Individual Variation in Alcohol Intake Predicts Reinforcement, Motivation, and Compulsive Alcohol Use in Rats

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Background: Alcohol is one of the most commonly used psychoactive substances. Prolonged alcohol use can result in alcohol use disorder (AUD), characterized by excessive and compulsive alcohol consumption. Importantly, however, the development of AUD only happens in a minority of individuals who consume alcohol. To understand the individual vulnerability for AUD, models that capture both the individual variability in alcohol consumption and the transition from casual to compulsive alcohol use are essential.

Methods: Individual variability in voluntary alcohol intake and the preference for alcohol were assessed under continuous alcohol access (CAA) and intermittent-every-other-day alcohol access (IAA) schedules in the home cage using outbred Lister Hooded rats. Subsequently, the reinforcing properties of alcohol were tested in an operant setting. In subsequent experiments, we performed a quinine adulteration experiment to assess inflexible alcohol consumption and blood alcohol levels (BALs) were assessed after voluntary alcohol consumption.

Results: We found marked individual differences in alcohol consumption and preference under both access schedules, whereby subgroups of high- and low-alcohol-drinking rats (HD and LD) could be identified. HD with IAA increased their alcohol intake over days in the first month, whereas LD did not. Moreover, when alcohol access time was extended from 7 to 24 h/d for rats with IAA, alcohol intake profoundly increased in HD with IAA, whereas LD with IAA maintained low levels of alcohol intake. Furthermore, HD earned more alcohol than LD under both fixed ratio and progressive ratio schedules of reinforcement. We further found that HD continued their intake of a quinine-adulterated alcohol solution to a larger extent than LD and HD showed higher BALs after 30 minutes of alcohol consumption.

Conclusions: These profound individual differences in alcohol intake, reinforcement, motivation, and AUD-like behavior provide a promising tool to unravel the neurobehavioral underpinnings of individual vulnerability for AUD.

Key Words: Addiction-Like Behavior, Alcohol Intake, Individual Differences, Rats, Reinforcement.

WITH APPROXIMATELY 2 billion current users worldwide, alcohol is among the most widely used substances of abuse (Anderson, 2006; WHO, 2011). Prolonged alcohol use can result in alcohol use disorder (AUD), a chronic relapsing disorder that is characterized by excessive alcohol intake and a compulsive engagement in alcohol use (American Psychiatric Association, 2013). Importantly, the development of AUD happens in a subpopulation of 3 to 5% of people who consume alcohol, affecting 76 million people worldwide (Anderson, 2006; Effertz and Mann, 2013; Rehm et al., 2009; WHO, 2011). This individual variability in the development of AUD is considered to result from an interaction between prolonged alcohol use, genetic predisposition, and psychosocial, cognitive, and environmental risk factors (Anderson, 2006; Chassin et al., 2002; Enoch, 2013; Goudriaan et al., 2011). Given its medical, societal, and economic burden (Effertz and Mann, 2013) and the limited number of effective treatment strategies for AUD (van den Brink, 2012; Pierce et al., 2012), it is critical to investigate the mechanisms that underlie individual vulnerability for AUD.

An increasing number of preclinical models have been developed to assess AUD-like behavior in rodents (Crabbe et al., 2009; Lesscher et al., 2009; Simms et al., 2008; Wolffgramm and Heyne, 1991). Rodents voluntarily consume

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more alcohol in paradigms with intermittent alcohol access (IAA) or repeated alcohol deprivations, compared to models with continuous alcohol access (CAA) (Cippitelli et al., 2012; Hwa et al., 2011; Loi et al., 2010; Sabino et al., 2013; Simms et al., 2008; Wise, 1973). Moreover, IAA induces a transition from moderate to escalated alcohol intake, a critical feature of AUD. Another important hallmark of human AUD is the continued use of alcohol despite adverse consequences (American Psychiatric Association, 2013); this has been captured in preclinical models of continued use in the face of adversity in which (conditioned) footshocks, bitter taste, or lithium chloride-induced sickness serve as aversive stimuli (Chen et al., 2013; Hopf and Lesscher, 2014; Hopf et al., 2010; Turyabahika-Thyen and Wolffgramm, 2006; Vanderschuren and Ahmed, 2013). For example, rats and mice with extended exposure to IAA develop resistance to quinine modulation of alcohol intake, indicative of inflexible alcohol consumption (Hopf et al., 2010; Lesscher et al., 2010; Wolffgramm and Heyne, 1991).

Individual differences in alcohol use have been documented in human and preclinical studies (Chassin et al., 2002; Goudriaan et al., 2007; Hayton et al., 2012; Hwa et al., 2011; Sabino et al., 2013; Simms et al., 2008) and several rodent lines have been bred for their differences in alcohol consumption (Colombo et al., 1995; Crabbe et al., 2009; Le et al., 2001; Li and McBride, 1995; Sinclair et al., 1989). For example, studies have examined whether individual differences in anxiety-related behaviors in outbred populations predict high alcohol consumption or vice versa (Bahi, 2013; Hayton et al., 2012; Sharko et al., 2013; Spanagel et al., 1995). However, individual differences in alcohol consumption have not been related to individual differences in alcohol reinforcement and AUD-like behavior in outbred rodents. In this study, we therefore assessed individual differences in alcohol intake in outbred rats under IAA and CAA conditions. Subsequently, we assessed whether individual variability in alcohol intake relates to operant alcohol self-administration, as well as the resistance to quinine modulation of alcohol intake. Knowledge about alcohol reinforcement and AUD-like behavior in selected high- versus low-alcohol-drinking rats will facilitate the investigation of the neurobehavioral mechanisms underlying the individual risk for AUD.

MATERIALS AND METHODS

Animals

Male Lister Hooded rats, obtained from Harlan (Horst, the Netherlands; Experiment 1) or Charles River (Sulzfeld, Germany; Experiment 2 and 3), weighing 220 to 250 g (~7 to 9 weeks old) on arrival in our laboratory were used. Rats were housed individually under controlled temperature and humidity conditions and a reversed 12-hour light/dark cycle (lights off 7.00 AM) with ad libitum access to water and chow. Rats were acclimatized to the housing conditions for 2 weeks and were weighed and handled at least once per week. All experiments were approved by the Animal Ethics Committee of Utrecht University and conducted in agreement with Dutch laws (Wet op de dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Experiment 1

