Inflexible and Indifferent Alcohol Drinking in Male Mice

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Background: Alcoholism is characterized by compulsive alcohol intake, but this critical feature of alcoholism is seldom captured in preclinical studies. Here, we evaluated whether alcohol-preferring C57BL/6J mice develop compulsive alcohol drinking patterns, using adulteration of the alcohol solution with quinine, in a limited access choice paradigm. We assessed 2 independent aspects of compulsive drinking: (i) inflexible alcohol intake by testing whether mice would drink bitter alcohol solutions if this was their only source of alcohol and (ii) indifferent drinking by comparing intake of aversive and nonaversive alcohol solutions.

Methods: Male C57BL/6J mice consumed alcohol for 2 or 8 consecutive weeks. The alcohol solution was then adulterated with graded quinine concentrations, and the effect on alcohol intake was determined.

Results: C57BL/6J mice rapidly developed compulsive alcohol drinking patterns. Adulteration of the alcohol solution with an aversive quinine concentration failed to reduce intake, indicative of inflexible drinking behavior, after only 2 weeks of alcohol experience, although quinine adulteration did suppress the acquisition of alcohol drinking in naïve mice. After 8 weeks of alcohol consumption, the mice also became indifferent to quinine. They consumed an aversive, quinine-containing alcohol solution, despite the simultaneous availability of an unadulterated alcohol solution. Prolonged alcohol ingestion did not alter the sensitivity to the bitter taste of quinine itself.

Conclusion: These findings demonstrate the staged occurrence in mice of 2 distinct behavioral characteristics of alcoholism, i.e., inflexible and indifferent alcohol drinking.

Key Words: Mouse, Alcohol Drinking, Compulsion, Alcoholism, Quinine.

ALCOHOLISM IS ONE of the most widespread addictions, affecting approximately 140 million people worldwide. Alcoholism is characterized by compulsive alcohol intake, which can be apparent as continued drinking despite adverse consequences (American Psychiatric Association, 2000). Treatment strategies for alcoholism that directly target its compulsive properties are therefore expected to be highly effective. To facilitate the development of such therapies, the neurobehavioral underpinnings of compulsive alcohol use need to be elucidated. However, there are only a few examples of genuine addiction-like behavior in rodents (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Wolffgramm, 1991) using the symptoms for addiction or alcohol abuse in DSM-IV (American Psychiatric Association, 2000) as criteria for addiction-like behavior. It has, for example, been shown that rats with extensive cocaine self-administration experience show persistent drug seeking in the face of adversity, such as presentations of footshocks or stimuli associated with them (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004). Persistent alcohol seeking and taking has been demonstrated in both rats and mice. Thus, consumption of alcohol or operant responding for alcohol was not reduced when the alcohol solution was adulterated with the bitter tastant quinine (Fachin-Scheit et al., 2006; Wolffgramm, 1991) or when alcohol was devalued using lithium chloride (Dickinson et al., 2002; but see Samson et al., 2004).

Recently, limited access choice paradigms for alcohol drinking have been developed, in which C57BL/6J mice display high levels of alcohol intake (up to 3 g/kg within 2 hours) and high alcohol preference (up to 90%) (Ford et al., 2008; Lesscher et al., 2009b). Limited access choice paradigms may therefore be very well suited to study the mechanisms underlying excessive alcohol drinking and alcohol preference. However, the question remains whether and how excessive alcohol intake gains alcoholism-like characteristics. Using quinine adulteration, we here show the rapid, staged development of 2 distinct behavioral characteristics of alcoholism in C57BL/6J mice, i.e., inflexible and indifferent drinking. Inflexible drinking was operationally defined as continued intake of an alcohol solution that had been rendered aversive by adding quinine, when this was the only source of alcohol. Drinking was termed indifferent when mice continued to drink an aversive, quinine-containing alcohol solution, despite the simultaneous availability of unadulterated alcohol.

