Face validity of a pre-clinical model of operant binge drinking: just a question of speed

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ABSTRACT

Binge drinking (BD) is often defined as a large amount of alcohol consumed in a ‘short’ period of time or ‘per occasion’. In clinical research, few researchers have included the notion of ‘speed of drinking’ in the definition of BD. Here, we aimed to describe a novel pre-clinical model based on voluntary operant BD, which included both the quantity of alcohol and the rapidity of consumption. In adult Long–Evans male rats, we induced BD by regularly decreasing the duration of ethanol self-administration from 1-hour to 15-minute sessions. We compared the behavioral consequences of BD with the behaviors of rats subjected to moderate drinking or heavy drinking (HD). We found that, despite high ethanol consumption levels (1.2 g/kg/15 minutes), the total amounts consumed were insufficient to differentiate HD from BD. However, consumption speed could distinguish between these groups. The motivation to consume was higher in BD than in HD rats. After BD, we observed alterations in locomotor coordination in rats that consumed greater than 0.8 g/kg, which was rarely observed in HD rats. Finally, chronic BD led to worse performance in a decision-making task, and as expected, we observed a lower stimulated dopaminergic release within nucleus accumbens slices in poor decision makers. Our BD model exhibited good face validity and can now provide animals voluntarily consuming very rapidly enough alcohol to achieve intoxication levels and thus allowing the study of the complex interaction between individual and environmental factors underlying BD behavior.

Keywords animal model, binge drinking, decision making, fast cyclic voltammetry, operant self-administration.

INTRODUCTION

Alcohol use disorders (AUDs) have widespread negative consequences for public health. For two decades, an increasingly globalized, pervasive culture of intoxication has emerged among young people. Binge drinking (BD) is one subtype of problematic alcohol use, but to date, no exact, unique definition has been accepted worldwide, either for diagnostic or for research purposes (Rolland & Naassila 2017). BD is mostly defined clinically in terms of drinking frequencies, typical drinking quantities, frequencies of heavy drinking (HD) and alcohol-related aftereffects. Indeed, the National Institute on Alcohol Abuse and Alcoholism (NIAAA) defines BD as a pattern of drinking that raises the blood ethanol concentration (BEC) levels to 0.8 g/l (the legal intoxication level in the United States) in a short period (2 hours). This BEC can typically be achieved by consuming 60 or 70 g of pure ethanol for women and men, respectively. The World Health Organization (WHO) has defined BD as heavy, episodic alcohol use. Heavy episodic drinking is defined as six or more WHO standard-drinks per occasion (World_Health_Organization 2014). Of note, a recent study has shown that, compared with the WHO criteria, the NIAAA criteria for BD identify subjects with more severe drinking patterns and alcohol aftereffects (Rolland et al. 2017). In the research context, the impact of BD on brain and cognitive functioning has been evaluated based on a more behavioral definition of BD. In research, the BD score includes both the quantity of alcohol consumed and the speed of consumption (Townshend & Duka 2002; Smith et al. 2017). In this context, brain...
damage and cognitive alterations were shown to be more severe with BD compared with social drinking. Those findings revealed that both the level of intoxication and drinking speed, which is possibly the most important factor, were crucial factors in BD behavior.

Animal models are meant to parallel the human condition; it is very interesting to note that the NIAAA definition of BD in humans has also been used in different studies on animals (Crabbe, Harris, & Koob 2011). However, rodents do not readily consume sufficient amounts of ethanol to achieve intoxicating relevant BEC. Moreover, pre-clinical research on AUD is limited by the lack of a clear definition of BD behavior. Developing relevant animal models of BD is a priority for gaining a better characterization of the neurobiological and psychobiological mechanisms underlying this dangerous and harmful behavior. Moreover, this research could lead to better pharmacotherapies.

