Brief exposure to a quinine (QHCl) solution immediately before sampling ethanol (EtOH) increases the acceptance of the taste of EtOH. Initial brief-access licking responses (**A**) of naïve rats that sampled either H_2O or 0.03 mM QHCl before randomized concentrations of EtOH and the subsequent licking responses (**B**) of rats either still rinsing with H_2O or a log-step higher concentration of QHCl (0.3 mM). Brief-access exposure to EtOH was sufficient to drive a rightward shift in the acceptability of the aversive orosensory properties of EtOH similar to that seen in Experiment 1 as evidenced by the increase in acceptance seen in rats rinsing with H_2O. Beyond that, rinsing with QHCl immediately prior to tasting EtOH resulted in an increase in the acceptance of the aversive orosensory properties of EtOH. On the initial exposures to EtOH, rats that were rinsing with QHCl prior to EtOH licked significantly more to the 20% concentration relative to rats rinsing with H_2O. On the subsequent exposure, the QHCl rats, now rinsing with 0.3 mM, licked significantly more, to the 40% concentration with a trend for the 20% concentration. Further, on the subsequent exposure, QHCl-rinsing rats did not significantly decrease their licking until the 40% concentration, as opposed to the decrease seen at 20% in H_2O-rinsing rats (#,*ps < 0.05).

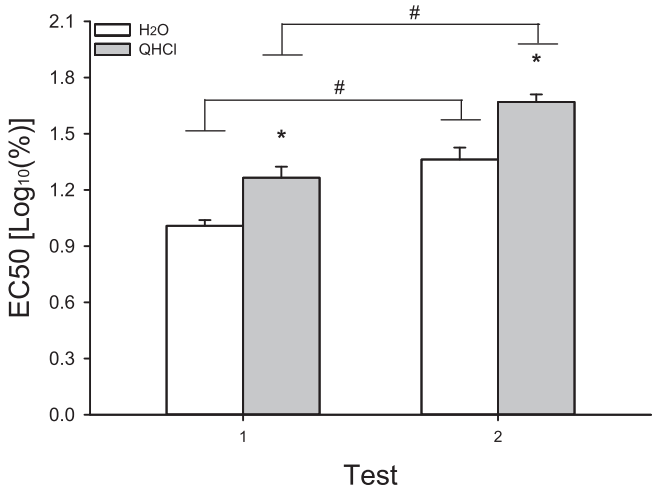

Fig. 9. Brief exposure to ethanol (EtOH) increases subsequent EtOH acceptability and is further increased by brief exposure to a quinine (QHCl) rinse immediately before sampling of EtOH. EC_{50}s (log_{10}[%]) for the initial (Test 1) and subsequent (Test 2) brief-access EtOH licking tests. In the H_2O-rinsing rats, repeated brief-access exposures to EtOH were sufficient to result in a significant rightward shift in the acceptance of the orosensory properties of EtOH, thus replicating findings from Experiment 1. For both tests, rats rinsing with QHCl (0.03 mM: Test 1; and 0.3 mM: Test 2) displayed a significant rightward shift in the EC_{50} to EtOH relative to rats that were rinsing with H_2O prior to tasting EtOH, indicating that they found the aversive orosensory properties of EtOH to be more acceptable (#, *ps < 0.05).

served to limit initial alcohol consumption (Duffy et al., 2004; Lanier et al., 2005; Thibodeau et al., 2017). A number of studies have identified a predictive relationship between bitter-sensitive genotypes and decreased alcohol consumption (Beckett et al., 2017; Dotson et al., 2012; Duffy et al., 2004; Hayes et al., 2011). It has previously been proposed that genetic sensitivity to bitterness is likely most protective against initiation of drinking behavior as opposed to total alcohol intake within a drinking session (Dotson et al., 2012; Hayes et al., 2011). Again, this is a relationship that is supported by our current findings, namely that a lower EC_{50} for acceptance of QHCl was positively correlated with lower acceptance of EtOH (Fig. 2) in naïve animals. Upon repeated EtOH exposures, we found a robust increase in the acceptance of the taste of EtOH with no effect on the acceptance of the taste of QHCl (Fig. 5), suggesting that ongoing drinking likely has little impact on systems that are responsible for signaling the orosensory properties of QHCl and that differences in bitter-related orosensory responding are likely more important for initiation of alcohol drinking as opposed to maintenance of drinking behavior. Further, the lack of an increase in the acceptance of the taste of QHCl following CIA strongly supports the previous conclusion that the development of resistance to the effects of QHCl adulteration on EtOH consumption following similar EtOH access procedures is not due to an adaptation in orosensory sensitivity to bitter stimuli (Hopf et al., 2010).

