Evaluation of alcohol use disorders pharmacotherapies in a new preclinical model of binge drinking

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ABSTRACT

Binge drinking is defined as a pattern of drinking leading to intoxication in a single short session and is a serious but preventable public health problem. Only few animal models of voluntary binge drinking using an operant paradigm are available in outbred animals and in general they do not display good face validity. We recently set up a new model of binge drinking behavior using an operant self-administration paradigm in which rats drink to intoxication level in 15-min daily session. Here we tested the current pharmacotherapies of alcohol use disorder: Acamprosate, (R)-Baclofen, gamma-hydroxybutyric acid, Nalmefene and Naltrexone. Our results show that all drugs are effective in reducing ethanol drinking. All drugs except Acamprosate also reduced the motivational properties of ethanol (breakpoint). (R)-Baclofen and gamma-hydroxybutyric acid were effective on ethanol intake at doses devoid of side effects. Among the tested drugs only (R)-Baclofen, gamma-hydroxybutyric acid and Naltrexone reduced reacquisition after a period of abstinence. Interestingly, the efficacy of all drugs except Nalmefene to reduce ethanol drinking was slightly and positively correlated with the basal level of drinking thus revealing heavy drinking as a predictive factor. In summary, all current alcohol use disorder pharmacotherapies were effective in our model of binge drinking behavior thus bringing new data regarding its good predictive validity. The tested drugs display some specificity regarding their effect on motivation, reacquisition and also in terms of individual factors such as basal drinking level. Our new model opens promising perspectives about the development of pharmacotherapies targeting binge drinking behavior.

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1. Introduction

Binge drinking (BD) behavior has no universal definition but is defined as a pattern of drinking leading to intoxication in a single short session (Rolland and Naassila, 2017). This drinking pattern is associated with significant morbidity and mortality and contributes to alcohol-attributable burden of disease thus becoming a public health issue. The most precise definition has been given by the National Institute on Alcohol Abuse and Alcoholism (NIAAA), 2018 as a pattern of drinking that brings blood alcohol concentration (BAC) levels to 80 mg%, typically occurring after the consumption of 56 g pure ethanol for women and 70 g pure ethanol for men in about 2 h. Most of epidemiological surveys on BD in young people are using simple definitions such as at least 4 drinks for women and 5 drinks for men in a single occasion (World Health Organisation, 2014) and at least once the past month (SAMHSA), 2016. One concern about the BD definition is the duration of the occasion that can be very short (in a row) or can last within a couple of hours and thus not leading to the same adverse events. A shorter duration may be predictive of more adverse consequences. For example, a recent study has shown that, compared with the WHO criteria, the NIAAA criteria for BD identify individuals with more severe alcohol consequences (Rolland et al., 2017).

Young people are drinking less regularly than adults but are drinking more on each occasion making BD behavior the dominant type of alcohol misuse in adolescents. However recent study also pointed out the fact that BD may also be of concern among adults aged ≥50 since 14.4% reported past-month BD (Han et al., 2018). Thus BD behavior should not be explored only in young populations. Another important point is that BD is a heterogeneous...
phenotype. Consequently, binge drinkers should not be considered as a unitary group, but rather as a heterogeneous population of individuals displaying particular gender and personality dimensions (Gierski et al., 2017), as well as differences in drinking motives and impulsivity (Lanniey et al., 2017).

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 that may also lead to alcohol addiction. Animal models of BD behavior may also be useful to find effective pharmacotherapies (Crabbe et al., 2017). Numerous preclinical models of BD behavior are currently used with passive exposure to ethanol or voluntary ethanol intake (see our recent review (Jeanblanc et al., 2018a)) We have proposed seven criteria for a relevant animal model of BD (Jeanblanc et al., 2018a). An ideal animal model of BD should display fast and voluntary ethanol intake to reach BAC >80 mg%, visible signs of intoxication, cognitive and somatic consequences, large inter-individual variability and intoxications should be repeated with intermittent cycles of consumption (Jeanblanc et al., 2018a). We published recently a new procedure to facilitate the induction of BD behavior in Long Evans rats (Jeanblanc et al., 2018b). In this procedure rats have been exposed to intermittent access to 20% ethanol in a 2-bottle choice paradigm for 4 weeks followed by 15-min daily sessions of 20% ethanol operant self-administration for several months (Jeanblanc et al., 2018b). With this procedure rats drink very quickly and display ataxia. Once established they maintain their BD behavior for months and display alterations in a decision-making task (Jeanblanc et al., 2018b) as seen in human binge drinkers (Goudrian et al., 2007). They also display signs that are characteristic of the post-dependent state, such as withdrawal (aggressive behavior and vocalizations), liver damage and increased motivation to consume ethanol, even at high ethanol concentrations (e.g., 30% ethanol)(Jeanblanc et al., 2018a).

In addition, we demonstrated in this BD model that the anti-craving drug N-acetylcysteine is effective in reducing ethanol intake, motivation and reacquisition of ethanol intake (Lebourgeois et al., 2018a).