Wise (1973) first described an every-other-day access procedure for facilitating escalation of alcohol intake using highly concentrated alcohol solution (20 percent) creating an animal model of high levels of consumption. This procedure is now largely used by several laboratories (Simms et al. 2008; Hopf et al. 2010; Carnicella, Ron, & Barak 2014; Jeanblanc et al. 2014; Jeanblanc et al. 2015; Spoelder et al. 2016; Lebourgeois et al. 2017; Spoelder et al. 2017). Previously, human observations showed that the ‘happy hour’ facilitated BD (Kuo et al. 2003); moreover, animal studies showed that scheduled availability was useful in getting rodents to consume sufficient amounts of ethanol to achieve human drinking behavior (Holloway, Bird, & Devenport 1984). Based on those findings, we created a BD model by combining every-other-day access with very short time periods of ethanol availability in operant ethanol self-administration sessions. Indeed, it was previously suggested that, in 30-minute sessions of operant self-administration of a 20 percent ethanol solution, animals consumed 70 percent of the ethanol within the first 10 minutes, and BECs ranged from 0.25 to 0.85 g/l (mean 0.60 g/l) (Carnicella et al. 2014).

The present study aimed to set up a new animal model of voluntary operant BD, where animals displayed high-speed drinking and signs of intoxication. In order to model different patterns of consumption, we chose to use different procedures inducing different levels of ethanol self-administration. Until the mid-2000s, the gold standard for ethanol self-administration studies was the use of long-lasting (1 hour) session with a 10 percent ethanol solution to lead to moderate levels of alcohol consumption (usually around 0.3–0.4 g/kg). With the revival of the use of the intermittent access paradigm by the Bartlett’s lab first and then the Ron’s lab and others, self-administration paradigms shifted to shorter sessions (30 minutes) with higher concentration of ethanol (20 percent) leading to higher consumption (0.6–0.7 g/kg). In order to mimic what is happening with human binge drinkers, we decided to reduce the accessibility to the drug to even shorter sessions (15 minutes) similarly to the ‘happy hours’ phenomenon, which leads to BD behavior. Our objectives were to achieve good face validity regarding high-speed alcohol consumption; to determine the levels of intoxication and a BEC threshold; and to identify the level of motivation (reinforcing efficacy of ethanol) for consuming highly concentrated ethanol solutions. Furthermore, we explored the negative consequences of BD by evaluating two hallmarks of AUDs and alcohol dependence: performance in a decision-making task and potential alterations in dopaminergic (DA) transmission within the nucleus accumbens (NAcc).

**MATERIALS AND METHODS**

**Animals**

Male Long–Evans rats (initial weight: 280–300 g; Janvier Laboratories, France) were individually housed under a light : dark cycle of 12 hours, with lights on at 7:00 AM. Food and water were available ad libitum. All animal procedures were in accordance with the European Community Regulations for Animal Use in Research (CEE No. 86/609) and approved by the local ethics committee (#260912-10).

**Reagents**

Ethanol (96 percent) was purchased from VWR (France), and sucrose was purchased from Sigma-Aldrich (France).

**Moderate drinking**

Initiation of moderate ethanol drinking (MD) was introduced with an ethanol 10 percent/sucrose two-bottle choice with a sucrose fading paradigm adapted from a previous study (Samson 1986) and described recently (Jeanblanc et al. 2014). Next, this first cohort of rats was trained to self-administer ethanol under a fixed ratio of 3 (three lever presses to obtain one reward of 0.1 ml of a 10 percent ethanol solution) in daily 1-hour sessions. For a more detailed description, see the Supporting Information.

**Heavy drinking**

To generate HD behavior, another cohort of rats was first submitted to a two-bottle choice protocol for 4 weeks with intermittent access to 20 percent ethanol, as described previously (Wise 1973). Then, rats were trained to self-administer ethanol (20 percent ethanol solution v/v) under a fixed ratio of 3 in daily 30-minute sessions.
For a more detailed description, see the Supporting Information.

**Binge drinking**

Binge drinking behavior was generated with a protocol similar to that described for HD. A third cohort of rats was used to generate BD rats. After the rats reached a stable baseline of ethanol self-administration (approximately 3 weeks), the session time was reduced to 15 minutes. Rats continued to self-administer the 20 percent ethanol solution (0.1 ml per delivery under a fixed ratio of 3).