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MATERIALS AND METHODS

Animals

Male C57BL/6J mice were bred in the Department of Neuroscience and Pharmacology at the University Medical Center Utrecht, from parents obtained from Jackson Laboratory (Bar Harbor, ME). Experimental animals were 8 to 10 weeks old at the onset of testing. The mice were group-housed and were acclimatized to a reversed 12-hour light/dark cycle (lights off at 7:00 AM) for at least 2 weeks prior to testing. Food and water were available ad libitum, and environmental conditions were controlled (20 ± 2°C and 50 to 70% humidity). The experimental procedures were approved by the Animal Ethics Committee of Utrecht University and were conducted in agreement with Dutch laws (Wet op de dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Quinine Taste Avoidance Test

Quinine is a bitter tastant that is perceived as aversive by mice. In models for alcoholism-like behavior for mice and rats, quinine concentrations ranging from 12 μM (Fachin-Scheit et al., 2006) to 500 μM (Wolffgramm, 1991) have been used. Taste preference tests, with gradually decreasing quinine concentrations, show that quinine is aversive for mice at concentrations of 10 μM and higher with a high–low quinine concentration curve, reflected by reduced preference over water at these concentrations (Whitney and Harder, 1994). To determine the optimal concentration range for our quinine modulation studies, the aversive concentration of quinine for C57BL/6J was first determined in a 24-hour 2-bottle choice setup. C57BL/6J mice ($n = 7$) were offered access to 1 bottle filled with tap water and 1 bottle filled with graded concentrations of quinine hemisulfate (Sigma, Zwijndrecht, The Netherlands) (0, 25, 50, 100, and 250 μM). All bottles were weighed daily to calculate fluid intake. Each concentration was offered for 2 consecutive days, and bottle positions were changed every day. Across these days, the average avoidance of each concentration of quinine was calculated as a percentage of the total fluid intake per individual animal. Quinine concentrations were presented in ascending order. This same analysis was also performed in separate groups of mice that had previously consumed alcohol for 2 or 8 weeks.

Limited Access Alcohol Consumption

Mice were trained to voluntarily consume alcohol using an adapted limited access paradigm, the procedures for which were based on previous studies (Ford et al., 2008; Lesscher et al., 2009b; Lopez and Becker, 2005; Rhodes et al., 2005). For daily drinking sessions, mice were placed into a separate test cage for 2 hours each day starting at 10:00 AM, i.e., 3 hours after the onset of the dark phase. The mice had access to 2 drinking tubes, i.e., 10-ml polystyrene pipettes fitted with a stainless steel ball-bearing sipper tube. One tube delivered tap water and the other 15% alcohol (v/v in tap water). During the initial 7 days of training, the water and alcohol bottles were presented at fixed locations. Thereafter, the bottle positions were switched daily to avoid side-preference. After 2 weeks of training, mice were tested 5 d/wk for the duration of the experiment. The mice were never food- or water-deprived. Fluid volumes were measured to the nearest 0.05 ml prior to and after each drinking session by reading the scale of the 10-ml pipette. During the sessions, the drinking tubes were fixed to the cages using clips to prevent spillage. Using this setup, mice develop stable alcohol drinking patterns after approximately 3 weeks of training (intake ~2.5 g/kg/2 h; preference ~70 to 90%, resulting in blood alcohol levels of ~100 mg/dl, see Tables 1–3 and Lesscher et al., 2009b).

Assessment of Inflexible Alcohol Intake

After 2 and 8 weeks of daily limited access alcohol consumption, the 15% alcohol solution was adulterated with graded concentrations of quinine (25, 100, 250, and 500 μM) on 4 consecutive days ($n = 8$). Apart from the adulteration, the procedures were similar to those described earlier. Fluid volumes were measured, and alcohol intake was normalized to the average baseline level of alcohol intake per experimental group (2 or 8 weeks), defined as the average alcohol intake over the last 3 days prior to quinine modulation. All individual data points—for each mouse and each quinine concentration—were expressed as the percentage of the ratio against the baseline intake for the respective group. The group averages for the normalized intake data were then calculated.