In the present study, we used the same animal model of BD behavior to test its predictive validity using all the current pharmacotherapies used in patients: Acamprosate, (R)-Baclofen, gamma-hydroxybutyric acid (GHB), Nalmefene and Naltrexone. We tested the efficacy of the different drugs on ethanol intake, the motivational properties of ethanol (breakpoint) and reacquisition of ethanol intake after abstinence. It is worthy of note that, to our knowledge, it is the first study testing all the current alcohol use disorder (AUD) pharmacotherapies with the possibility to compare their efficacy in the same animal model. Because the identification of good responders is of high priority in patients we also looked at the individual response depending on the basal level of intake. This point has been raised for example in a recent study and a meta-analysis on baclofen showing that higher daily ethanol intake could be associated with a greater effect of baclofen (Reynaud et al., 2017; Rose and Jones, 2018). In addition, reduction of ethanol consumption is now recognized as a valid primary outcome and the individual response depending on the basal level of consumption the greater the drug efficacy.

2. Materials and methods

2.1. Subjects

Twenty-one male Long-Evans rats (461–530 g at the beginning of the dose-response experiment) were obtained from Charles River (L’Arbresle, France). Animals were individually housed under a light/dark cycle of 12 h (lights on at 7:00 a.m.) with food and water available ad libitum. Experiments were carried out in accordance to the guidelines for Care and Use of Laboratory Animals (National Institutes of Health) and the European community regulations for animal use in research (CEC no 86/609) and were approved by the local research ethics committee (CREMEAP; no 260912-10).

2.2. Drug solutions

Ethanol solution for operant alcohol self-administration experiments was prepared by diluting ethanol 96% (VWR-ProLabo, Fontenay-sous-Bois, France) to 20% (v/v) in tap water. Lundbeck Pharmaceuticals, D&A Pharma and Merck generously donated Nalmefene, GHB and Acamprosate (France) while (R)-Baclofen and Naltrexone were obtained from Tocris (Tocris, Bio-Techne, Lille Cedex, France). (R)-Baclofen was preferred to (R/S)-Baclofen because it has been shown to be more effective on ethanol intake than (S)-Baclofen or (R/S)-Baclofen (Lorrain et al., 2016). All drugs were dissolved in 0.9% sterile saline except GHB, which was dissolved in distilled water. Drug administration was given 30 min before the start of operant alcohol self-administration experiments via i.p. injections except for Naltrexone, which was administered subcutaneously (s.c.). Nalmefene was administered at the doses of 0.025, 0.05 and 0.1 mg/kg in a volume of 2 mL/kg of body weight; (R)-Baclofen at 0.5, 1 and 2 mg/kg, Acamprosate at 50, 100, 200 and 400 mg/kg, and Naltrexone at 0.1, 0.5 and 1 mg/kg in a volume of 1 mL/kg of body weight; finally, GHB was administered at the doses of 50, 100, 200 and 300 mg/kg in a volume of 5 mL/kg of body weight. All solutions were used at room temperature and doses and routes of administration were chosen accordingly to the literature (Colombo et al., 2012, 2003; Czachowski et al., 2001; Czachowski and Delory, 2009; Heyser et al., 1998; Maccioni et al., 2008; Walker and Koob, 2008, 2007; Williams and Broadbridge, 2009).

2.3. Self-administration of high levels of ethanol

In order to facilitate high level of ethanol intake, rats were trained to consume 20% ethanol solution in a 2-bottle-choice drinking procedure followed by several weeks of operant ethanol self-administration. Specifically, individually housed naïve rats (n = 21) were given intermittent access to one bottle of 20% ethanol and one bottle of tap water for three 24-h sessions per week (Mondays, Wednesdays and Fridays). Rats had unlimited access to two bottles of water between the ethanol-access periods. The placement of the ethanol bottle was alternated each ethanol drinking session to control for side preferences. This procedure has been shown to induce escalation of ethanol consumption in several strains of rats (Jeanblanc et al., 2018b, 2013; Simms et al., 2008; Wise, 1973). It should be noticed that individual differences have been demonstrated using this procedure in Wistar rats (Fredriksson et al., 2017). Rats were maintained on the 20% ethanol intermittent-access (IAE) 2-bottle-choice drinking procedure for 4 weeks.
2.3.1. Operant ethanol self-administration

Following 4 weeks of the IAE, operant ethanol self-administration training started (Jeanblanc et al., 2018b). Rats were randomly assigned to one operant conditioning chamber and were trained to press one of the two levers (the active lever) for 20% ethanol solution delivery. Rats were initially trained in two 12-h overnight sessions under a fixed ratio 1 (FR-1) schedule of reinforcement (i.e. each response on the active lever resulted in delivery of 0.1 mL of ethanol) followed by four 60-min daily sessions. After three 30-min daily sessions, the response requirement was raised to 3 lever presses to get the same quantity of ethanol (FR-3) and the length of the session was gradually decreased from four 60-min daily sessions to three 30-min daily sessions, and finally to 15-min daily sessions for 2 months. This paradigm induces high level of ethanol intake of about 1.5 g/kg/15min. This level of ethanol consumption leads to a blood ethanol concentration above 80 mg% (Jeanblanc et al., 2018b). Training sessions were run at the same time of the day, 5 days per week. Finally, when stable levels of responding were observed over at least three consecutive self-administration sessions (±20%), drug treatment started. Two groups of rats were constituted as described on Fig. 1: Group A received Nalmefene then Acamprosate while Group B received (R)-Baclofen then Naltrexone, in order to avoid the administration of the two opioid receptor antagonists (Naltrexone and Nalmefene) in the same animals. Thereafter rats from the two groups were used to constitute Group C that received GHB.