IAA Versus CAA. Rats were given either continuous ($n = 20$) or intermittent ($n = 20$) access to alcohol and water in a 2-bottle choice setup in the home cage. For CAA, alcohol was presented for 24 h/d, 7 days a week for 2 consecutive months. For IAA, alcohol was presented 3 days a week (Monday–Wednesday–Friday) for 7 h/d between 9.00 AM and 16.00 PM (i.e., during the dark phase) in the first month; access was extended to 24 h/d in the second month (Fig. 1). On alcohol drinking days, the rats were presented with 2 bottles, fitted with stainless steel dual ball-bearing drinking spouts, containing 20% alcohol (v/v) (Klinipath, Duiven, the Netherlands) or water. Bottles were weighed before and after each session. In addition, the bottles of rats with CAA were weighed on Monday–Wednesday–Friday after 7 hours of access in the first month, to compare their intake with rats with IAA. Alcohol intake and preference were calculated per rat per session and averaged per week, that is, 3 sessions per week for IAA and 7 sessions per week for CAA, or per month, that is, 12 sessions for IAA and 28 sessions for CAA. Alcohol was freshly diluted with tap water once per week to a final concentration of 20% (v/v). Bottle positions were switched between sessions (IAA) or days (CAA) to avoid side bias. After 2 months, the rats were divided into low-, medium-, and high-alcohol-drinking rats. To select rats that consistently consumed low or high levels of alcohol throughout the experiment, rats were ranked from low to high based on the animals' average alcohol intake per week and were assigned ranking scores. These weekly ranking scores were summed over the 2 months of the experiment to calculate a total ranking score. This was performed separately for the IAA and CAA groups. Rats within the lower and upper 25% of the total ranking score were designated as low- and high-alcohol-drinking rats (LD and HD), respectively. The median 50% of the population (medium-alcohol-drinking rats; MD) were used in other experiments (not presented here).

Alcohol Self-Administration Under Fixed Ratio and Progressive Ratio Schedules of Reinforcement. HD and LD were subsequently trained and tested in operant conditioning chambers (29.5 cm L, 24 cm W 25 cm H; Med Associates, Georgia, VT), situated in light- and sound-attenuating cubicles equipped with a ventilation fan. Each chamber was equipped with two 4.8-cm-wide retractable levers, placed 11.7 cm apart and 6 cm from the grid floor. A cue light (28 V, 100 mA) was present above each lever, a liquid dipper was in a magazine between the levers and a house light (28 V, 100 mA) was located on the opposite wall. The position of the active and inactive levers was counterbalanced between rats. Pressing the active lever raised the dipper cup containing alcohol (0.1 ml, 20% v/v), illuminated the cue light above the active lever, and switched off the house light. Access to alcohol was terminated 10 seconds after a head entry into the magazine, the cue light was turned off, and after a 5-second interval, a new trial started. Pressing the inactive lever was recorded, but had no programmed consequences. The rats were tested 5 days a week. Alcohol consumption during operant behavior was calculated by weighing the alcohol container underneath the dipper cup before and after each 60-minute session; the alcohol solution was refreshed before each session of each rat. Experimental events and data recording were controlled using MED-PC (Med Associates) for Windows.

Rats were habituated to the operant chamber for two 30-minute sessions during which 15 alcohol rewards were freely available every other minute. Thereafter, the rats were trained under a fixed ratio 1 (FR1) schedule of reinforcement. As soon as the animals had acquired responding (i.e., at least 10 rewards in 3 subsequent ses-

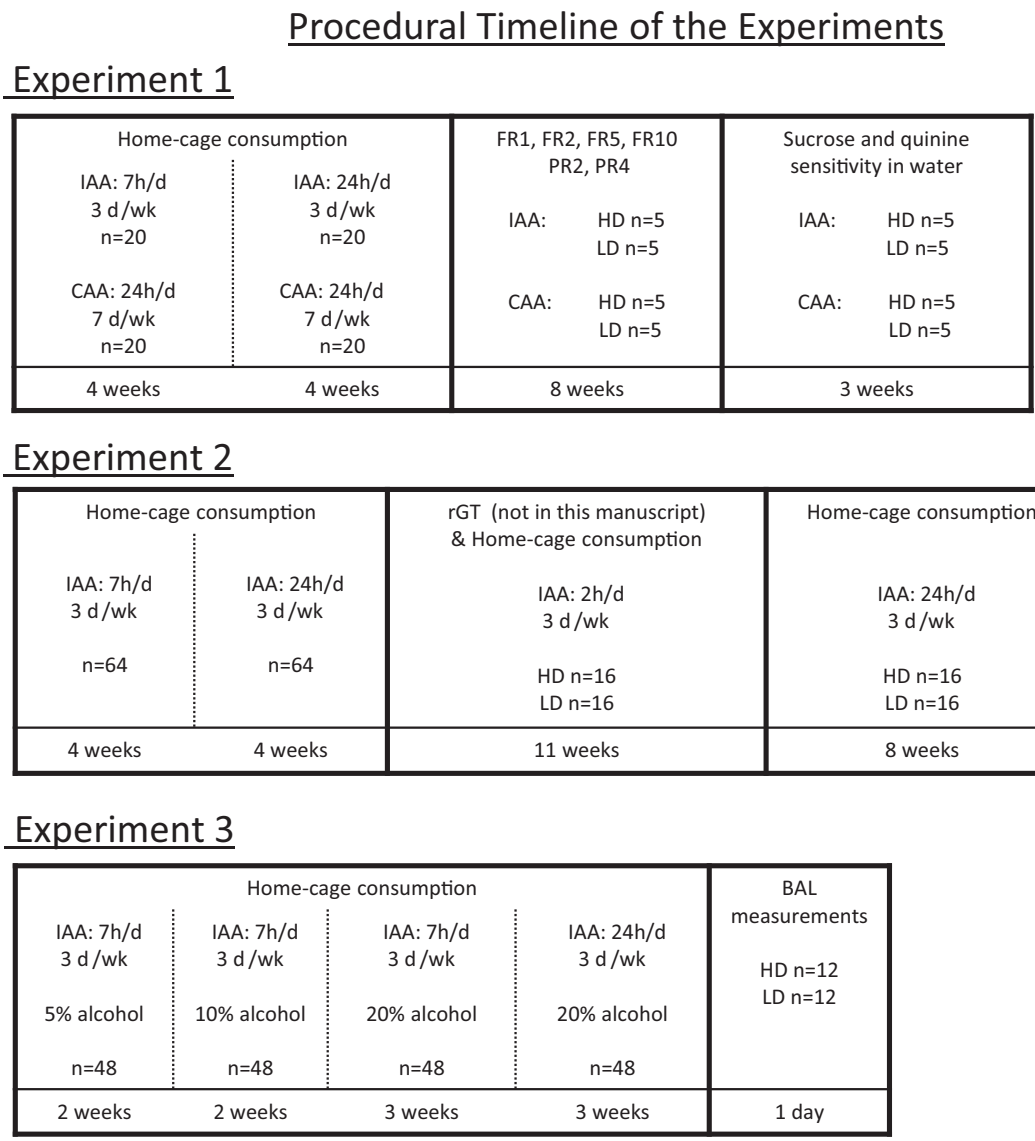

Fig. 1. Subgroups of high- and low-alcohol-drinking rats (HD and LD) (25% of upper and lower part of distribution) were selected based on the level of alcohol intake in the home cage during 8 weeks (Experiments 1 and 2) or 10 weeks (Experiment 3).