Assessment of Indifferent Alcohol Intake

These tests were performed in the week subsequent to the tests for inflexible alcohol intake. The procedures are similar to those described before, but now the mice were offered 2 bottles filled with alcohol during regular daily limited access drinking sessions. One of the alcohol solutions was adulterated with quinine at graded concentrations (25 to 500 μM) on 4 consecutive days. Aversion for the quinine-adulterated solution was calculated as a percentage of the ratio (quinine-adulterated alcohol/total fluid intake).

Assessment of Quinine's Effects on Acquisition of Alcohol Intake

Two groups of naïve C57BL/6J mice ($n = 8$) were trained to voluntarily consume alcohol using an adapted limited access paradigm. One group received a choice between water and alcohol (15% v/v), while the other group received a choice between water and alcohol (15% v/v) that was adulterated with 250 μM quinine. This concentration of quinine is aversive for mice (Fig. 1*A*), but alcohol that is adulterated with this concentration of quinine is tolerated by mice with 2-week experience with alcohol consumption when this is the only source of alcohol (Fig. 1*B*). Alcohol consumption and preference were determined for 2 consecutive weeks.

Table 1. Mean Daily Alcohol Intake, Alcohol Preference, and Total Fluid Intake for the 2-Week and the 8-Week Groups in Daily 2-Hour Sessions During the Assessment of Inflexible Alcohol Intake (Fig. 1*B*)

	Quinine concentration (μM)				
	0	25	100	250	500
Intake (g/kg/2 h)					
2 weeks	1.46 ± 0.28	1.63 ± 0.27	1.32 ± 0.37	1.13 ± 0.27	0.57 ± 0.13
8 weeks	2.42 ± 0.24	2.36 ± 0.20	0.19 ± 0.31	1.73 ± 0.30	1.31 ± 0.30
Alcohol preference (%)					
2 weeks	56.7 ± 7.8	54.9 ± 6.4	56.5 ± 14.8	43.5 ± 12.3	20.9 ± 6.2
8 weeks	71.9 ± 7.8	78.6 ± 7.3	75.5 ± 8.5	65.7 ± 13.2	49.8 ± 11.7
Total V (ml/kg/2 h)					
2 weeks	35.5 ± 6.7	39.9 ± 7.1	32.4 ± 5.2	36.8 ± 4.5	38.3 ± 5.0
8 weeks	30.1 ± 3.6	26.4 ± 2.8	21.3 ± 3.0	18.6 ± 4.2	23.4 ± 2.9

Table 2. Mean Daily Alcohol Intake, Quinine Avoidance, and Total Fluid Intake for the 2-Week and the 8-Week Groups in Daily 2-Hour Sessions During the 5 Sessions the Assessment of Indifferent Alcohol Intake (Fig. 3*A*)

	Quinine concentration (μM)				
	0	25	100	250	500
Intake (g/kg/2 h)					
2 weeks	2.11 ± 0.29	1.89 ± 0.36	1.81 ± 0.29	2.02 ± 0.40	2.11 ± 0.41
8 weeks	2.40 ± 0.37	2.79 ± 0.33	2.71 ± 0.40	2.37 ± 0.24	0.45 ± 0.10
Quinine avoidance (%)					
2 weeks	49.4 ± 6.3	33.1 ± 5.3	23.4 ± 7.3	20.3 ± 5.8	0.7 ± 0.7
8 weeks	39.3 ± 7.9	50.4 ± 9.6	41.7 ± 3.4	52.2 ± 10.3	16.7 ± 3.5
Total V (ml/kg/2 h)					
2 weeks	17.9 ± 2.5	16.0 ± 3.1	15.3 ± 2.4	17.1 ± 3.4	17.9 ± 3.5
8 weeks	20.3 ± 3.1	23.6 ± 2.8	22.9 ± 3.4	20.1 ± 2.1	21.2 ± 3.6

The mice were given access to 2 bottles, 1 filled with 15% v/v alcohol and the other filled with 15% v/v alcohol that was adulterated with graded quinine concentrations (0 to 500 μM). Alcohol intake was calculated from the total volume consumed from both alcohol bottles. Quinine avoidance was calculated as the percentage of adulterated alcohol the mice consumed relative to the total volume of alcohol consumed.