Operant conditioning for ethanol reinforcement was conducted in twelve standard operant conditioning chambers housed in sound attenuated and ventilated cubicles (Bioseb, Vitrolles, France), as previously described (Alaux-Cantin et al., 2013; Jeanblanc et al., 2018b; Simon O'Brien et al., 2011). Briefly, each experimental chamber was equipped with one lever on the left and right side (designated as active or inactive) of the walls. A liquid dipper, placed on the center of the left and the right sides was fitted to receive fluid deliveries from an ethanol reservoir into the dipper cup. Responding in the active lever resulted in the delivery of a 0.1 mL volume of a 20% ethanol solution paired with a 2-s light-cue and 2-s time-out period following the lever press (in this time-out period, lever pressing was without consequences). During the whole session, inactive lever pressing was recorded, but had no consequences (i.e. neither light-cue nor ethanol deliveries were made available). Number of lever presses and ethanol solution deliveries were automatically recorded (PackWin software).

2.3.2. Progressive ratio

After determination of the dose of each drug that induces about 50% decrease in ethanol intake ("effective dose"), we then assessed its effect on motivation for ethanol in the progressive ratio (PR) schedule of reinforcement. In this schedule, the number of lever presses required for each ethanol reward increases after each ethanol reward; it is a task that measures an animal’s motivation to continue working for a reward as the effort requirement increases. Two 15-min PR sessions were conducted, in which the sequence of response requirement was increased by a step size of 1, 2 or 3. Concretely, animals had to press the active lever two- or three-times more than the previous event to receive a consecutive delivery of ethanol solution. The ratio sequence employed was as following: 3, 4, 5, 7, 9, 12, 15, 18, 20, 23, 25, 28, 30, 33, 35, 38, 40 ... 53. The last ratio completed in the 15 min session was defined as the breakpoint (BP). Performance in the PR sessions also followed the same protocol conducted in the dose-effect experiments (i.e. 3-days baseline, test on Wednesday and a "recovery" session). Two PR sessions were conducted, in which the injections (vehicle or the "effective" dose of each drug) were counterbalanced.

2.3.3. Reacquisition of operant ethanol self-administration

Rather than extinguishing the self-administration behavior with daily ethanol-free sessions, rats were removed from the self-administration situation for a prescribed amount of time. Thus, at the time of reacquisition testing the ethanol-reinforced associations including lever press, behavior and environmental stimuli in the self-administration situation are fully intact (Reichel and...
Bevins, 2009). Following approximately 9 days of abstinence (i.e., rats were kept in their home cage in the colony room), the effect of contingent ethanol solution delivery was assessed in all animals. To trigger memory retrieval of operant responding for ethanol, an ethanol prime (0.1 mL of 20% ethanol) was non-contingently delivered at the beginning of the session over the first 60 s (Carnicella et al., 2009, 2008; Peana et al., 2010). Afterwards, 3 active lever presses resulted in the delivery of ethanol solution, as described for the training of operant ethanol self-administration (FR-3, 15 min session). In detail, after rats reached a stable baseline, a first abstinence period was performed and a first reacquisition experiment occurred. Two reacquisition tests were conducted, in which the injections (vehicle or the “effective” dose of each drug) were counterbalanced. In this abstinence period, animals were handled and received standard care.

24. Drug injections

After operant responding stabilized in the procedures described above, rats received either i.p. or s.c. injections of either the vehicle (saline or distilled water) or the drug treatment 30 min before the start of the operant session. Injections were performed with an interval of one week between doses in a random order via a Latin square design, except GHB and Acamprosate, which was tested in increasing dose order due to some health concerns about high doses (e.g. dose-dependent weight loss) (Bowers et al., 2007).

24.1. Experiment 1: dose-response of nalmefene and (R)-Baclofen on operant ethanol consumption

Rats were randomly divided into Nalmefene (n = 11) and (R)-Baclofen (n = 10) groups. Thus, each drug was tested once per week (every Wednesday), taking 4 weeks to complete the dose-effect curves. On the other days of the week, operant sessions were conducted in the presence of saline injections; data from these last three days (Friday, Monday and Tuesday) were considered baseline days. Thursday was excluded from baseline days and considered as a “recovery” session.

24.2. Experiment 2: dose-response of Acamprosate and Naltrexone on operant ethanol consumption

When Experiment 1 was completely finished (i.e. dose-response, progressive ratio and reacquisition), Experiment 2 started. Rats from the Nalmefene group were assigned to the Acamprosate group (n = 11) and those from the (R)-Baclofen group to the Naltrexone group (n = 10). The same protocol as in Experiment 1 was followed, with the only exception that it took 5 weeks to complete the dose-effect curves for the Acamprosate group.

24.3. Experiment 3: dose-response of GHB on operant ethanol consumption

Finally, for Experiment 3, 18 rats from the Acamprosate and the Naltrexone groups were assigned to the GHB group (n = 10 rats per dose) and the experimental protocol was exactly the same as for Experiments 1 and 2. The completion of the dose-effect curves also required 5 weeks.