sions under the FR1 schedule), the response requirement was increased to FR2, FR5, and FR10 during which the animals had to earn at least 10 rewards for 2 to 3 sessions before progressing to the next FR or progressive ratio (PR) schedule. Based on the results of previous studies, a linear PR schedule of reinforcement was used, in which 2 (PR2; i.e., 2, 4, 6, 8, 10, etc.) and subsequently 4 (PR4; i.e., 4, 8, 12, 16, 20, etc.) additional lever presses were required for each subsequent reward (Brown et al., 1998; Ritz et al., 1994; Rodd et al., 2003). Responding under the PR schedules was deemed stable when there was <25% variation in reward deliveries over 3 subsequent sessions. The breakpoint under the PR schedules was defined as the maximum number of presses performed in the last, successfully completed ratio in either the 1-hour session or when no reward had been obtained in 20 minute. Responding for alcohol was analyzed in 10-minutes bins to investigate the pattern of responding during the operant session.

Quinine Avoidance and Sucrose Preference Tests. To assess taste sensitivity of the HD and LD, the rats received 2-bottle choice tests. For sucrose preference, the rats were offered 1 bottle containing tap water and 1 bottle with graded concentrations of sucrose (0 to 5% w/v) in tap water for 2 hours. For quinine avoidance, the rats were presented with 1 bottle containing tap water and 1 bottle with graded concentrations of quinine (0 to 1.0 g/l; Sigma-Aldrich, Schnelldorf, Germany) in tap water for 24 hours and measurements were taken after 2 and 24 hours. The bottles were weighed prior to and after each session that started at 9:00 AM. Each concentration was offered for 2 consecutive days, and bottle positions were switched between sessions to avoid side bias. Sucrose preference and quinine avoidance were calculated as the percentage of sucrose/quinine consumption of the total fluid intake.

Experiment 2

Quinine Modulation of Alcohol Intake. The effects of quinine adulteration were assessed as previously described (Lesscher et al., 2010) in a second group of HD ($n = 16$) and LD ($n = 16$). Subgroups were selected as described above. These rats received IAA for 2 months, subsequently served as subjects in a decision-making task where they received IAA for 2 hours access/session, and were

thereafter re-exposed to IAA with 24 hours access/session for 8 weeks before the onset of the quinine modulation experiment (Fig. 1). The alcohol solution was adulterated with increasing concentrations of quinine (0 to 1.0 g/l). Each concentration was tested once, bottle positions were switched between sessions to avoid side bias, and bottles were weighed after 24 hours.

Experiment 3

Blood Alcohol Levels. Blood alcohol levels (BALs) following voluntary alcohol intake were determined in a third group of HD ($n = 12$) and LD ($n = 12$) (Fig. 1). Subgroups were selected as described above, with the exception that these animals were exposed to graded alcohol concentrations in the IAA paradigm over 10 consecutive weeks: 2 weeks 5% v/v (7 h/d), 2 weeks 10% v/v (7 h/d), and 20% v/v (3 weeks 7 h/d and 3 weeks 24 h/d). Blood samples were collected from the lateral tail vein, immediately after 30 minutes access to alcohol (20% v/v) in the home cage, into EDTA-coated capillary tubes (Sarstedt, Numbrecht, Germany) and immediately stored on ice. Blood samples were spun at 3,000 rpm for 20 minutes (at 4°C), and plasma was stored at −20°C until blood alcohol analysis. BALs (mg/dl) were determined using an NAD-ADH reagent kit (Sigma-Aldrich) and a standard curve for quantitation (Lesscher et al., 2009).

Statistical Analysis

Two rats failed to maintain responding for alcohol during operant training, blood collection was unsuccessful for 4 rats, and 3 rats had 1 unreliable measurement of water/alcohol consumption during the quinine adulteration experiment; these rats were excluded from the concerning analyses. For analyses of operant behavior, the alcohol intake, number of lever presses, and breakpoints were averaged over the 3 sessions during which the rat reached the response criteria as described for Experiment 1. Data were analyzed using 1-, 2-, and 3-way repeated-measures analyses of variance with time, quinine, and sucrose concentrations as within-subject variables and alcohol access condition (IAA vs. CAA) and/or subgroup (HD vs. LD) as between-subject variables. Each parameter was tested for normality with a Kolmogorov–Smirnov test. Mauchly's test of sphericity was used to test whether variances of the differences between treatment levels were equal. If the assumption of sphericity was violated, degrees of freedom were corrected using Huynh–Feldt estimates of sphericity. When appropriate, post hoc analyses were conducted using Student's *t*-tests and paired *t*-tests. A nonparametric Mann–Whitney *U*-test for group comparisons was used when a certain variable was not normally distributed. The threshold for statistical significance was set at $p < 0.05$. All data are presented as mean ± SEM. Statistical analyses were conducted using SPSS 20.0 for Windows (IBM Corp., Armonk, NY).

RESULTS

Experiment 1

Home Cage Alcohol Intake and Preference—IAA Versus CAA. Alcohol intake and preference changed over the course of the first 4 weeks, but differently for rats with IAA and CAA (intake: $F(3, 105)$ week × access = 14.56, $p < 0.001$; preference: $F(3, 114)$ week × access = 7.17, $p < 0.001$) (Fig. 2*A*,*B*). Rats with CAA consumed more alcohol compared to rats with IAA during the first 2 weeks, but both groups consumed similar levels of alcohol in weeks 3 and 4. Because rats with IAA had access to alcohol for 7 h/d, and rats with CAA for 24 h/d, we also measured alcohol intake and preference for the rats with CAA over the first 7 hours of each session on Monday–Wednesday–Friday, in parallel to the IAA group. During these 7 hours, rats with IAA consumed a similar amount of alcohol in weeks 1 to 2 but consumed more alcohol than rats with CAA in weeks 3 to 4, $F(3, 114)$ week × access = 6.42, $p < 0.001$ (Fig. 2*A*,*B*). Moreover, rats with IAA showed a higher preference for alcohol in weeks 2 to 4, $F(3, 114)$ week × access = 6.67, $p < 0.001$, compared to rats with CAA. Rats with IAA consumed more alcohol, $F(1, 38)$ access = 8.17, $p = 0.007$, and showed a greater preference for alcohol, $F(1, 38)$ access = 7.52, $p = 0.009$, during the second month, when both groups had access to alcohol for 24 h/d (Fig. 2*A*,*B*).