Using a brief-access licking paradigm that serves to minimize postingestive consequences, we show that brief, repeated exposures to the taste of EtOH are sufficient to increase the acceptance of its taste as evidenced by a rightward shift in the EC_{50} of the taste-guided licking curves in response to randomly presented EtOH concentrations. Behavioral and neurophysiological studies demonstrate that the orosensory properties of EtOH likely resemble a mixed taste profile representing both appetitive, sweet-like qualities and aversive, bitter-like qualities as well as trigeminal-mediated oral irritation (Bachmanov et al., 2003; Dilorenzo et al., 1986; Kiefer and Mahadevan, 1993; Lemon et al.,

2004; Trevisani et al., 2002). Adaptive changes in the salience of any of these orosensory components could result in the increased acceptance observed here. In contrast to a previous study examining prenatal exposure (Youngentob and Glendinning, 2009), we did not find a correlative increase in the acceptance of the bitterness of QHCl following exposure to EtOH in adulthood. Here, no rats displayed a shift in response to the taste of QHCl upon their subsequent experience. This may simply indicate that the level of EtOH access used in the present study is not sufficient to result in an increased acceptance of QHCl, or that whichever mechanisms that are driving the increase in QHCl acceptance following prenatal exposure are not engaged following exposure in adulthood. Other studies demonstrate exposure-induced increases in the acceptance of bitter stimuli (London et al., 1979; Torregrossa et al., 2014; Zellner et al., 1985) and so the present findings do not simply represent a ceiling effect in the amount of licking that a rat will direct to a bitter-like taste stimulus. Thus, the underlying mechanisms resulting in the rapid increase in the acceptance of EtOH, relative to QHCl, following repeated exposure in adulthood remain to be determined. However, these data suggest that the rapid increase in acceptance of EtOH following exposure in adulthood is likely not simply due to a generalized increased in the acceptance of bitter-like taste stimuli or necessarily dependent on substantial experience with the postingestive consequences of EtOH. While more extensive access ultimately resulted in a larger shift in the acceptance of EtOH (Fig. 4), these data demonstrate that simply being repeatedly exposed to the taste of EtOH is sufficient to increase the acceptance of its taste. Because we did not measure blood EtOH concentrations in this study, we cannot be certain that the rats were not experiencing any postingestive consequences of brief-access EtOH exposure, albeit similarly designed brief-access licking tests in a previous report have shown that the marginal volume of EtOH consumed in these tests does not result in measurable changes in blood EtOH concentration (Brasser et al., 2012). With that being said, the substantially larger increases in the acceptance of the taste of EtOH seen following a free-access drinking paradigm (CIA), relative to brief access alone, are likely the result of a combination of the rapid increases in acceptance seen following brief-access EtOH access and conditioning effects due to the reinforcing pharmacological and caloric postingestive consequences of EtOH consumption (Ackroff and Sclafani, 2003).

Interestingly, in the present study, rats that were placed on the CIA procedure following brief access to the taste of EtOH did not show the gradual increase in either the free-access consumption of, or the preference for, EtOH that is typical of these procedures (e.g., Bito-Onon et al., 2011; Carnicella et al., 2011; Simms et al., 2008). Here, rats initiated free-access EtOH drinking at a relatively high volume of consumption (Fig. 7*A*) above that of rats that were previously naïve to EtOH, and ultimately, a level of consumption comparable to that which is usually seen toward the end of the CIA procedure in studies employing similar schedules of access. This suggests that the brief-access exposures to EtOH in the Davis rig were sufficient to familiarize the animals with EtOH in a manner that may potentially ameliorate the aversive orosensory properties of EtOH. Further support for such a conclusion is provided by our finding that brief access in the Davis rig alone was sufficient to instigate a significant rightward shift in the concentration–response function for the taste of EtOH (i.e., Figs 3, 8, and 9).

Ultimately, we were surprised that we did not find a correlative increase in the acceptance of QHCl following extensive access to EtOH in adulthood as would be predicted based on the available data concerning prenatal EtOH exposure (Glendinning et al., 2017; Youngentob and Glendinning, 2009), particularly in light of the positive correlation observed in the acceptance of EtOH and QHCl upon initial exposure. Many of these effects following prenatal exposure were found to be transient in that they disappeared when the animals were tested in adulthood, with the exception of the decrease in responsiveness to QHCl. This could mean that the developmental changes that drive the increased acceptance observed in adolescence are lost by adulthood. Another potential explanation is that the effects may be lost in the absence of subsequent EtOH exposures, because all adults in these previous studies were abstinent with regard to EtOH prior to testing, following the initial prenatal exposure (Glendinning et al., 2017; Youngentob and Glendinning, 2009). Despite the substantial increase in acceptance of the taste of EtOH following repeated exposure, and the lack thereof for QHCl, we found that the sensitivity to the taste of QHCl was still predictive of the sensitivity to the taste of EtOH (Fig. 6). This sustained positive relationship between these 2 measures further indicates that there is some common mechanism for the processing of the tastes of EtOH and QHCl that serves to limit the avidity for the tastes of 2 stimuli, but that these overlapping mechanisms are not substantially impacted by repeated EtOH exposure in adulthood. There was no relationship between either measure and the amount of EtOH consumed during CIA, again suggesting that the reinforcing pharmacological and caloric effects of EtOH serve to facilitate ongoing drinking and that these sensitivities are likely more influential in the initiation of drinking as was observed during the various brief-access tests. Regardless, Experiment 2 was conducted to further explore any potential interaction between the bitter taste system and palatability for concentrated EtOH solutions.