25. Locomotor activity

The effect of high doses of baclofen and GHB were tested at the end of the study on rats from the GHB group and thus in rats that have already been tested for three drugs. The activity monitoring chamber consists in a 40 × 40 × 30 cm box with opaque acrylic walls, transected with infrared photocell beams 2 cm above the floor at 16 sites along each side. Activity chambers were illuminated with indirect white light (20 lux). Horizontal locomotion was measured from photocell beam interruptions using ActiTrack software (Bioseb, Vitrolles, France) as we previously described (Lebourgeois et al., 2018b). Results are expressed as the total distance travelled (cm) during 15 min. In order to study the effect of (R)-Baclofen or GHB on locomotor activity, two groups of rats were exposed to saline or (R)-Baclofen (1 and 2 mg/kg) in counterbalanced order on non-consecutive days. On both days, rats received an i. p. injection of vehicle (saline) or (R)-Baclofen 30 min before being placed into the open field and allowed to freely explore the apparatus for 15 min. The same procedure was used to test the effect of GHB 200 or 300 mg/kg. For each animal, the total distance traveled during the 15-min test period was quantified using ActiTrack software (Bioseb).

2.6. Statistical analyses

Statistical analyses were performed using SigmaPlot version 11.0 for Windows. Data were analyzed using paired t-tests, one- and two-way analysis of variance (ANOVA) with repeated measures (RM-ANOVA) and followed by Student-Newman-Keuls (SNK) post hoc tests when appropriate. For each drug, a mixed-model analysis of covariance (ANCOVA) was used to evaluate the influence of baseline level on decrease in ethanol consumption. A mixed-model ANCOVA was also conducted to identify the most effective drug in reducing ethanol consumption after adjusting for the basal level of drinking. Finally, correlation analyses were performed to explore the dose-related changes in ethanol consumption. Results are expressed as mean ± SEM or mean [95%CI] throughout the text and significance criteria was set at p < 0.05.

3. Results

3.1. Effect of Nalmefene on ethanol intake, motivation and reacquisition

Results are presented on Fig. 2. Nalmefene (n = 11) was effective in reducing ethanol consumption as revealed by a significant dose effect (F(3,30) = 19.535, p < 0.001). Post hoc SNK comparisons indicated that both 0.05 and 0.1 mg/kg doses of Nalmefene significantly reduced ethanol consumption (M = 0.799, SD = 0.483 and M = 0.772, SD = 0.385, respectively) relative to vehicle treatment (dose 0) (M = 1.409, SD = 0.630; p < 0.001) (Fig. 2a). The paired t-test revealed that 0.05 mg/kg of Nalmefene significantly lowered breakpoint compared with saline control injection (16 ± 1 versus 22 ± 1) (t10 = 3.602, p = 0.005) (Fig. 2b). Nalmefene (0.05 mg/kg; n = 11) failed to alter ethanol consumption during reacquisition relative to saline control injection (t10 = 2.115, p = 0.060) (Fig. 2c).

3.2. Effect of Naltrexone on ethanol intake, motivation and reacquisition

Results are presented on Fig. 3. The results of the 1-way RM-ANOVA calculated on the effects of Naltrexone (n = 10) on ethanol consumption revealed a significant main effect of dose (F(3,27) = 27.362, p < 0.001) (Fig. 3a). Post hoc SNK tests confirmed that Naltrexone dose-dependently reduced operant ethanol drinking even at the lowest dose tested (M = 0.680, SD = 0.344 for 0.1 mg/kg; M = 0.398, SD = 0.200 for 0.5 mg/kg and M = 0.220, SD = 0.112 for 1 mg/kg) when compared to vehicle treatment (dose 0) (M = 1.032, SD = 0.262; p = 0.001 vs 0.1 mg/kg and p < 0.001 vs 0.5 and 1 mg/kg). Naltrexone (0.5 mg/kg) reduced the breakpoint relative to saline control injection (10 ± 1 versus 21 ± 1) (t9 = 11.985, p < 0.001) (Fig. 3b). The paired t-tests revealed that Naltrexone (0.5 mg/kg; n = 9) decreased ethanol consumption during reacquisition when compared with vehicle control injection.
(t_{(8)} = 10.225, p < 0.001) (Fig. 3c).

3.3. Effect of Acamprosate on ethanol intake, motivation and reacquisition

The dose effect of Acamprosate (n = 11) on ethanol consumption using four different doses is illustrated in Fig. 4. Rats drank significantly less ethanol when pretreated with Acamprosate as revealed by a significant main effect of dose (1-way RM-ANOVA, F_{(4,40)} = 19.627, p < 0.001) (Fig. 4a). Post hoc SNK comparisons indicated that 100, 200 and 400 mg/kg of Acamprosate significantly decreased ethanol consumption (M = 0.921, SD = 0.355; M = 0.797, SD = 0.290 and M = 0.147, SD = 0.312, respectively) when compared to vehicle treatment (dose 0) (M = 1.274, SD = 0.659;

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Fig. 2. Effect of Nalmefene on ethanol intake, motivation (breakpoint) and reacquisition. a) Effects of Nalmefene treatment using three different doses (0.025, 0.05 and 0.1 mg/kg; n = 11) on operant ethanol self-administration (g/kg/15min, FR-3, 15min). b) Effect of Nalmefene (0.05 mg/kg; n = 11) on motivation (breakpoint value) as a function of vehicle (black bar) and drug (grey bar) pretreatment. c) Effect of Nalmefene (0.05 mg/kg; n = 11) on reacquisition (ethanol consumption after 9 days of abstinence). **p < 0.01 and ***p < 0.001 compared to vehicle (Dose 0).