Table 3. Quinine Avoidance (% of Total Volume Consumed) and the Total Volume Consumed per 24 Hours in Mice That Consumed Alcohol for 2 or 8 Preceding Weeks, Corresponding to Fig. 3*B*

	Quinine concentration (μM)					
	0	25	50	100	250	500
Quinine avoidance (%)						
2 weeks	48.9 ± 2.6	45.7 ± 2.9	40.7 ± 2.8	30.6 ± 3.8	19.9 ± 3.2	8.2 ± 0.5
8 weeks	57.6 ± 3.7	45.9 ± 4.0	41.9 ± 3.6	29.8 ± 4.1	18.6 ± 3.6	8.8 ± 1.4
Total volume consumed (ml/kg/24 h)						
2 weeks	145 ± 5.9	151 ± 5.9	153 ± 5.2	148 ± 4.0	163 ± 3.7	150 ± 6.0
8 weeks	161 ± 16.5	156 ± 10.1	158 ± 8.9	147 ± 8.3	165 ± 10.5	144 ± 9.3

Statistical Analysis

All results are shown as mean ± SEM values. The taste avoidance data were analyzed by 1-way repeated measures ANOVA with quinine as the repeated measures within-subjects factor. The concentration curve data for assessment of indifferent and inflexible alcohol intake (intake, preference, and total fluid intake) were analyzed by 2-way repeated measures ANOVA with group—referring to mice that consumed alcohol daily for 2 or 8 preceding weeks—as the between-subjects factor and quinine concentration as the repeated measures within-subjects factor. The acquisition data were also analyzed by 2-way repeated measures ANOVA, with quinine as the between-subjects factor and time as the repeated measures within-subjects factor. Posthoc analysis was performed by 2-tailed *t*-tests where appropriate. Differences between pairs of means were considered significant at alpha < 0.05.

RESULTS

We first assessed inflexible drinking, i.e., continued intake of an aversive, quinine-containing alcohol solution, when this was the only source of alcohol. After 2 or 8 weeks of alcohol consumption, C57BL/6J mice were given the choice between quinine-adulterated alcohol and water, to assess whether they were willing to endure an aversive taste during alcohol drinking. Inflexible alcohol drinking was already apparent in the mice after 2 weeks of alcohol consumption in the limited access choice paradigm, when mice showed average daily intake levels of 1.4 ± 0.3 g/kg; see Table 1 for mean intake, preference, and total fluid intake prior to data normalization. Quinine was aversive for C57BL/6J mice at concentrations of ≥250 μM (Fig. 1*A*). However, adulteration with an aversive quinine concentration (250 μM) failed to reduce alcohol consumption in C57BL/6J mice with only 2 weeks of daily alcohol experience (Fig. 1*B*). Only the highest quinine concentration of 500 μM significantly reduced alcohol intake (Fig. 1*B*). The degree of inflexibility did not change with prolonged alcohol experience, because mice with 8 weeks of alcohol experience, with average daily intake levels of 2.4 ± 0.2 g/kg/2 h, also showed reduced alcohol intake with a quinine concentration of 500 μM while alcohol consumption was not affected by adulteration with the lower quinine concentration of 250 μM (Fig. 1*B* and Table 1 for mean intake, preference, and total fluid intake prior to data normalization).