Individual Differences in Home Cage Alcohol Consumption. We observed marked individual differences in alcohol intake and preference between the animals, which were most pronounced in rats subjected to IAA. The alcohol intake of rats with IAA in Experiment 1 ranged from 0.64 to 2.32 g/kg/7 h (mean ± SEM: 1.39 ± 0.10) and 0.50 to 4.84 g/kg/24 h (mean ± SEM: 1.93 ± 0.27), whereas the alcohol intake of rats with CAA ranged from 1.33 to 2.23 g/kg/24 h (mean ± SEM: 1.65 ± 0.06) and 0.52 to 1.99 g/kg/24 h (mean ± SEM: 1.09 ± 0.12) in the first and second month, respectively. Analyses of the alcohol intake and preference of the HD, MD, and LD confirmed differences between the selected subgroups in rats with IAA, $F(2, 17)$ group = 30.60, $p < 0.001$; $F(2, 17)$ group = 20.69, $p < 0.001$, respectively, and CAA, $F(2, 17)$ group = 24.50, $p < 0.001$; $F(2, 17)$ group = 25.40, $p < 0.001$, respectively (Fig. 2*C*–*F*). When comparing the alcohol intake of the first month to the second month, the subgroups with IAA responded differently to the increase in alcohol access duration (7 to 24 h/d), $F(2, 17)$ month × group = 12.70, $p < 0.001$; HD with IAA increased their intake when access time was extended, while the LD and MD subgroups did not (Fig. 2*C*). Alcohol preference was not changed in HD with IAA, while MD and LD showed a reduction in alcohol preference upon the increment in session duration, $F(2, 17)$ month × group = 8.14, $p = 0.003$ (Fig. 2*D*). Rats with CAA showed a trend for differential alcohol consumption between subgroups over time, $F(2, 17)$ month × group = 3.14, $p = 0.069$, and overall alcohol intake declined in the second month, $F(1, 17)$ month = 48.62, $p < 0.001$ (Fig. 2*E*). The alcohol preference of rats with CAA declined over time in LD but not in MD and HD, $F(2, 17)$ month × group = 4.61, $p = 0.025$ (Fig. 2*F*). There were no differences in total fluid intake between the subgroups with IAA, $F(2, 17)$ group = 0.33, $p = 0.726$, but total fluid intake was different between CAA subgroups, $F(2, 17)$ group = 4.54, $p = 0.026$; HD consumed less fluid than MD ($p < 0.05$) (data not shown).

Alcohol Self-Administration Under FR and PR Schedules of Reinforcement. After 2 months of home cage alcohol con-

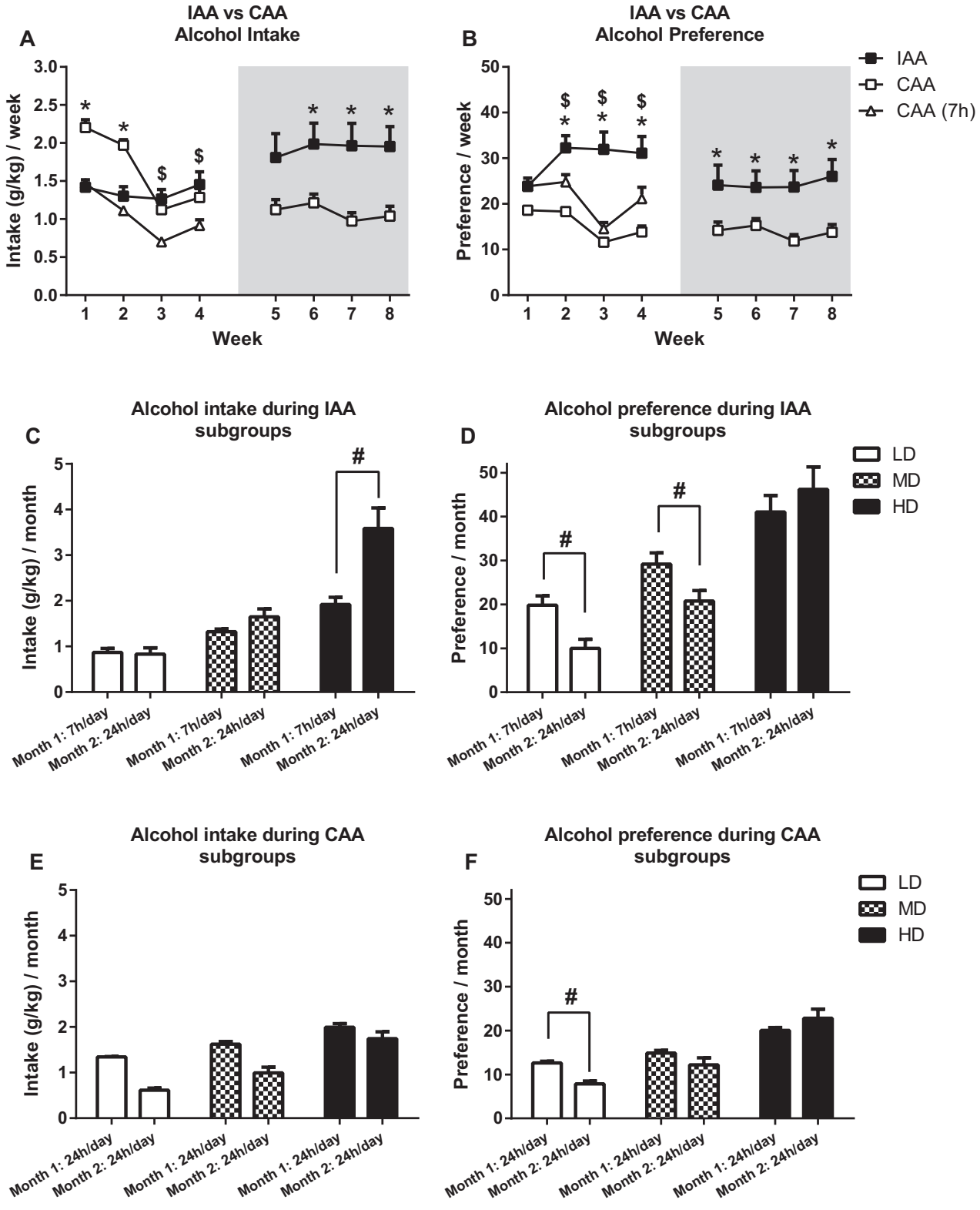

Fig. 2. Alcohol intake and preference during intermittent alcohol access (IAA) versus continuous alcohol access (CAA) in the home cage. (**A, B**) Alcohol intake (**A**) and preference (**B**) differed between rats with IAA and CAA, in both the first month (white area) and second month (gray area). (**C, D**) High-alcohol-drinking rats (HD) with IAA increased their alcohol intake when access to alcohol was extended (**C**) and retained similar alcohol preference over 2 months (**D**). (**E, F**) All subgroups with CAA reduced their alcohol intake from the first to the second month (**E**), but HD and medium-alcohol-drinking rats retained a similar alcohol preference over both months (**F**). Data are shown as mean ± SEM average alcohol intake and preference per week (**A, B**) or month (**C**–**F**). *Significant differences between IAA and CAA (24-hour measurement); $^{\$}$significant differences between IAA and CAA (7-hour measurement) (post hoc Student's *t*-tests, $p < 0.05$); $^{\#}$significant differences within the subgroup (post hoc paired *t*-tests, $p < 0.05$).