**Blood ethanol concentration**

Blood was collected 10 minutes after the end of the self-administration sessions for all rat groups. An additional group of HD rats was used in order to evaluate the BECs 15 and 30 minutes after the beginning of the self-administration session to compare with the BECs of the BD (15-minute sessions). Rats were moderately sedated (5 percent isoflurane for 2 minutes); then, we collected 200-μl blood from the lingual vein in heparinized tubes. Samples were centrifuged and stored on ice. Serum alcohol concentrations were determined with an ANALOX system (Imlab SARL, Lille, France).

**Rotarod**

Rotarod training was performed in the morning at least 3 hours prior to self-administration sessions. In two sessions (one per day), BD rats were trained to remain on a rotating rod (7.62-cm diameter, Rotarod, IITC, Woodland Hills, CA, USA), which rotated at a fixed speed of 5 rpm. Then, rats were submitted to five sessions: one session at 10 rpm, one session at 15 rpm and finally three sessions, where the speed increased from 5 to 25 rpm over 300 seconds. The day after training, this same series of tests was performed immediately after a BD session. The times that rats remained on the rotating rod during the test session were subtracted from the times achieved in the last session of training.

**Progressive ratio**

The reinforcing efficacy of ethanol was evaluated with a progressive ratio (PR) paradigm. In this paradigm, the ratio of lever presses to one reward (i.e. the effort required for a single portion of ethanol) was successively increased after each reward delivery, as follows: 3, 4, 5, 7, 9, 12, 15, 17, 20, 22, 25, 28, 30, 33 and 35 (Jeanblanc et al., 2015). At some point, the animals stopped pressing the lever without receiving a reward. This point was called the breaking point, and it served as an index of the animal’s motivation to consume ethanol. We choose to test the reinforcing efficacy with sessions of the same duration of the self-administration ones, 15 and 30 minutes, keeping the level of ethanol consumed relatively moderate avoiding any interference of the total amount of ethanol consumed and the behavior. In addition, we already observed a plateau effect after 15 minutes in the 30-minute sessions of PR with HD (Jeanblanc et al. 2015).

**Motivation for high ethanol concentrations**

Rats were submitted to changes in the ethanol concentration between different self-administration sessions (FR3 schedule, 15 minutes), and we evaluated the amount of ethanol consumed. They had access to the regular 20 percent ethanol solution, then the solution was switched to a 10 percent ethanol solution for three consecutive sessions and finally a 30 percent ethanol solution for another three consecutive sessions. The data from the last session of each of the ethanol concentrations were used for the analysis.

**Rat Gambling Task**

This experiment was performed as described by Rivalan, Ahmed, & Dellu-Hagedorn (2009). For a detailed description, see the Supporting Information. Rats stopped completely the BD sessions before the Rat Gambling Task (RGT) started and were not re-exposed to any alcohol during the whole RGT experiment. Briefly, naïve (control) and BD rats were trained to nose-poke to obtain a sucrose pellet from four different holes, with no specific rules during the entire training phase (7–12 days). On the test day, a rule was attributed to each hole. Two holes led to advantageous choices for the rats; they received one pellet immediately, and a short time-out was imposed before they could obtain the next reward. The two other holes led to disadvantageous choices; they received two pellets immediately, but the time-out was long. A score was calculated as the number of advantageous choices per 10-minute segment, over a 1-hour test session. For a detailed description, see the Supporting Information.

**Ex vivo fast-scan cyclic voltammetry**

After performing the RGT experiment (BD and alcohol-naïve groups), rats were euthanized, and brains were collected. Fast-scan cyclic voltammetry experiments were performed, as described previously (Gibb et al. 2011). Briefly, coronal slices of the NAcc core were collected to measure electrically stimulated DA release. For a detailed description, see the Supporting Information.