The C57BL/6J mice developed inflexible alcohol consumption after 2 weeks of alcohol experience. This is even before stable baseline levels of alcohol intake are reached, which usually takes approximately 3 weeks (Lesscher et al., 2009a). To determine whether C57BL/6J mice do display sensitivity to quinine at the onset of alcohol drinking, an acquisition experiment was performed. The control group of mice was offered—during acquisition—a free choice between water and 15% alcohol (v/v) while the quinine group was offered water and quinine-adulterated alcohol (250 μM in 15% v/v

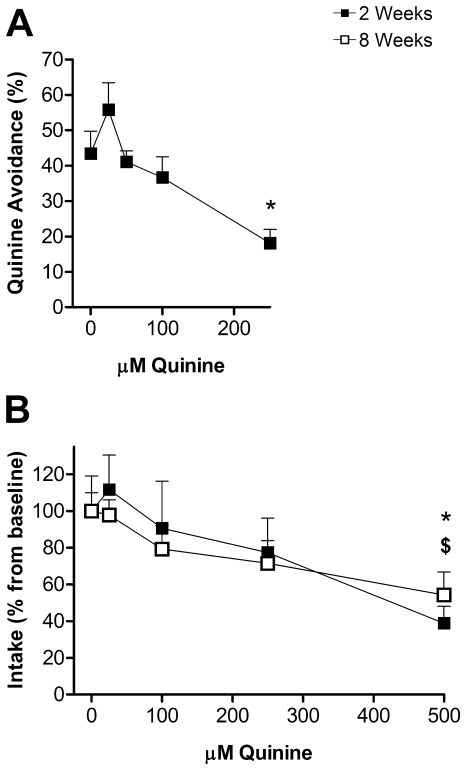

Fig. 1. C57BL/6J mice develop inflexible alcohol drinking. (**A**) Alcohol-naïve C57BL/6J mice showed taste aversion for ≥250 μM quinine in water [$F_{(quinine)4,24} = 5.8$, $p < 0.01$]. *$p < 0.05$ from 0 μM quinine by *t*-test. (**B**) The mice developed inflexible alcohol drinking in that they drank an aversive alcohol solution (250 μM) when this was the only source of alcohol. Only adulteration with 500 μM quinine significantly reduced alcohol intake [$F_{(quinine)4,48} = 14$, $p < 0.001$], independent of the duration of alcohol experience [$F_{(quinine \times time)4,48} = 0.88$, N.S.]. Alcohol intake levels were normalized to average baseline alcohol intake per experimental group as described in the methods section. *$^{\$}p < 0.05$ from 0 μM quinine by *t*-test after 2 or 8 weeks of daily alcohol consumption, respectively.

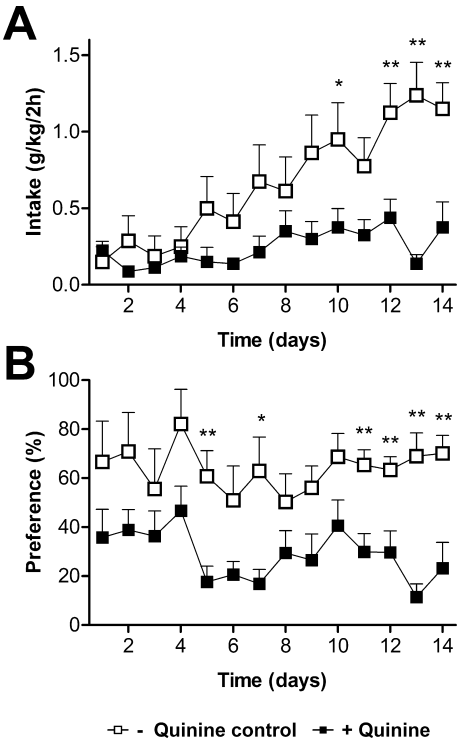

Fig. 2. Quinine adulteration inhibits acquisition of alcohol consumption in alcohol-naïve C57BL/6J mice. (**A**) Alcohol intake was lower for mice that were given a choice between water and quinine-adulterated alcohol (15% v/v) when compared to control mice that were given a choice between water and nonadulterated alcohol (15% v/v) [$F_{(quinine)1,13} = 6.1$, $p < 0.05$]. Moreover, quinine adulteration inhibited the progressive increase in alcohol intake [$F_{(quinine \times time)13,169} = 4.6$, $p < 0.001$]. (**B**) Preference for alcohol over water was significantly lower in the group that received a choice between water and quinine-adulterated alcohol when compared to the control group [$F_{(quinine)1,6} = 36$, $p < 0.01$], which was not dependent on time [$F_{(quinine \times time)13,78} = 1.5$, N.S.). *$p < 0.05$ **$p < 0.01$ quinine group differs from the control group by *t*-test.