sumption under IAA or CAA conditions, HD and LD were trained to self-administer alcohol. LD required more FR training sessions (15 ± 1.0) to fulfill the response requirements to proceed to the PR schedules than HD (11 ± 0.5), $F(1, 14)$ group = 6.70, $p = 0.021$, independent of access condition (IAA or CAA, $F(1, 14)$ access = 1.14, $p = 0.304$) (data not shown). Responding under the FR1, $F(1, 14)$ access = 0.14, $p = 0.712$; $F(1, 14)$ access × group = 2.07, $p = 0.172$, PR2, $F(1, 14)$ access = 0.35, $p = 0.567$; $F(1, 14)$ access × group = 0.42, $p = 0.530$, and PR4 schedules, $F(1, 14)$ access = 0.04, $p = 0.849$; $F(1, 14)$ access × group = 0.21, $p = 0.654$, as well as breakpoint under the PR2 and PR4 schedules, PR2: $F(1, 14)$ access = 0.35, $p = 0.566$; PR4: $F(1, 14)$ access = 0.08, $p = 0.783$, and the alcohol consumed during the operant sessions, FR1: $F(1, 14)$ access = 0.33, $p = 0.575$; PR2: $F(1, 14)$ access = 0.15, $p = 0.704$; PR4: $F(1, 14)$ access = 0.36, $p = 0.559$, did not differ between CAA and IAA rats (data not shown); data from these groups were therefore collapsed. Under an FR1 schedule of reinforcement, HD made more active lever presses than LD, $F(1, 16)$ group = 6.54, $p = 0.021$. Responding declined in a similar manner for both HD and LD during the session, $F(3, 40)$ group × time = 0.74, $p = 0.515$ (Fig. 3*A*). Likewise, under a PR2 schedule of reinforcement, HD showed higher response levels than LD, $F(1, 16)$ group = 7.44, $p = 0.015$, and lever pressing declined in a similar manner for both groups, $F(4, 69)$ group × time = 1.65, $p = 0.167$ (Fig. 3*B*). Analysis of the PR4 data revealed an interaction between group and session time, $F(5, 80)$ group × time = 4.32, $p = 0.002$, but no main effect of HD versus LD, $F(1, 16)$ group = 2.32, $p = 0.148$. Post hoc analyses showed that HD made more lever presses compared to LD in the first 10 minutes of the task ($p < 0.05$) (Fig. 3*C*). HD reached higher breakpoints than LD under the PR2 schedule of reinforcement, $F(1, 16)$ group = 6.85, $p = 0.019$, and there was a trend toward a higher breakpoint in HD under the PR4 schedule, $F(1, 16)$ group = 4.08, $p = 0.060$ (Fig. 3*E*,*F*). The number of rewards obtained correlated with the amount of alcohol consumed for the FR1 schedule of reinforcement (Fig. 3*D*), but not for PR2 and PR4 schedules