**Statistical analysis**

When a significant effect was observed, normally distributed data with equal variances were analyzed (SigmaPlot
11.0, Systat Software, Inc.) San José, CA, USA with one-way or two-way analyses of variance (ANOVAs), with or without repeated measures, followed by a Tukey multiple comparison test. When a normal distribution was not observed, data were analyzed with a non-parametric analysis (Kruskal Wallis and Dunn’s tests). For single comparisons,
we used a Student’s t-test (two-tailed). Correlations were evaluated with Pearson’s correlation test. Significance was set at $P < 0.05$.

**RESULTS**

Comparison of different models of ethanol self-administration

First, we recorded the numbers of active lever presses (Fig. 1a) in the three groups of rats (MD, HD and BD). The BD group performed the greatest number of active lever presses. A one-way ANOVA revealed a significant effect of the group: $F_{(2, 51)} = 8.29, P < 0.001$. The post hoc analysis indicated a significant difference between the MD and BD groups ($P < 0.01$). Because the session durations were different for each group, we represented the lever-pressing rates for each group of rats (Fig. 1b). Again, a one-way ANOVA indicated a main effect of the group: $F_{(2, 51)} = 54.94, P < 0.001$. The post hoc analysis revealed no significant difference between the MD and HD groups. However, the pressing rate of the BD group was significantly different from both the MD and HD groups ($P < 0.001$).

We then studied the amounts of ethanol consumed in each group. We observed that both the HD and BD groups consumed greater amounts of ethanol than the MD group (Fig. 1c). The equal variances criterion was not fulfilled in these data; therefore, we performed a Kruskal Wallis analysis and found a significant effect of the group ($H = 30.06, P < 0.001$). The post hoc analysis (Dunn’s multiple comparison test) indicated a significant difference between the MD group and both the HD and BD groups ($P < 0.001$). We then analyzed the increase in the rate of ethanol consumed for each group (Fig. 1d). A one-way ANOVA conducted on these data revealed a significant effect of the group: $F_{(2, 51)} = 71.17, P < 0.001$. The post hoc analysis indicated a significant difference between the MD group and both the HD and BD groups ($P < 0.01$ and $P < 0.001$, respectively) and between the HD and BD groups ($P < 0.01$).

Blood samples were collected 10 minutes after the self-administration sessions and analyzed to determine BECs (Fig. 1e). A one-way ANOVA revealed a significant effect of the group $F_{(2, 16)} = 25.96, P < 0.001$. The post hoc analysis indicated a significant difference between the MD group and both the HD and BD groups ($P < 0.01$ and $P < 0.001$, respectively) and between the HD and BD groups ($P < 0.01$). The rates of increase in BECs were analyzed with a one-way ANOVA (Fig. 1f); this analysis revealed a significant effect of the group: $F_{(2, 36)} = 47.50, P < 0.001$. The post hoc analysis indicated a significant difference between the BD group and both the MD and HD groups ($P < 0.001$ for both).

The cumulative lever-press analysis (Fig. 2a) indicated a slow increase in the number of lever presses observed in the MD group, up to about 60 presses for the 10 percent ethanol solution. In contrast, the rate of lever presses was higher in the HD group, and a plateau was reached at about 15 minutes after the beginning of the session. Only a few presses were observed during the last 15 minutes, and the maximum was about 80 presses for the 20 percent ethanol solution. In the BD group, the rate of lever pressing increased steadily during the 15-minute session, and the maximum was 120 presses for the 20 percent ethanol solution. Typical examples of ethanol consumption showed that the MD and HD groups consumed in small bouts, every 5–10 minutes; in contrast, the BD group exhibited continuous consumption during the whole session of self-administration (Supporting Information Fig. S1a).

The BECs were directly correlated with the amounts of ethanol consumed in each group (Fig. 2b; Pearson’s test: MD, $r = 0.69, P < 0.05$; HD, $r = 0.77, P < 0.01$; and BD, $r = 0.94, P < 0.001$).