alcohol). Adulteration with quinine during acquisition inhibited alcohol intake and impaired the time-dependent increase in alcohol intake that is normally observed in the limited access choice paradigm (Fig. 2*A*). These data demonstrate that C57BL/6J mice are initially sensitive to quinine. Preference for the alcohol solution was significantly lower in the group that was offered quinine-adulterated alcohol when compared to the control group (Fig. 2*B*). The total fluid volume consumed was not affected by quinine adulteration [$F_{(quinine)1,13} = 0.03$, N.S.; $F_{(quinine \times time)13,169} = 1.1$, N.S., data not shown].

We next determined whether the mice would also develop indifferent alcohol drinking, i.e., intake of an aversive, quinine-containing alcohol solution, despite the simultaneous availability of unadulterated alcohol, after 2 or 8 weeks of alcohol consumption. After 2 weeks of alcohol experience (intake 1.8 ± 0.4 g/kg/2 h, preference 68 ± 12%, total fluid intake 21 ± 2 ml/kg/2 h), the mice avoided quinine at concentrations of 100 μM and higher, when they were given the choice between an alcohol solution with and without quinine (Fig. 3*A*, Table 2). However, after 8 weeks of alcohol consumption (intake 2.4 ± 0.2 g/kg/2 h, preference 72 ± 7.8%, total fluid intake 30 ± 4 ml/kg/2 h), C57BL/6J mice no longer discriminated between alcohol and quinine (100 or 250 μM)-adulterated alcohol (Fig. 3*A*, Table 2). Thus, prolonged alcohol consumption produced indifference to the aversive taste of quinine-adulterated alcohol. Total fluid intake during the test for indifferent alcohol drinking was not affected by quinine adulteration [$F_{(quinine)4,56} = 0.29$, N.S.] and was similar between the groups [$F_{(quinine \times group)4,56} = 2.2$, N.S., $F_{(group)1,14} = 1.4$, N.S.]. Moreover, the indifference to quinine observed after 8 weeks of alcohol consumption was not related to altered taste sensitivity, because quinine itself (when comparing intake of water vs. quinine-adulterated water) was equally aversive for mice that had consumed alcohol for 2 or 8 weeks (Fig. 3*B* and Table 3; baseline alcohol intake 2.6 ± 0.1 and 2.2 ± 0.2 g/kg/2 h, baseline alcohol preference 91 ± 5.2 and 94 ± 2.4%, and total fluid intake 29 ± 6.4 and 29 ± 2.4 ml/kg/2 h for mice after 2 and 8 weeks of alcohol consumption, respectively) and alcohol-naïve mice (Fig. 1*A*).