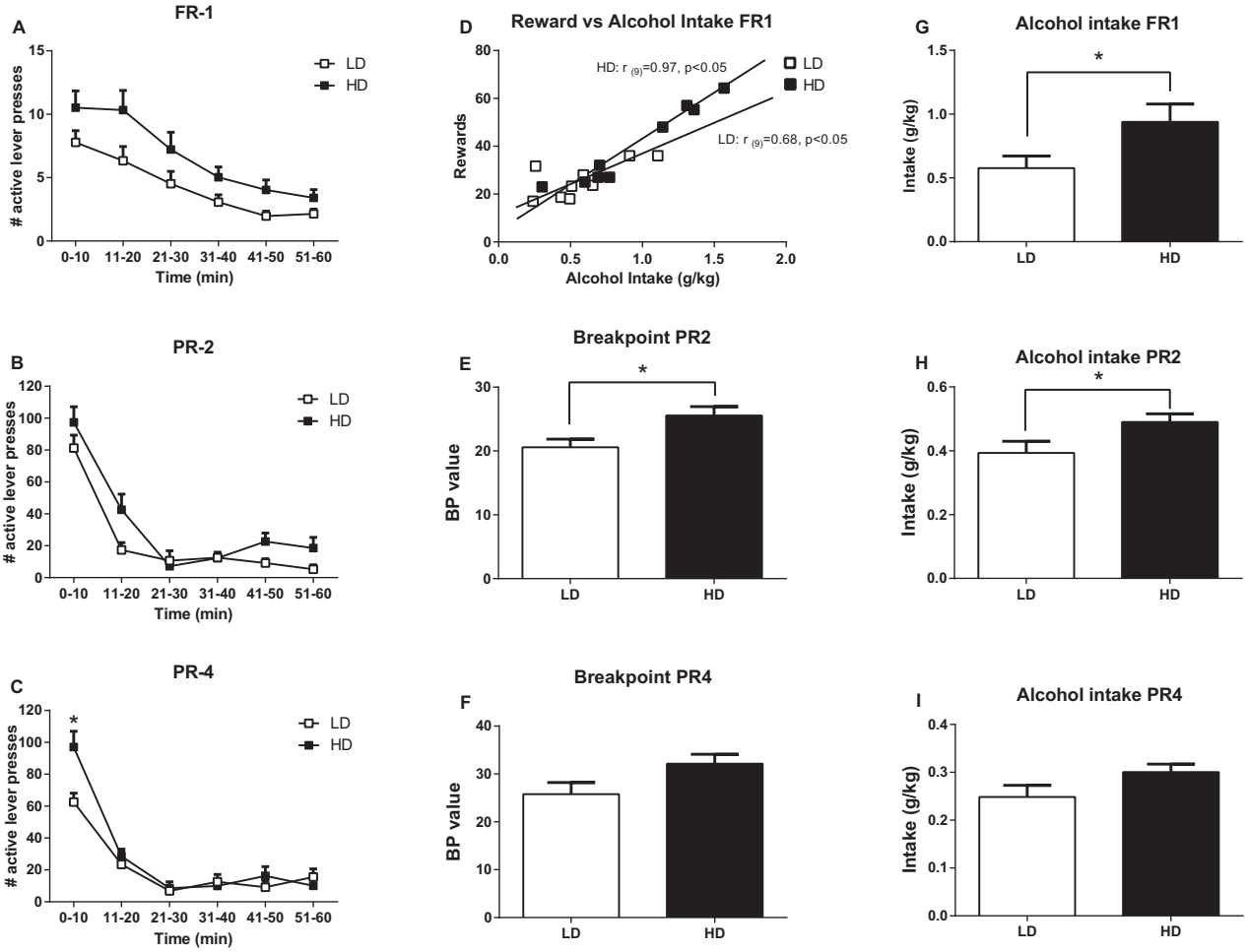

Fig. 3. Operant responding for alcohol by LD and HD under FR1 and PR schedules of reinforcement. (**A**–**C**) Number of active lever presses under the FR1 and PR schedules. (**D**) The number of earned rewards correlated with alcohol intake (g/kg) during the FR1 sessions. (**E, F**) HD showed a higher breakpoint during the PR2 schedule, and a trend toward a higher breakpoint under the PR4 schedule. (**G**–**I**) HD consumed more alcohol than LD under the FR1 and PR2 schedules, but not under the PR4 schedule. Data are shown as mean ± SEM. *Significant between-subgroup differences (post hoc Student's *t*-tests, $p < 0.05$). FR1, fixed ratio 1; HD, high-alcohol-drinking rats; LD, low-alcohol-drinking rats; PR, progressive ratio.

(data not shown). Analysis of alcohol intake during operant self-administration showed higher alcohol intakes in HD versus LD under the FR1 and PR2 schedule, FR1: $F(1, 16)$ access = 4.56, $p = 0.049$; PR2: $F(1, 16)$ access = 4.74, $p = 0.045$, but not for the PR4 schedule, $F(1, 16)$ access = 3.15, $p = 0.095$ (Fig. 3*G–I*). Active lever press and reward collection latencies did not differ between groups on any of the reinforcement schedules (Table S1).

Sucrose Preference and Quinine Avoidance. To rule out the possibility that the differential alcohol intake and motivation in HD and LD is a result of altered taste sensitivity, the animals were subsequently tested for sucrose preference and quinine avoidance in a 2-bottle choice test. Sucrose preference did not differ between HD and LD, $F(6, 95)$ concentration × group = 0.42, $p = 0.856$, nor between rats with previous IAA and CAA exposure, $F(3, 95)$ concentration × access = 0.44, $p = 0.713$ (Table 1). Quinine aversion was not different for HD and LD, after 2 hours: $F(6, 102)$ concentration × group = 0.46, $p = 0.835$; after 24 hours: $F(3, 59)$ concentration × group = 1.25, $p = 0.300$ (Table 1). Quinine aversion was comparable for rats with previous IAA or CAA exposure after 2 hours exposure, $F(3, 102)$ concentration × group = 1.35, $p = 0.262$, although there was a significant interaction with the subgroups after 24 hours exposure, $F(2, 59)$ concentration × group = 3.41, $p = 0.049$. However, post hoc tests only revealed a significant difference between access groups for the 0 g/l quinine concentration ($p < 0.05$).

Experiment 2

Quinine Modulation of Alcohol Intake. To determine whether HD show inflexible alcohol consumption, that is, continued intake of an aversive, quinine-containing alcohol solution, we performed a quinine adulteration experiment in a separate group of rats with a history of IAA. Analysis of the alcohol intake of LD and HD in the first 2 months with IAA indicated that HD increased their alcohol over days in the first month with 7 hours alcohol access/d, while LD did not, $F(7, 207)$ day × group = 8.73, $p < 0.001$ (Fig. 4*A*). Consistent with the first experiment, HD increased their alcohol intake to a larger extent than LD when comparing alcohol intake between the first and second month, $F(1, 30)$ month × group = 95.13, $p < 0.001$. Subgroup differences in alcohol intake persisted during the subsequent 2-hour IAA sessions, $F(1, 30)$ group = 70.11, $p < 0.001$, as well as during 24-hour IAA re-exposure, $F(1, 30)$ group = 46.59, $p < 0.001$, prior to the start of the adulteration experiment (data not shown).

Analysis of the quinine adulteration data showed a significant interaction between quinine concentration and subgroup for both alcohol intake, $F(6, 155)$ concentration × group = 11.31, $p < 0.001$, and alcohol preference, $F(6, 162)$ concentration × group = 4.84, $p < 0.001$, indicative of a differential sensitivity to quinine adulteration in HD and LD (Fig. 4*B*,*C*). LD decreased their alcohol intake and preference at quinine concentrations of 0.01 g/l and higher ($p < 0.03$), whereas HD only decreased their alcohol intake and preference at 10-fold higher quinine concentrations (i.e., 0.1 g/l and higher, $p < 0.002$). During the experiment, HD retained higher levels of alcohol intake and alcohol preference compared to LD, intake: $F(1, 27)$group = 30.99, $p < 0.001$, preference: $F(1, 27)$group = 23.32, $p < 0.001$, at all, except for the 2 highest, quinine concentrations.

Experiment 3

BALs in HD and LD. BALs after alcohol consumption were assessed in a third group of LD and HD. HD increased their alcohol intake to a larger extent compared to LD upon the increment in alcohol concentration during the 7-hour sessions, $F(10, 217)$ day × group = 6.25, $p < 0.001$ (Fig. 5*A*). Similar to the previous experiments, the alcohol intake of the 20% alcohol concentration increased to a larger extent in HD as compared to LD as

Table 1. Sucrose Preference and Quinine Avoidance (%) in High- and Low-Alcohol-Drinking Rats (HD and LD). Volumes were measured 2 or 24 Hours After Presentation of the Bottles

		Sucrose			
Group	Substance: Concentration (% w/v):	0	0.1	1.0	5.0
HD	2 hours	50.1 ± 5.1	49.9 ± 3.3	81.3 ± 2.7*	95.1 ± 0.8*
LD	2 hours	46.2 ± 4.1	55.5 ± 3.0	78.0 ± 4.2*	95.1 ± 0.9*
		Quinine			
	Substance: Concentration (g/l):	0	0.1	0.3	1.0
HD	2 hours	52.7 ± 4.2	37.0 ± 3.4*	30.6 ± 2.8*	31.7 ± 4.7*
LD	2 hours	52.9 ± 2.6	40.2 ± 1.7*	32.2 ± 2.7*	34.2 ± 2.5*
HD	24 hours	54.2 ± 3.3	19.0 ± 1.1*	16.3 ± 1.0*	15.6 ± 0.8*
LD	24 hours	52.9 ± 3.8	18.4 ± 1.0*	14.1 ± 0.8*	14.9 ± 0.7*

Values represent mean ± SEM.