Within the group HD, we performed an analysis of the BECs 15 and 30 minutes after the beginning of the self-administration. We found that, in two groups of 10 rats that self-administer similar amount of alcohol (Supporting Information Fig. S1b: t-test, $P > 0.05$), there was no difference in the BECs at 15 and 30 minutes (Supporting Information Fig. S1c: t-test, $P > 0.05$) despite the small number of presses performed at the end of the 30-minute session.

It is noteworthy that the latency to receiving the first reward (Fig. 2c) was not significantly different among the three groups ($F_{(2, 51)} = 2.75, P = 0.07$). In contrast, the delays between 1st and 20th rewards (Fig. 2d) were significantly shorter in both the HD and BD groups, compared with the MD group. The one-way ANOVA conducted on these data revealed a significant effect of the group: $F_{(2, 15)} = 85.86, P < 0.001$. The post hoc analysis indicated a significant difference between the MD group and both the HD and BD groups ($P < 0.001$).

We then analyzed the intervals between two consecutive deliveries (Fig. 2e). We observed an interval of 10 seconds for about 15, 50 and 60 percent of deliveries in the MD, HD and BD groups, respectively. A two-way ANOVA analysis with repeated measures revealed a significant effect of the intervals ($F_{(6, 306)} = 115.93, P < 0.001$) and the group ($F_{(2, 106)} = + \text{inf}, P < 0.001$). Moreover, we found a significant interaction between these two factors ($F_{(12, 106)} = 25.95, P < 0.001$). The post hoc test indicated that the first interval duration was significantly different between the MD group and both the other groups ($P < 0.001$ for both) and between the HD and BD groups ($P < 0.05$). The last interval duration was also significantly different between the MD group and both the other groups ($P < 0.001$ for both).
Binge drinking alters the reinforcing efficacy of ethanol

Next, we evaluated the motivation to self-administer ethanol. The HD and BD groups underwent a progressive ratio paradigm. The previous sessions lasted 15 minutes for the BD group and 30 minutes for the HD group. To facilitate comparisons, we conducted one 15-minute and one 30-minute session for the BD group and one 30-minute session for the HD group (Fig. 3a). The analysis revealed a main effect of the group ($F(2, 48) = 14.83, P < 0.001$). The multiple comparison test indicated a significant difference between the performances of the HD and BD groups in both the 15-minute ($P < 0.05$) and 30-minute ($P < 0.001$) sessions. Of note, when the BD group was allowed to press the active lever for 30 minutes, they exhibited higher motivation than they exhibited in the 15-minute session ($P < 0.05$).

We then evaluated the motivation in the BD group for consuming different solutions of ethanol, one 10 percent and one 20 percent (Fig. 3b). We found that these rats exhibited a higher breaking point for the 20 percent ethanol solution than for the 10 percent ethanol solution ($P < 0.01$). Finally, rats were tested for three different concentrations of ethanol solutions (10, 20 and 30 percent). A one-way ANOVA with repeated measures revealed a significant effect of the ethanol solution ($F_{1,20} = 14.83, P < 0.001$).
The post hoc test indicated a significant difference between the BD group that received 10 percent ethanol and both of the other groups ($P < 0.001$). No difference was found between the BD groups that received 20 and 30 percent ethanol ($P > 0.05$). We analyzed the cumulative active lever presses (Fig. 3d) performed for the 20 and the 30 ethanol solution, the two different solutions leading to the same level of pure alcohol consumption (~1 g/kg/15 minutes, Fig. 3c). The two-way with repeated measures ANOVA indicated a main effect of the factor ethanol solution ($F_{(1, 88)} = 14.01, P < 0.001$), of the factor time ($F_{(4, 88)} = 116.56, P < 0.001$) and of an interaction between both factors ($F_{(4, 88)} = 22.43, P < 0.001$). The post hoc test first revealed a significant difference between the two groups (20 and 30 percent ethanol) for the timepoints 6–9 ($P < 0.01$), 9–12 and 12–15 ($P's < 0.001$). On the other hand, the within ethanol...
concentration groups comparison revealed within the 20 percent ethanol group a significant difference between all timepoints but except for the 9–12 and 12–15 timepoints, meaning the last two. In regard to the 30 percent ethanol group, the absence of difference between timepoints appears earlier at the timepoints 6–9 and for the three last timepoints. This within comparison indicates that when the rats have access to the 30 percent ethanol solution, they reach the 1 g/kg faster than when they have the 20 percent ethanol solution delivered.