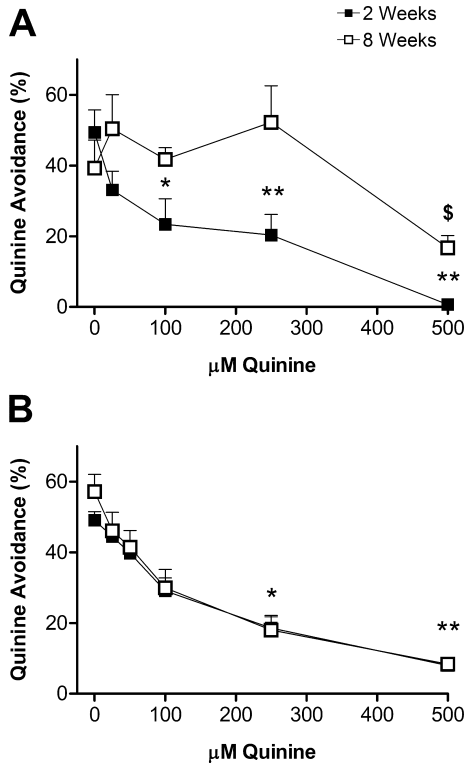

Fig. 3. C57BL/6J mice develop indifferent alcohol drinking. (**A**) C57BL/6J mice developed indifferent alcohol drinking in that they consumed aversive, quinine-adulterated alcohol, despite availability of unadulterated alcohol after 8 weeks alcohol experience. Quinine reduced alcohol intake [$F_{(quinine)4,56} = 9.9$, $p < 0.001$], but higher quinine concentrations were required to reduce alcohol intake in mice that had 8 weeks of alcohol experience when compared to mice with only 2 weeks of alcohol experience [$F_{(quinine \times time)\ 4,56} = 2.9$, $p < 0.05$]. Significant from 0 μM quinine by *t*-test: $^{*}p < 0.01$, $^{**}p < 0.05$ after 2 weeks and $^{\$}p < 0.05$ after 8 weeks of alcohol experience. (**B**) Quinine was equally aversive when provided in plain tap water after 2 or 8 weeks of alcohol experience [$F_{(quinine)5,75} = 72.3$, $p < 0.001$; $F_{(quinine \times time)5,75} = 0.84$, N.S.], $^{*}p < 0.05$, $^{**}p < 0.01$ from 0 μM quinine by *t*-test.

DISCUSSION

Alcoholism is characterized by compulsive alcohol intake, which can be apparent as continued drinking despite adverse consequences (American Psychiatric Association, 2000). To determine the genetic, behavioral, and neurobiological processes involved in the development of alcoholism, it is essential to implement rodent models with face validity to the human disease. Here, we demonstrate the rapid, staged development of 2 distinct behavioral characteristics of alcoholism, i.e., inflexible and indifferent alcohol drinking, in C57BL/6J mice.

Already after 2 weeks of daily alcohol consumption in the limited access choice paradigm, C57BL/6J mice develop inflexible drinking behavior. Rodents were previously shown to become insensitive to quinine adulteration, and we hypothesized that C57BL/6J mice would develop this aspect of alcoholism-like behavior, reflected by willingness to endure the bitter taste of quinine, after prolonged ethanol consumption in the limited access choice paradigm also. Indeed, C57BL/6J mice developed inflexible alcohol drinking, although the onset was more rapid than predicted from the literature (Fachin-Scheit et al., 2006; Wolffgramm, 1991). In addition to inflexible drinking behavior, prolonged alcohol consumption resulted in another behavioral characteristic of alcoholism that has not been previously reported for rodents: indifferent drinking. After 8 weeks of alcohol consumption, the mice no longer discriminated between alcohol and aversive, quinine-adulterated alcohol, even though their taste sensitivity was not altered as the mice still showed aversion for quinine when dissolved in water. This phenomenon shows remarkable parallels to the behavior of human alcoholics, who drink nonbeverage alcohol (e.g., eau de colognes and mouthwash) with a very aversive taste but a high alcohol content (Leon et al., 2007; Soo Hoo et al., 2003). The consumption of aversive tasting nonbeverage alcohols is illustrative of the persistent craving for and intake of alcohol in alcoholics, despite the negative consequences.