Fig. 3. Effect of Naltrexone on ethanol intake, motivation (breakpoint) and reacquisition. a) Effects of Naltrexone treatment using three different doses (0.1, 0.5 and 1 mg/kg; n = 10) on operant ethanol self-administration (g/kg/15min, FR-3, 15min). b) Effect of Naltrexone (0.5 mg/kg; n = 10) on motivation (breakpoint value) as a function of vehicle (black bar) and drug (grey bar) pretreatment. c) Effect of Naltrexone (0.5 mg/kg; n = 10) on reacquisition (ethanol consumption after 9 days of abstinence). **p < 0.01 and ***p < 0.001 compared to vehicle (Dose 0).
3.4. Effect of (R)-Baclofen on ethanol intake, motivation, reacquisition and locomotor activity

A 1-way RM-ANOVA revealed a significant main effect of (R)-Baclofen dose (F_{3,27} = 18.826, p < 0.001) on ethanol intake (Fig. 5a). Post hoc SNK comparisons revealed that (R)-Baclofen (1 and 2 mg/kg) significantly reduced ethanol intake (M = 0.658, SD = 0.604 and M = 0.00312, SD = 0.00986, respectively) relative to vehicle treatment (dose 0) (M = 1.366, SD = 0.530; p = 0.003 vs 1 mg/kg and p < 0.001 vs 2 mg/kg). (R)-Baclofen (1 mg/kg; n = 10) reduced the breakpoint relative to saline control injection (12 ± 2 versus 23 ± 1) (t_{(9)} = 4.124, p = 0.003) (Fig. 5b). A paired t-tests revealed that (R)-Baclofen (1 mg/kg; n = 10) decreased ethanol consumption during reacquisition when compared with vehicle control injection (t_{(9)} = 7.789, p < 0.001) (Fig. 5c).

As shown in Fig. 5d, a paired t-test revealed that 1 mg/kg of (R)-Baclofen (n = 11) had no significant effect on locomotor activity compared with saline control injection (t_{(10)} = -1.386, p = 0.196). However, 2 mg/kg of (R)-Baclofen (n = 11) significantly decreased the locomotor activity in rats (t_{(10)} = 4.775, p < 0.001). The results confirm that the effective 1 mg/kg dose of (R)-Baclofen failed to produce any significant effects on the locomotor activity of the rats compared to saline. Therefore, the effects of 1 mg/kg of (R)-Baclofen on operant ethanol consumption were not due to locomotor side effects.

3.5. Effect of GHB on ethanol intake, motivation, reacquisition and locomotor activity

The Fig. 6a illustrates the dose effect of GHB (n = 10) on operant ethanol consumption using four different doses. A 1-way RM-ANOVA revealed a significant main effect of GHB dose (F_{4,43} = 16.131, p < 0.001). Post hoc SNK comparisons revealed that both the medium-high and the highest dose of GHB (200 and 300 mg/kg) significantly reduced ethanol intake (M = 0.536, SD = 0.448 and M = 0.000, SD = 0.000, respectively) relative to vehicle treatment (dose 0) (M = 1.108, SD = 0.293; p = 0.006 vs 200 mg/kg and p < 0.001 vs 300 mg/kg). A paired t-test revealed that GHB (200 mg/kg, n = 10) significantly decreased the breakpoint compared with distilled water control injection (9 ± 2 versus 20 ± 1) (t_{(9)} = 4.287, p = 0.002) (Fig. 6b). A paired t-tests revealed that GHB (200 mg/kg; n = 10) decreased ethanol consumption during reacquisition when compared with vehicle control injection (t_{(9)} = 7.029, p < 0.001) (Fig. 6c).

A paired t-test showed that the 200 mg/kg dose of GHB (n = 11) had no significant effect on total traveled distance compared to distilled water control injection (t_{(10)} = 0.182, p = 0.859) (Fig. 6d). Whereas the dose of 300 mg/kg significantly decreased the locomotor activity in rats (t_{(10)} = 4.650, p < 0.001). These results confirm that the 200 mg/kg dose of GHB failed to produce any significant effects on the locomotor activity of the rats compared to their corresponding distilled water control injection. Hence, the effects of 200 mg/kg of GHB on operant ethanol consumption are not associated with locomotor side effects.

3.6. Comparison of the effectiveness of the different drugs and efficacy of the different drugs depending on the basal level of ethanol drinking

The results on the effectiveness of each drug is presented on Table 1A. It is important to notice that a complete recovery of ethanol intake (no difference with baseline level) was observed the day after treatment by each drug. Since the effectiveness may be dependent upon the basal level of ethanol intake we analyzed this possibility. We found that the
effectiveness of all drugs except Nalmefene is dependent upon the basal level of drinking (see Table 1B), even though there is only a slight correlation. After adjustment depending on the basal level of drinking, the analysis of effectiveness demonstrates that Naltrexone is more effective than GHB, Nalmefene and Acamprosate (Naltrexone > GHB > Nalmefene > Acamprosate) (see Table 2). The effectiveness of (R)-Baclofen tends to better than for Naltrexone but is not significant (p = 0.2744) with a difference in intake evolution of −0.12 [-0.34; 0.10].