We next changed the accessibility to the 20 percent ethanol solution and evaluated the effects. First, after 10 days of abstinence, BD rats were allowed to re-access to self-administration. The abstinence led to a 20 percent increase in the number of active lever presses, compared to self-administration. The abstinence led to a 20 percent increase in the number of active lever presses, compared to self-administration.

The results showed that BD rats made less advantageous choices than control rats. The data from the last 20 minutes of the RGT were used to categorize the

Loss of motor coordination

We tested the time intervals that HD and BD rats remained on the rotarod before falling, under ethanol and sober conditions (Fig. 4a). The difference in times was significantly correlated to the total amount of ethanol consumed during one BD session ($P < 0.001$), whereas no correlation between these parameters was observed in the HD group. Next, we separated these data into two groups; one group comprised rats that consumed less than 0.8 g/kg in 30 (HD) or 15 minutes (BD), and the other group comprised rats that consumed more than 0.8 g/kg in 30 (HD) or 15 minutes (HD). The two-way ANOVA revealed a main effect of the factor levels of alcohol consumed ($<0.8$ g/kg versus $>0.8$ g/kg): $F_{(1, 50)} = 7.44$, $P < 0.01$, no effect of the factor group (HD versus BD) $F_{(1, 50)} = 1.59$ not significant and no interaction between both factors $F_{(1, 50)} = 2.84$, $P = 0.098$ (Fig. 4b). The multiple comparison test (Tukey) indicated a significant difference between the groups HD and BD within the condition $>0.8$ g/kg ($P < 0.05$) and between the groups $<0.8$ and $>0.8$ g/kg within the BD group ($P < 0.001$).

Binge drinking alters decision making

A new cohort of rats trained in BD behavior was generated for the RGT experiment. Over the three sessions prior to the RGT, this group displayed an average ethanol self-administration of $0.92 \pm 0.08$ g/kg (range: 0.52 to 1.67 g/kg). Each sequence of choices made by the control and BD rats was recorded and expressed as the ratio of good/bad choices, in blocks of 10 minutes (Rivalan et al. 2009) (Fig. 5a). A two-way repeated measures ANOVA revealed a main effect of the group: $F_{(1, 191)} = 14.42$, $P < 0.001$, but no effect of the time: $F_{(5, 191)} = 1.47$, $P = 0.20$. We found no interaction in a Groups × Time analysis: $F_{(5, 191)} = 0.09$, $P = 0.99$.

The results showed that BD rats made less advantageous choices than control rats. The data from the last 20 minutes of the RGT were used to categorize the

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distribution of choices made by each of the groups. When less than 33 percent of the choices were advantageous, rats exhibited poor decision-making (PDM) behavior; when between 33 and 66 percent of choices were advantageous, rats exhibited neutral behavior; and when 66 percent or more choices were advantageous, rats exhibited good decision-making (GDM) behavior. We found different proportions of decision making between the control and BD groups. In particular, a higher proportion of rats exhibited PDM in the BD group than in the control group ($\chi^2 = 5.719, P = 0.057$, Fig. 5b). In the control group, 10, 40 and 50 percent exhibited PDM, neutral and GDM behaviors, respectively. In the BD group, 43, 14 and 43 percent exhibited PDM, neutral and GDM behaviors, respectively.