Inflexible alcohol drinking has previously been observed in rodents, albeit after very long episodes of ethanol consumption. Insensitivity to quinine adulteration was observed after 9 months of ethanol consumption followed by a 40-week deprivation interval in rats (Wolffgramm, 1991), and more recently, inflexible drinking behavior was reported for Swiss mice after 10 weeks of ethanol consumption using a 24-hour 2-bottle choice task (Fachin-Scheit et al., 2006; Ribeiro et al., 2008). Inflexible alcohol intake may be preceded by a phase where seeking of the drug is insensitive to devaluation, but its actual ingestion is not. It has been shown that responding for alcohol is insensitive to lithium-induced devaluation of alcohol after 2 weeks of operant training (Dickinson et al., 2002). However, the main difference from the quinine studies is that the animals responded in extinction, whereas the quinine adulteration studies assessed intake of the devalued ethanol solution itself. Indeed, in the lithium study, alcohol intake was sensitive to devaluation during acquisition and re-acquisition (Dickinson et al., 2002). Procedural differences (mice vs. rats, quinine vs. lithium, limited access drinking vs. operant responding) preclude a straightforward comparison of the different studies. However, when combined, they suggest that there are distinct phases in the development of alcoholism-like behavior with increasing alcohol experience: inflexible seeking followed by inflexible intake followed by indifferent intake of alcohol.

The present findings show that C57BL/6J mice very rapidly become insensitive to devaluation of the alcohol solution itself. Quinine adulteration inhibited the acquisition of alcohol consumption in alcohol-naïve C57BL/6J mice, indicating that C57BL/6J mice show initial sensitivity to quinine adulteration when this is the only source of alcohol, but this sensitivity to quinine was lost after only 2 weeks of alcohol drinking experience. Substantial differences in alcohol consumption between mouse inbred strains have been reported, and it is likely that inbred strains also differ in their susceptibility to develop compulsive drinking patterns. C57BL/6J mice are among the highest alcohol consuming and preferring mouse inbred strains (Belknap et al., 1993; Rhodes et al., 2007; Yoneyama et al., 2008), and by restricting access to alcohol to daily

2-hour sessions in a limited access choice paradigm, C57BL/6J mice show particularly high levels of alcohol intake and preference (Lesscher et al., 2009a,b). This implicates that C57BL/6J mice, using the limited access choice paradigm, require less time to reach high levels of exposure to alcohol compared to other inbred strains. In addition to their high alcohol intake, we here show that C57BL/6J mice are also highly susceptible to develop compulsive alcohol drinking patterns, i.e., inflexible and indifferent alcohol drinking. Inbred strains show marked differences in both basal and alcohol-induced gene expression (Kerns et al., 2005; Misra and Pandey, 2003; Treadwell and Singh, 2004), which may contribute to inter-strain variations in alcohol consumption. Moreover, multiple murine quantitative trait loci have been identified for ethanol intake and/or ethanol preference (Belknap and Atkins, 2001; Lesscher et al., 2009a). Further research is required to determine the genetic and neurobehavioral mechanisms that determine the innate susceptibility of C57BL/6J mice for compulsive alcohol drinking.

In summary, we demonstrate the subsequent development of 2 behavioral characteristics of alcoholism, i.e., inflexible and indifferent drinking. The emergence of 2 behavioral characteristics over time and more alcohol exposure suggests that the development of compulsive alcohol drinking occurs in several distinct phases. Perhaps this represents a gradual worsening of the addiction syndrome with increasing alcohol experience. The development of addiction is thought to be the consequence of drug-induced neural changes in limbic-corticostriatal circuits resulting in altered emotion processing, craving, and aberrant decision-making that together lead to compulsive patterns of drug intake (Everitt and Robbins, 2005; Kalivas and O'Brien, 2008; Koob and Volkow, 2009; Vanderschuren and Everitt, 2005). Indeed, chronic exposure to alcohol causes functional adaptations in the prefrontal cortex, nucleus accumbens, dorsal striatum, and amygdala that underlie critical aspects of the addiction syndrome (Grusser et al., 2004; Koob, 2003; Li et al., 2009; Makris et al., 2008; Martinez et al., 2005; Nestby et al., 1999; Schneider et al., 2001; Stephens et al., 2005; Volkow et al., 1996; Wrase et al., 2008). Our demonstration of rapid development of 2 distinct behavioral characteristics of alcoholism in mice therefore opens new avenues to study the neurobehavioral underpinnings of alcoholism.

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