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Is R(+)-Baclofen the best option for the future of Baclofen in alcohol dependence pharmacotherapy? Insights from the preclinical side

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Abstract

For several decades, studies conducted to evaluate the efficacy of RS(±)-Baclofen in the treatment of alcohol dependence yielded contrasting results. Human and animal studies recently questioned the use of the racemic drug in patients since a potential important role of the different enantiomers has been revealed with an efficacy thought to reside with the active R(+)-enantiomer. Here we conducted experiments in the postdependent rat model of alcohol dependence to compare the efficacy of R(+)-Baclofen or S(−)-Baclofen to that of RS(±)-Baclofen on ethanol intake, seeking, and relapse. R(+)-Baclofen was more effective than RS(±)-Baclofen in reducing ethanol intake and seeking during acute withdrawal and during relapse after abstinence. We also used an original population approach in order to identify drug responders. We found a significant proportion of responders to S(−)-Baclofen and RS(±)-Baclofen, displaying an increase in ethanol intake, and this increasing effect on alcohol intake was not seen in the R(+)-Baclofen group. At an intermediate dose of R(+)-Baclofen, devoid of any motor side effects, we identified a very large proportion of responders (75%) with a large decrease in ethanol intake (90% decrease). Finally, the response to RS(±)-Baclofen on ethanol intake was correlated to plasma level of Baclofen. R(+)-Baclofen and RS(±)-Baclofen were effective in reducing sucrose intake. Our study has important clinical implication since it suggests that the wide variability in the therapeutic responses of patients to RS(±)-Baclofen may come from the sensitivity to the R(+)-Baclofen but also to the one of the S(−)-Baclofen that can promote an increase in ethanol intake.

KEYWORDS

alcohol use disorder, GABAB receptor, operant oral alcohol self-administration, R(+)-Baclofen, RS(±)-Baclofen, S(−)-Baclofen

Victor Echeverry-Alzate and Jérôme Jeanblanc contributed equally to this work.

1 | INTRODUCTION

Alcohol dependence (AD) is a chronic disease associated with high rates of mortality and morbidity. The 1-year prevalence of AD in the European Union was estimated at 3.4% among people 18 to 64 years of age (women 1.7%, men 5.2%), resulting in close to 11 million affected people.[1] In France, the prevalence is also estimated to 3.4% and a recent study demonstrated that alcohol is the first cause of hospitalization.[2] Despite the high prevalence of AD and its substantial impact on mortality and disease, it has the widest treatment gap at 92% in Europe.[3] Several kinds of medication are currently available for the treatment of AD with different goals and specific mechanisms of action. Medications are used either to maintain abstinence (ie, disulfiram, acamprosate, naltrexone, GHB, and RS(±)-Baclofen) or to reduce drinking (ie, nalmefene and RS(±)-Baclofen). However several studies and meta-analyses of drug clinical trials for AD demonstrate consistent effectiveness with modest effect sizes for efficacy in reducing heavy drinking or maintaining abstinence. For example, the numbers needed to treat for benefit for acamprosate and naltrexone have been calculated around 11 to 20.[4]

Among the AD pharmacotherapies, RS(±)-Baclofen, a selective gamma-aminobutyric acid-B (GABA-B) receptor agonist, has a very special status and its use remains highly controversial. Open-label studies and clinical trials have shown that RS(±)-Baclofen can increase rates of abstinence and reduce alcohol craving and anxiety, with good tolerance and few side effect leading to its widespread off-label use in the treatment of AD, particularly in relapse prevention. A recent study in 30 patients with AD showed that RS(±)-Baclofen (30-75 mg) for at least 2 weeks attenuates alcohol cue reactivity in prefrontal brain regions associated with no changes in craving and decrease in the % of heavy drinking days.[5] The promising results suggested by some studies and an impressive and intriguing lobbying by patient associations and clinicians insensitive to evidence-based medicine led in France to a "Temporary Recommendations for Use" in AD in 2014 and a Marketing Authorization Approval for doses up to 80 mg daily in 2018.[6] Some recent publications failed to demonstrate a superior clinical outcome of RS(±)-Baclofen vs. placebo to maintain abstinence and reported that the current increasing use of RS(±)-Baclofen as a treatment for AD is premature.[7,8] Some studies have reported concerns about adverse effects of RS(±)-Baclofen especially at high doses and particularly when taken with alcohol.[9,10] The Cagliari Consensus statement suggested that RS(±)-Baclofen's superiority over placebo is yet to be established, and further knowledge of dose-response relationships is required.[11] Controversial data about RS(±)-Baclofen efficacy in AD may come from numerous parameters such as the optimal dose, highly variable plasma levels of RS(±)-Baclofen, levels of comorbid anxiety, clinical endpoints (abstinence versus intake reduction), lack of identification of responder characteristics, and AD severity.[12-15] Regarding highly variable plasma levels of RS(±)-Baclofen in AD patients, no study has been able to identify factors explaining interindividual variability.[16,17]