In the control and BD groups that previously performed the RGT experiment (Fig. 5c & d), we measured DA released in the NAcc core slices induced by electrical stimulation. The peak levels of DA (200 nM) were similar between the control and BD groups ($n = 10$ in each group, $t$-test, $P = 0.86$). Similarly, no difference in the area under the curve was detected between groups ($t$-test, $P = 0.50$). We then combined the control and BD groups and divided them into groups based on RGT performance (GDM versus PDM). The peak DA values indicated a marginal effect of performance on DA release ($n = 7$ in the PDM group, $n = 9$ in the GDM group, $t$-test, $P = 0.11$). However, the areas under the curve revealed a significant difference between the two groups ($n = 7$ in the PDM group, $n = 9$ in the GDM group, $t$-test, $P = 0.013$; Fig. 5d). It is noteworthy that on average, our groups of rats started the 20 percent intermittent access at 1.17 ± 0.19 g/kg/24 hours to finish at 4.57 ± 0.28 g/kg/24 hours at the end of the protocol. The analysis we performed show no correlation between the levels of alcohol consumption at the end of the 20 intermittent access protocol and none of the subsequent parameters studied (BD levels, decision making and DA release).

**DISCUSSION**

For about 10 years, researchers have developed different animal models of BD. However, most BD models were developed in mice, and often, the administration of alcohol was forced, rather than voluntary (for review, see Crabbe...
ethanol with an intraperitoneal injection (Pascual et al. 2009; Alaix-Cantin et al. 2013; Pascual et al. 2014) or an inhalation procedure (Izzo et al. 2007), to obtain rapid increases in BEC that mimicked the BECs observed in humans with BD-like behavior. Models that tested voluntary intake were mostly based on the drinking-in-the-dark paradigm in mice (McBride et al. 2010; Thiele, Crabbe, & Boehm 2nd 2014) and, more recently, in rats (Holgate et al. 2017).

Although, these non-operant paradigms are very useful, simple to set up and inexpensive, they have some limitations. For example, in those models, it is actually difficult to evaluate some important parameters of drug consumption, such as motivation and seeking out the drug.

Here, we demonstrated that our operant procedure could effectively train rats to drink, in a very short period of time, sufficient alcohol to induce ataxia. The rapid intake of ethanol associated with signs of intoxication provided good face validity of the model. Of note, we obtained the same BEC threshold as that used by the NIAAA to define BD. This threshold is typically difficult to obtain in models based on voluntary drinking. On average, in less than 15 minutes, our rats self-administered >1.2 g/kg, and even more (>1.5 g/kg), when we removed three outliers (Fig. 1c). In addition, the BECs were obtained only in 15 minutes; in other models, similar BECs were observed, but typically after 2-hour sessions. Because HD reached a kind of plateau in their active lever presses after 20 minutes of self-administration, we evaluated the BECs after 15 and 30 minutes in the HD group. These BECs obtained after 15 minutes were half of those obtained after 15 minutes of our BD group and remained stable until the end of the 30-minute sessions. This indicates that their ethanol consumption behaviors are completely different and the differences observed in the effect of such consumption are not due to different timepoints of blood collection.

Human studies suggested that the deleterious effects of BD may due to the rapid increase in BEC. Thus, it is important for animal models of BD to consume large amounts of ethanol, but in addition, they must take into account the rate of drinking. In our model, the BEC increased at a mean rate of 5 mg/dl/minute; thus, the 80-mg/dl threshold was achieved after only 15 minutes. In humans, the rapid rise in BEC, typically observed after gulping drinks and/or drinking on an empty stomach, was shown to be a key predictor of the occurrence of amnesia, also known as ‘black-outs’ (Goodwin et al. 1970; White 2003; Perry et al. 2006).