Rinsing with QHCl prior to EtOH resulted in a substantial increase in the initial acceptance of the taste of EtOH, further suggesting that EtOH and QHCl share some physiological mechanisms that contribute to the hedonic evaluation of their tastes. One such mechanism could be that the 2 substances share a receptor responsible for transduction of their respective taste signals. The TAS2R family of mammalian genes encode ~30 broadly tuned receptors that respond to a wide variety of bitter-tasting compounds (Meyerhof, 2005). If the T2Rs responsible for the transduction of the bitter taste of EtOH are the same as those of QHCl, then rinsing

the oral cavity with QHCl may contribute to receptor adaptation or some other form of competitive inhibition wherein QHCl has bound these putative receptors, thus preventing activation by EtOH. Such an interpretation of these data is tempered by the fact that, at least in humans, QHCl and EtOH do not bind overlapping T2Rs (Allen et al., 2014; Nolden et al., 2016); though, to the best of our knowledge, there is not adequate information regarding the potential receptors of EtOH and QHCl in rodents to make such a claim. Regardless, reducing any bitter component of the taste of EtOH would likely increase its palatability through enhancement of the salience of the established sweet-like component in the taste of EtOH (Lemon et al., 2004). Such an outcome would likely result in a behavioral profile similar to that observed in the present study. Because brief-access exposure alone was sufficient to increase the acceptance of the orosensory properties of EtOH upon a second test, we cannot make a direct claim that the increase in acceptance following a QHCl oral rinse was concentration dependent. Rather, simply exposing the rats to the taste of QHCl prior to EtOH was sufficient to increase the acceptance of EtOH, relative to rats tasting H_2O, and this was true for both concentrations of QHCl tested (0.03 and 0.3 mM; Figs 8 and 9).

While peripheral interactions between the 2 stimuli at the receptor level would be sufficient to explain the observed increase in the acceptance of the taste of EtOH, this explanation is not the only relevant hypothesis. Dynamic contrast between the taste of the adapting stimulus, here QHCl, and that of the test stimulus, EtOH, may be equally likely to result in the observed enhancement. Within-meal variability in consumed stimuli enhances the hedonic responding to these stimuli and thus elevates intake (Rolls et al., 1982). One proposed contributing factor to this enhanced intake is the inherent contrast between variable intraoral stimulations that subsequently lead to an increase in perceived palatability (Hyde and Witherly, 1993). Likewise, successive positive contrast through presenting a less rewarding, or more aversive taste stimulus prior to a test stimulus results in an increased avidity for the subsequently presented stimulus and has been demonstrated across a number of sensory modalities and reinforcers (Candido et al., 2002; Cuenya et al., 2015; Hall et al., 1997; Suarez et al., 2014). Of particular importance for the present report, presenting rats with a more concentrated QHCl solution prior to a less concentrated QHCl solution results in an increase in the number of classically defined appetitive taste reactivity responses and a decrease in the aversive-like responses even when the 2 stimuli were not presented in close temporal proximity (Suarez et al., 2017). Ultimately, while we did observe an increase in the acceptance of the taste of EtOH through preexposure to the taste of QHCl, the mechanism responsible for this effect is not known and is a topic for future study.

Regardless of the mechanisms responsible for driving the enhancement in the acceptance of the taste of EtOH, these findings demonstrate that orosensory factors play a substantial role in the initial acceptance of EtOH solutions. Of particular importance for problematic drinking in humans is the potential role of the addition of bitter orosensory activating compounds such as nicotine and caffeine to ongoing drinking bouts (Dahl et al., 1997; Fritz et al., 2016; Le et al., 2003; Miller and Gold, 1998; Oliveira-Maia et al., 2009; Simons et al., 2006). As both nicotine and caffeine are capable of activating bitter- and trigeminal-related orosensory pathways, it follows that adapting the oral cavity with either of these stimuli may result in an enhancement of the acceptance of the taste of EtOH as was observed here with oral adaptation to QHCl; these questions are the focus of ongoing research. These potential enhancement effects would be above and beyond that expected from the reinforcing pharmacological properties of both stimuli. Nevertheless, in light of these reported effects, interactions between the orosensory properties of substances commonly coadministered with EtOH merit further exploration. In summation, rodents rapidly adapt to the taste of EtOH resulting in an increased acceptance of more concentrated EtOH solutions which may drive increased EtOH consumption. Moreover, sensitivity to the aversive orosensory properties of EtOH and QHCl is positively correlated and lends support to findings in humans that increased bitter sensitivity may play a protective role against initiation of alcohol consumption. Decreasing the salience of the aversive orosensory properties of EtOH results in increased acceptance of EtOH which may promote development of alcohol use disorders.

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