The effectiveness of the different drugs on motivation are as follows: Naltrexone > (R)-Baclofen > GHB > Nalmefene > Acamprosate (see Table 1C).

The effectiveness of the different drugs on reacquisition are as follows: GHB > (R)-Baclofen > Naltrexone > Acamprosate > Nalmefene (see Table 1D).

4. Discussion

In AUD, psychosocial intervention is recommended while pharmacotherapy is not recommended as a first-line treatment for non-dependent patients (i.e. for harmful use of alcohol or alcohol abuse or BD) and especially for adolescents and young adults (Rolland et al., 2016b; Rolland and Naassila, 2017). Each of the medications used here has been shown to have beneficial effects on AUD in humans. Medications are used either to maintain abstinence (i.e. Acamprosate, Naltrexone, GHB and Baclofen) or to reduce drinking (i.e. Nalmefene and Baclofen) but exclusively in patients with alcohol dependence. It is noteworthy that some recent publications reported that the current increasing use of Baclofen as a treatment for AUD is premature (Rose and Jones, 2018) and that Nalmefene may have limited efficacy in reducing alcohol consumption (Palpacuer et al., 2015).

Among all treatments only Naltrexone has been shown to be particularly effective in non-dependent heavy drinkers (Tidey et al., 2008). Our aim was to evaluate current treatments of alcohol addiction in a relevant preclinical model of BD behavior. We analyzed the efficacy on reacquisition after abstinence and on ethanol intake that are classical endpoints in clinical studies and we also measured the efficacy on the motivational properties of ethanol. Given the only moderate effect sizes of AUD drugs more recent research tried to identify “target population” and predictive factors such as genetic polymorphisms. Thus we also investigated if the effectiveness of drugs can be linked to basal drinking level because reducing the drinking risk level in patients has been recognized as a target for developing new medications of AUD by the EMA in its 2010 guideline (Rolland et al., 2016a). Here, all drugs are efficacious in reducing alcohol intake in our BD model. However, regarding motivation three drugs were very effective ((R)-Baclofen, Naltrexone and GHB), Nalmefene was moderately effective and Acamprosate was ineffective. In general, we found that drugs that are the most effective on motivation are also the most effective to reduce drinking during reacquisition after abstinence.

In the present study we used an animal model of BD behavior displaying fast (15min) and high level of ethanol intake (between 1.0 and 1.5 g/kg) to test all the current AUD pharmacotherapies (Jeanblanc et al., 2018b; Lebourgeois et al., 2018a). A major interest of our model is that we can see if the tested drug is effective in reducing ethanol intake below the threshold of intoxication (80 mg kg/15min, FR-3, 15min). b) Effect of (R)-baclofen (1 mg/kg; n = 10) on motivation (breakpoint value) as a function of vehicle (black bar) and drug (grey bar) pretreatment. c) Effect of (R)-baclofen (200 mg/kg; n = 10) on reacquisition (ethanol consumption after 9 days of abstinence). **p < 0.01 and ***p < 0.001 compared to vehicle (Dose 0). d) Effect of (R)-baclofen (1 and 2 mg/kg; n = 10) on locomotor activity (total distance travelled, cm/15min). ***p < 0.001 compared to respective vehicle.

Fig. 5. Effect of (R)-baclofen on ethanol intake, motivation (breakpoint), reacquisition and locomotor activity. a) Effects of (R)-baclofen treatment using three different doses (0.5, 1 and 2 mg/kg; n = 10) on operant ethanol self-administration (g/
Jeanblanc et al., 2018b), because the mean level of basal intake is relatively high (1.0–1.5 g/kg). All drugs were effective in reducing ethanol intake in a dose-response manner and at doses devoid of locomotor side effects especially for (R)-Baclofen and GHB. In general, all drugs, and all doses tested, had no effect the day after treatment and thus all rats recovered very quickly after a single injection. Interestingly we observed for most of drugs that their effectiveness was higher in “heavy drinkers”. The three drugs Naltrexone, (R)-Baclofen and GHB that were effective on motivation were also effective on reacquisition after abstinence. Nalmefene and Acamprosate displayed a limited or no effectiveness on motivation and had no effect on reacquisition.

It is important to keep in mind that we used an animal of BD behavior and even if rats have been trained to binge drink for months the neuro-adaptations induced by chronic ethanol intake may be different from those induced in other models such as the post-dependent one using ethanol inhalation procedure. We cannot rule out the possibility that some rats displaying BD behavior for months may also display some signs of dependence and this needs to be further investigated in future studies. Even though our results demonstrate that all drugs are effective on ethanol intake, they also demonstrate that the most effective ones are targeting the GABAB and the mu-opioid receptors. Acamprosate is recognized in both animal and human studies as a drug that may be more effective on negative reinforcement and that may counter an hyperglutamatergic state (targeting NMDA and mGlu5 receptors), i.e. in individuals displaying high motivation to drink ethanol to relieve withdrawal signs (Holmes et al., 2013; Mann et al., 2008). It is possible that the limited efficacy observed in our model of BD behavior may be linked to the low level of relief craving and of hyperglutamatergic state. In this regard, we previously suggested in the same animal model using N-acetylcysteine that was found to be more effective in animal displaying high level of craving (during reacquisition after abstinence but not extinction) that its efficacy may be dependent upon the hyperglutamatergic state (Lebourgeois et al., 2018a). In contrast with Acamprosate, Naltrexone has been suggested to be more effective in patients with reward craving (Mann et al., 2008) and thus the fact that Naltrexone is effective on all parameters in our BD model may support that it is counteracting the positive reinforcement in animals chronically engaged in BD behavior. In this regard we have demonstrated that rats displaying BD behavior display an increased motivation to work for highly concentrated ethanol solutions and may thus also display an increased sensitivity to the positive motivational properties of ethanol (Jeanblanc et al., 2018b).