*Significantly different from 0% w/v or 0 g/l (paired *t*-test), $p < 0.05$. Previous intermittent alcohol access (IAA) or continuous alcohol access (CAA) in the home cage did not interact with the subgroups; therefore, the results of the subgroups with previous IAA or CAA were pooled.

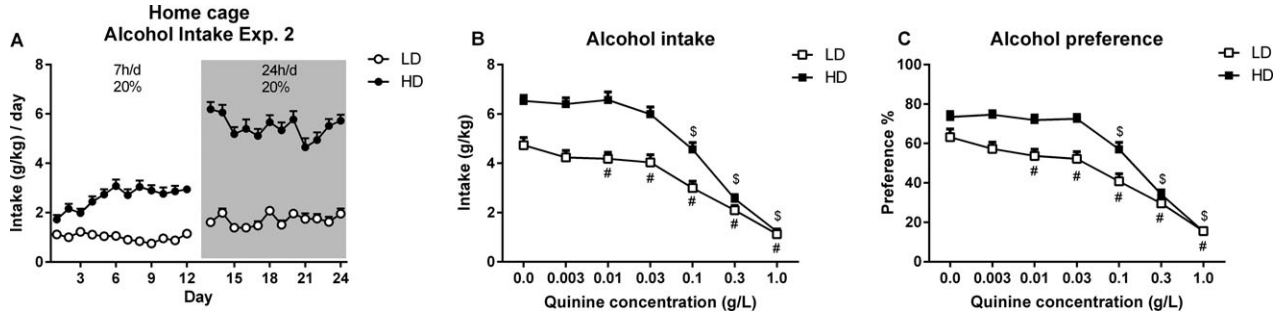

Fig. 4. Alcohol intake and preference of low- and high-alcohol-drinking rats (LD and HD) during the quinine adulteration experiment. (**A**) Alcohol intake in HD and LD during the first 2 months of home cage consumption. (**B, C**) LD decreased their alcohol intake and preference at lower quinine concentrations (i.e., >0.01 g/l) as compared to HD (i.e., >0.1 g/l). Data are shown as mean ± SEM. #Significantly different from 0.0 g/l in LD; $significantly different from 0.0 g/l in HD (post hoc paired *t*-test, $p < 0.05$).

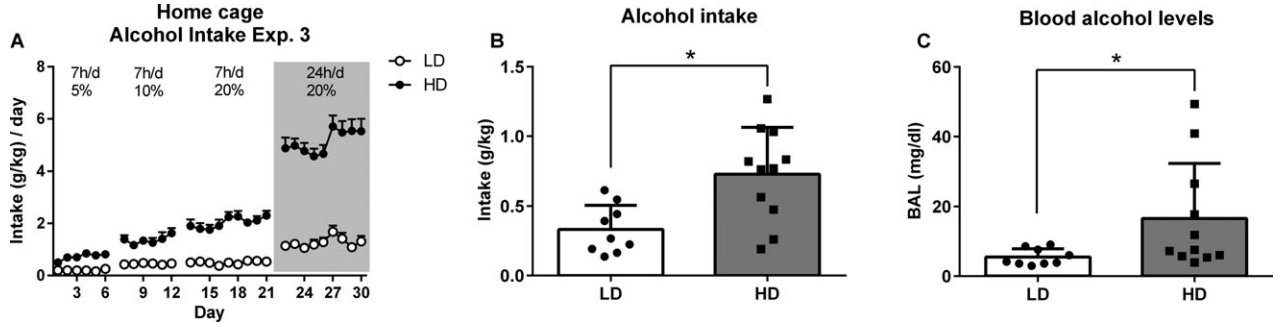

Fig. 5. Alcohol intake and corresponding blood alcohol levels (BALs) of low- and high-alcohol-drinking rats (LD and HD) after 30 minutes of home cage alcohol access. (**A**) Alcohol intake in HD and LD during 10 weeks of home cage consumption. (**B**) Alcohol intake and (**C**) BALs were higher in HD compared LD. Data are shown as mean ± SEM for **A**, and individual data points and the mean ± SEM are presented in **B** and **C**. *Significant group differences (Student's *t*-test (**B**) and Mann–Whitney *U*-test (**C**), $p < 0.05$).

session duration increased from 7 hours to 24 h/d, $F(7, 151)$ day × group = 16.41, $p < 0.001$ (Fig. 5*A*). HD showed a higher alcohol intake and preference than LD during the 30 minutes of alcohol access before blood sampling, $t(18)$ intake = 3.23, $p = 0.005$; $t(18)$ preference = 3.10, $p = 0.006$ (Fig. 5*B*), which also resulted in higher BALs in HD compared to LD, $U = 23$, $p = 0.046$ (Fig. 5*C*). Moreover, BALs correlated with alcohol intake, $r(18) = 0.60$, $p = 0.005$.

DISCUSSION

In this study, we observed marked individual differences in voluntary alcohol intake and preference in outbred Lister Hooded rats. The subgroup of the HD with IAA escalated their alcohol intake upon extension of the alcohol access duration. Moreover, HD showed greater alcohol reinforcement and motivation to obtain alcohol and they continued to consume alcohol despite an aversive taste to a greater extent than LD. These findings show that HD develop compulsive characteristics of alcohol use, a hallmark of AUD in humans.

Chronic Versus Intermittent Alcohol Exposure in Rodents

The IAA paradigm produces higher levels of alcohol intake in rodents in comparison with CAA (Cippitelli et al., 2012; Hwa et al., 2011; Loi et al., 2010; Sabino et al., 2013; Simms et al., 2008; Wise, 1973). Consistent with these studies, we found greater alcohol intake in rats with IAA compared to CAA. Rats with CAA reduce their alcohol intake after 2 weeks of alcohol access, which has been observed previously (Cippitelli et al., 2012), but not consistently so (Colombo et al., 1995; Loi et al., 2010; Sabino et al., 2013; Wise, 1973). It is assumed that intermittent exposure to alcohol increases the rewarding properties of alcohol, which may facilitate the development of AUD (Brown et al., 1998; O'Dell et al., 2004; Rodd et al., 2003). We observed no differences between rats with previous home cage IAA or CAA in operant responding for alcohol, which may be explained by the fact that during operant self-administration for 5 d/wk, rats were exposed to similar amounts of alcohol, thereby reducing potential group differences over sessions. Moreover, cumulatively, the rats with CAA have consumed more alcohol during home cage alcohol access compared to rats with IAA, which may explain why the motivation to obtain alcohol was not different for rats with IAA and CAA.