Reinforcing efficacy of ethanol is higher in BD rats as compared with HD rats. Moreover, when rats have a prolonged PR session, we observed a new increase in the breaking point value. The duration of a normal BD session is 15 minutes; thus, we decided to perform the PR experiment using first the same duration. However, because HD rats had access to the levers for 30 minutes, we added a condition in which BD rats had access to the levers for 30 minutes too during a PR experiment. The increase observed in this latter condition suggests that the breaking point observed during the 15-minute sessions is not due to a real lack of motivation to continue but rather due to the limited duration of the session. Indeed, when rats have access to the levers for a longer period, the breaking point increases as compared with the 15-minute sessions. In addition, we also found that BD rats show higher preference for a 20 versus 10 percent ethanol solution. BD rats maintained high response levels even when the ethanol concentration was increased to 30 percent. In addition, by the analysis of the cumulative active lever presses, we show that when rats had the 30 percent ethanol solution delivered, they urge to self-administer faster than when they have the 20 percent ethanol solution. It is striking to observe that our preclinical data resonate with clinical observations regarding the fact that adolescent’s binge drinkers are more prone to choose highly alcohol concentrated beverages to reach drunkenness/intoxication faster.

Our experiment on the alteration of locomotor coordination reveals that the speed of consumption is really the important features of the BD behavior. Indeed, there is no sign of intoxication in the HD rats consuming more than 0.8 g/kg (no difference with the rats consuming less than 0.8 g/kg), whereas a clear loss of locomotor coordination is observed in BD rats consuming more than 0.8 g/kg as compared with either the BD rats consuming less than 0.8 g/kg or the HD rats consuming more than 0.8 g/kg.

Taken together, the signs described previously are important features of BD behavior. It is notable that these rats could also voluntarily self-administer ethanol twice per day in a BD pattern. This finding was particularly relevant, because we recently showed that two binge-like ethanol exposures were sufficient to abolish long-term depression in the hippocampus after 48 hours (Silvestre de Ferron et al. 2017). Finally, the face validity of our model was further supported by our finding that, several weeks after chronic voluntary BD behavior, rat decision-making performance was reduced. Indeed, animals that consumed high levels were categorized as PDMs more frequently than animals that consumed low levels of ethanol. This last result was consistent with results obtained in humans (Goudriaan, Grekin, & Sher 2007; Johnson et al. 2008; Townshend et al. 2014).

Dopamine release in the NAcc was not altered by BD; however, we found reduced DA release in rats categorized as PDM compared with those categorized as GDMs,
consistent with previous studies (de Visser et al. 2011; Rogers 2011; Jones, Cservenka, & Nagel 2016). The absence of a significant difference between our control and BD groups could be due to one of three possible factors. First, there was a delay in measuring DA release (DA release was recorded at least 2 weeks after the end of the operant BD sessions). Second, the mix of GDMs and PDMs in both groups may have obscured the difference between groups. The last important hypothesis is that we used adult animals and that the current data in human BD have been obtained in adolescent individuals in which the brain may be more vulnerable to BD and thus to long-term effect of excessive alcohol intake during the critical period of adolescence. Future experiments are needed with a longitudinal approach to determine whether BD alters both decision making and DA release: also, DA release should be evaluated compared with the release observed at baseline, before starting voluntary BD.

Testing ways to reduce BD behavior might be very interesting and potentially useful in preventing the development of addiction. In this regard, we recently used this BD model to show that N-acetylcysteine, which has been used off-label to reduce craving in cocaine addiction, could also effectively reduce BD behavior (Lebourgeois et al. 2017). Thus indicating that our BD model has a good predictive validity. To further confirm this it might also be very interesting to use our model to investigate the efficacy of current AUD treatments and also find new neurobiological targets (construct validity). In regard to the gender, it is known that in Humans, the women start to consume also large amounts of alcohol and it was shown that they may be more sensitive to the negative effect of alcohol than men. For this reason, it will be very important but also complex to decipher the differences between male and female rats in our BD model. Finally, our voluntary operant BD model could be used to study both environmental and individual factors involved in BD behavior. Moreover, subpopulations might be identified based on distinct personality traits, as we recently demonstrated in a population of college students with BD behavior (Gierski et al. 2017).

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Authors Contribution

JJ designed and performed the experiments, analyzed the data and wrote the manuscript. SP and RL designed and performed part of the experiments and analyzed the data. JV, LS, MGM and EAV performed the experiments. MN designed the experiments and wrote the manuscript.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.