Numerous clinical trials on Acamprosate have demonstrated its effectiveness over placebo in controlling relapse, at least during the initial period of abstinence (Rösner et al., 2010). In our model and our particular paradigm of treatment, Acamprosate reduced ethanol intake but did not reduce motivation or reacquisition. It is noteworthy that in our paradigm, the treatments were administered as single injection the day of relapse and thus cannot mimic exactly what is done in humans. However, the lack of efficacy of Acamprosate on both motivation and reacquisition after abstinence and the lack of effect of Nalmefene on reacquisition support the
face validity of our BD model. Acamprosate has been shown to be efficacious to maintain abstinence (time to first relapse) but not heavy drinking during relapse in alcohol dependent patients while Naltrexone has been shown to maintain abstinence and to reduce return to heavy drinking (Maisel et al., 2013). In addition, Nalmefene is the first pharmacological intervention that is specifically indicated for alcohol reduction rather than abstinence. In our model, the effectiveness of (R)-Baclofen, Naltrexone and GHB on relapse is in accordance with results from animal and human studies (Agabio and Colombo, 2014; Caputo et al., 2016; Leone et al., 2010; Soyka and Müller, 2017).

Different types of neuro-adaptations between dependent individuals and whose with more episodic drinking (pattern of BD behavior) may also explain our results with Nalmefene. Nalmefene is like Naltrexone a mu-opioid antagonist but also a partial agonist of kappa-opioid receptors. Previous study has shown that at the dose of 0.1 mg/kg Nalmefene is more effective than Naltrexone in dependent animals compared to non-dependent animals and that the difference may be linked to a dysregulation of the dynorphin/kappa-opioid systems (Walker and Koob, 2008).

Because AUD drugs have only moderate effect sizes and because in general patients with AUD are very heterogeneous populations, a great challenge now in future clinical trials is to identify good responders and thus “target population”. Some studies have suggested that AUD drugs are likely to be more beneficial for more severe patients and/or patients with high or very high drinking risk level, for example for Baclofen and Nalmefene (Leggio et al., 2010). Nalmefene is approved in the European Union and other countries for the reduction of alcohol consumption in alcohol dependent patients with a high drinking risk level according to WHO (“target population”) (Mann et al., 2016). Identification of “target population” has led us to analyze our data on drug efficacy depending on the basal level of intake. We found that the sensitivity of animals to the different drugs is dependent upon their basal drinking level except for Nalmefene. Thus our results on Nalmefene do not fit those obtained in dependent patients but this discrepancy may be explained by the lack of dysregulation of the dynorphin/kappa-opioid system in rats displaying BD behavior in contrast to dependent animals.

This study has some limitations that have to be pointed out. We used only male rats, acute treatments and rats received multiple treatments. The latter point may not have impacted our results because we show that the recovery is complete and very quick. We have also carried out three sessions of recovery between two doses or two drugs (3 repeated baseline sessions, “washout”). It is noteworthy that even in patients, it is quite usual to test several treatments sequentially. For example, Baclofen may provide a pharmacotherapy for high need patients and those who have already failed to respond to other drug treatments (Rose and Jones, 2013).

### Table 1

Results of the analysis of the efficacy of the different drugs depending on ethanol intake (A), ethanol intake after adjustment on basal level of intake (B), breakpoint (C) and ethanol intake during reacquisition after abstinence (D).