Individual Differences in Alcohol Intake and Reinforcement

In this study, we consistently observed a high degree of individual variability in alcohol intake in outbred Lister Hooded rats, which was more pronounced under IAA than

under CAA conditions. These individual differences in alcohol intake were highly consistent across 3 batches of animals from 2 different vendors (Table S2). To assess individual differences within a population of Lister Hooded rats, rats were classified into subgroups of LD and HD based on their alcohol consumption in the home cage. A potential limitation of this approach is that by excluding the MD, the data may not be subjected to linear regression analyses. Nevertheless, the differences in alcohol reinforcement, motivation, and loss of control over alcohol use between the selected subgroups provide valuable information about individual differences in the risk of AUD.

During the first month with 7-hour IAA sessions, the HD gradually increased their alcohol intake over time, whereas the LD did not. Moreover, subsequent increases in alcohol access duration from 7 to 24 h/d in the second month led to a larger increase in alcohol intake in HD compared to LD. Thereafter, in agreement with other IAA studies, alcohol intake stabilized, which suggests that animals titrate their alcohol consumption to a preferred level of intoxication (Cippitelli et al., 2012; Loi et al., 2010; Sabino et al., 2013; Simms et al., 2008). Individual differences in alcohol intake in outbred rodent populations have been related to certain behavioral factors (e.g. anxiety and decision making) (Bahi, 2013; Hayton et al., 2012; McMurray et al., 2014; Sharko et al., 2013; Spanagel et al., 1995), but have not directly been related to alcohol reinforcement and AUD-like behaviors. Interestingly, the current data show that rats which have been selected on their high alcohol intake in the home cage (HD) obtained more rewards during FR1 and PR schedules of reinforcement than LD. Furthermore, the HD reached the response criteria to continue to PR schedules faster than the LD, illustrating their increased sensitivity to the reinforcing effects of alcohol. HD made more active lever presses during the entire FR1 and PR2 session, while under the PR4 schedule of reinforcement, the HD performed more active responses in the first 10 minutes of the session. These data suggest that the animals adjust the response requirement to the alcohol reward, which influences their responding for alcohol during the session, indicating that the animals primarily lever press in the beginning of the session where the response requirement is lower compared to the later stages of the PR session. The positive relationship between alcohol consumption in the home cage and alcohol reinforcement in an operant setting has been previously reported in animals selectively bred for high or low alcohol consumption (Files et al., 1997; Ritz et al., 1994; Samson et al., 1998; Vacca et al., 2002). However, it has also been shown that there is not a complete overlap between the genes that contribute to differences in home cage consumption and alcohol reinforcement (Ritz et al., 1994; Samson et al., 1998). In sum, the individual differences in alcohol intake observed in this study are related to the reinforcing properties of alcohol and may mimic the diversity in the propensity for alcohol consumption in humans, supporting the validity of our approach as a rodent model for AUD (Chassin et al., 2002; Goudriaan et al., 2007; Hill et al., 2000; Tucker et al., 2003).

Importantly, we observed higher BALs after 30 minutes of alcohol consumption in HD as compared to LD, which corresponded with the alcohol intake. The BALs after 30 minutes of voluntary alcohol consumption were comparable to the average BALs described by other studies using similar IAA procedures (Cippitelli et al., 2012; Loi et al., 2010; Sabino et al., 2013; Simms et al., 2008), where most rats show BALs between 20 and 40 mg/dl, with a few animals reaching BALs up to 80 mg/dl.

Aversion-Resistant Alcohol Intake

In general, loss of control over substance use emerges upon extended and excessive substance use (American Psychiatric Association, 2013) and this loss of control over substance use has been modeled in rodents (Ahmed and Koob, 1998; Deroche-Gamonet et al., 2004; Pelloux et al., 2007; Turyabahika-Thyen and Wolffgramm, 2006; Vanderschuren and Everitt, 2004; Wolffgramm and Heyne, 1991). For example, the continued use of alcohol in conflict situations, by adulterating the alcohol solution with quinine or by concurrently providing an attractive alternative, is considered to reflect the compulsive motivation for alcohol that is observed in humans with AUD (Hopf and Lesscher, 2014; Vengeliene et al., 2009). In the present study, we observed that HD exhibited a greater aversion resistance in alcohol intake compared to LD after a total of 6 to 7 months of IAA exposure, indicative of less flexible alcohol consumption in HD. This is in line with previous studies that reported quinine-resistant motivation for alcohol after at least 3 to 4 months of alcohol consumption (Hopf et al., 2010; Wolffgramm and Heyne, 1991). The current findings are in agreement with those of Turyabahika-Thyen and Wolffgramm (2006), who reported that individual rats which displayed continued intake of bitter-tasting alcohol solutions, had, in retrospect, previously consumed more alcohol compared to rats that showed flexible, quinine-sensitive, alcohol intake. Together, these findings reveal individual differences in rats in susceptibility to inflexible alcohol consumption, a hallmark of AUD.

The HD and LD did not differ in taste sensitivity for quinine or sucrose, which is in agreement with previous comparisons between selected high versus low or alcohol-experienced versus nonexperienced rats (Hopf et al., 2010; Loi et al., 2010; O'Dell et al., 2004; Turyabahika-Thyen and Wolffgramm, 2006). Importantly, this makes it less likely that differences in taste sensitivity between the subgroups explain the differences in alcohol intake, alcohol reinforcement, and flexibility of alcohol intake between HD and LD.

Further investigation is required to discern whether the enhanced motivation to obtain alcohol and the development of aversion-resistant alcohol intake in HD are the consequences of the amount of alcohol the HD consumed, their innate susceptibility for AUD-like behavior, or an interaction between these factors. Previous studies

have, for example, shown that the development of addiction-like behavior in a subgroup of animals, after extended cocaine self-administration, was not related to the amount of cocaine the animals had self-administered (Chen et al., 2013; Deroche-Gamonet et al., 2004; Pelloux et al., 2007). The HD in our study, however, increased their alcohol intake and, correspondingly, show a higher motivation to respond for alcohol and a greater aversion resistance in alcohol intake, which suggests that there is a predisposition to develop AUD-like behavior in this subgroup of animals.

CONCLUDING REMARKS

Our results indicate that a subgroup of Lister Hooded outbred rats escalate their alcohol consumption during home cage IAA. These individual variations concur with differences in alcohol reinforcement, motivation, and AUD-like behavior. The behavioral characteristics of these high-alcohol-drinking rats—escalated and compulsive alcohol use—capture key aspects of AUD. Therefore, the current model provides a framework for more in-depth analyses of the neurobehavioral mechanisms underlying individual vulnerability to AUD, which may facilitate the development of novel behavioral and pharmacological interventions for this devastating condition.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Average reward collection and active lever press latencies during the 1-hour self-administration session.

Table S2. Alcohol intake and preference of the used cohorts.