<table>
<thead>
<tr>
<th>Drug</th>
<th>p-value</th>
<th>Mean decrease in ethanol intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-Baclofen</td>
<td>p &lt; 0.0001</td>
<td>-0.73 [-0.93; -0.53]</td>
</tr>
<tr>
<td>Naltrexone</td>
<td>p &lt; 0.0001</td>
<td>-0.48 [-0.62; -0.34]</td>
</tr>
<tr>
<td>Nalmefene</td>
<td>p = 0.0003</td>
<td>-0.33 [-0.45; -0.21]</td>
</tr>
<tr>
<td>Acamprosate</td>
<td>p = 0.0042</td>
<td>-0.27 [-0.41; -0.13]</td>
</tr>
<tr>
<td>GHB</td>
<td>p = 0.9314</td>
<td>-0.005 [-0.11; +0.10]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>p-value</th>
<th>Mean decrease in baseline level of intake for a 1 point increase in ethanol intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naltrexone</td>
<td>p = 0.0008</td>
<td>-0.81 [-1.25; -0.37]</td>
</tr>
<tr>
<td>(R)-Baclofen</td>
<td>p = 0.0196</td>
<td>-0.67 [-1.23; -0.11]</td>
</tr>
<tr>
<td>GHB</td>
<td>p = 0.0323</td>
<td>-0.31 [-0.59; -0.03]</td>
</tr>
<tr>
<td>Nalmefene</td>
<td>p = 0.02</td>
<td>-0.29 [-0.53; -0.05]</td>
</tr>
<tr>
<td>Acamprosate</td>
<td>p = 0.1291</td>
<td>-0.17 [-0.39; +0.05]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>p-value</th>
<th>Mean decrease in breakpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naltrexone</td>
<td>p &lt; 0.0001</td>
<td>-10.6 [-12.4; -8.8]</td>
</tr>
<tr>
<td>(R)-Baclofen</td>
<td>p = 0.0026</td>
<td>-10.4 [-15.4; -5.4]</td>
</tr>
<tr>
<td>GHB</td>
<td>p = 0.002</td>
<td>-10.2 [-15.0; -5.4]</td>
</tr>
<tr>
<td>Nalmefene</td>
<td>p = 0.0048</td>
<td>-5.5 [-2.5; -8.6]</td>
</tr>
<tr>
<td>Acamprosate</td>
<td>p = 0.2763</td>
<td>-2.0 [-5.5; -1.5]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>p-value</th>
<th>Mean decrease in ethanol intake during reacquisition</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHB</td>
<td>p &lt; 0.0001</td>
<td>-0.92 [-1.21; -0.63]</td>
</tr>
<tr>
<td>(R)-Baclofen</td>
<td>p &lt; 0.0001</td>
<td>-0.89 [-1.14; 0.63]</td>
</tr>
<tr>
<td>Naltrexone</td>
<td>p &lt; 0.0001</td>
<td>-0.68 [-0.79; -0.52]</td>
</tr>
<tr>
<td>Nalmefene</td>
<td>p = 0.0591</td>
<td>-0.30 [-0.75; 0.19]</td>
</tr>
<tr>
<td>Acamprosate</td>
<td>p = 0.0065</td>
<td>-0.22 [-0.46; 0.01]</td>
</tr>
</tbody>
</table>

### Table 2

Results of the analysis of the efficacy of the different drugs depending on the basal level of ethanol drinking. Data are presented after adjustment depending on the basal level of drinking.

<table>
<thead>
<tr>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.1043</td>
<td>0.1083</td>
</tr>
<tr>
<td>Baseline level</td>
<td>-0.3546</td>
<td>0.07291</td>
</tr>
<tr>
<td>Acamprosate</td>
<td>0.2305</td>
<td>0.09939</td>
</tr>
<tr>
<td>(R)-Baclofen</td>
<td>-0.1166</td>
<td>0.0664</td>
</tr>
<tr>
<td>GHB</td>
<td>0.4192</td>
<td>0.1030</td>
</tr>
<tr>
<td>Nalmefene</td>
<td>0.2696</td>
<td>0.1070</td>
</tr>
<tr>
<td>Naltrexone</td>
<td>Reference group</td>
<td>—</td>
</tr>
</tbody>
</table>
Other animal studies have also used the same approach with multiple treatments [Moore et al., 2014; Walker and Koob, 2008]. It was not possible in our study using a within-subjects design to carry out dose-response analysis on all parameters (motivation and reacquisition). Thus, another limitation is that only the intermediate dose that induced about 50% of decrease in ethanol intake for each drug has been further tested on motivation and reacquisition.

It is also important to note that we cannot exclude that differences in treatment response are related to age-related pharmacokinetic differences since, for example, about 16 weeks elapsed between the beginning of the first treatment and that of the third treatment. During the 16 weeks the mean increase in body weight was 23%. Finally since we tested the effect of only single injection, the potential development of tolerance or sensitization after repeated drug injections remains unknown.

Altogether the present results suggest that our animal model of BD behavior displays a good predictive validity that does not seem to be achieved in other models. For example, the high drinking in the dark (HDID) mice model of binge-like drinking that uses the 2-bottle choice paradigm is sensitive to Acamprosate (300 mg/kg) and (R)-Baclofen (5−10 mg/kg) but not to Naltrexone (1−8 mg/kg) [Crabbé et al., 2017]. Compared to our model, the HDID model is sensitive to higher doses of these drugs and is insensitive to Naltrexone despite the fact that Naltrexone is known to be effective in reducing ethanol drinking in several animal models.

5. Conclusion

In summary, we provide here new and original data using an animal model of BD behavior showing that most of the AUD drugs yielded promising results with the reduction of BD behavior. Our animal model appears relevant for future studies aiming at developing and screening drugs to reduce drinking in non-dependent animals that display harmful use of alcohol.

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Conflicts of interest

MN has received lecture or expert fees from Merck-Serono, Lundbeck, Indivior and Bouchara-Recordati. MCGM, SL, MD and JJ reported no biomedical financial interests or potential conflicts of interest.

Author’s contribution

MN, MCGM and JJ designed the experiments. MCGM and SL contributed to the acquisition of animal data. MN, MCGM, MD, JJ and SL assisted with data analysis and interpretation of findings. MN and MCGM wrote the paper. All authors critically reviewed content and approved final version for publication.

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