Efficacy of racemic Baclofen (RS(±)-Baclofen) to reduce alcohol intake in rats has been demonstrated for more than three decades.[18-23] However, it is striking to note that the doses used in the studies are very variable, generally ranging from 1 to 10 mg/kg for the reduction of alcohol intake or even 40 mg/kg for the reduction of withdrawal symptoms.[22] Preclinical studies have also demonstrated mixed results using RS(±)-Baclofen since an increase in ethanol intake has also been shown in two-bottle choice and operant procedures.[24-26] For example, RS(±)-Baclofen reduces ethanol and sucrose responding at 5mg/kg but increases ethanol and decreases sucrose responding at 1.25mg/kg.[26]

Much less studies have explored the effects of the two enantiomers of RS(±)-Baclofen, the S(−)-Baclofen and the R(+)-Baclofen. The Baclofen that is used clinically is a racemic compound that breaks down into absolute configurations of R- and S- and positive (+) and negative (−) molecular rotations. The biological action of the racemic drug Baclofen is known to reside with the active R(+)-Baclofen.[27] We have recently demonstrated that R(+)-Baclofen reduced alcohol intake, motivation to consume alcohol and alcohol relapse in a relevant animal model of binge drinking.[28-30] Altogether these data demonstrated that R(+)-Baclofen is the most interesting enantiomer; however, the data have been obtained in inbred mice and selectively bred mice and rats and no study compared the effect of R(+)-Baclofen to those of RS(±)-Baclofen and no data are available regarding interindividual responses.

In the present study, we tested the efficacy of the different Baclofen enantiomers on operant alcohol self-administration and relapse in outbred Long Evans rats. The use of outbred rats may be more appropriate to reveal interindividual variability to alcohol intake and response to Baclofen that is also analyzed in the present study. In addition we used here the postdependent state model of AD that is a more relevant model regarding the induction of a more severe AD phenotype and behavioral traits of the human disease with loss of control over ethanol intake and compulsive use.[31,32] Because the identification of good responders is of high priority in AD patients, we looked at the individual response depending on the enantiomer and the dose. Identification of good responders is a critical issue, and this point has been raised for example in a recent study and a meta-analysis on Baclofen showing that higher daily alcohol intake could be associated with a greater effect of Baclofen.[7,33]

The effect of RS(±)-Baclofen, the racemic Baclofen that is used in patients, is mediated by both R(+)-Baclofen and S(−)-Baclofen. Because S(−)-Baclofen may have opposite effects compared with those of R(+)-Baclofen, we explored individual responses to each enantiomer in order to better understand the potential reason of the wide variability observed in patients in their therapeutic responses to RS(±)-Baclofen.[16] Finally, human pharmacokinetic studies have shown a wide interindividual variability.[13] Thus, since the efficacy may be linked to pharmacokinetic factors, we quantified plasmatic Baclofen concentrations.

2 | MATERIALS AND METHODS

2.1 | Subjects

Twenty-four male Long-Evans rats (weighing 250-290 g at the beginning of the two-bottle choice procedure) were obtained from Charles River (L'Arbresle, France). Animals were individually housed under a 12-hour light/dark cycle (lights on at 7:00 am) in a temperature and humidity-controlled environment (22 ± 1°C), 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 (CEE no. 86/609) and were approved by the local research ethics committee (CREMEAP; no. APAFIS#2145-201510051547534). All efforts were made to minimize animal suffering and to reduce the number of animals used.

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. R(+)-Baclofen, S(−)-Baclofen, and RS(±)-Baclofen were obtained from (Sigma Aldrich, Saint Quentin Fallavier, France). All drugs were dissolved in 0.9% sterile saline. Drug administration was given 30 minutes before the start of operant alcohol self-administration experiments via i.p. injections. Baclofen was administered at 0.5, 1, and 2 mg/kg, in a volume of 1 mL/kg of body weight. The dose of 1.5mg/kg of R(+)-Baclofen was also tested. All solutions were used at room temperature, and doses and routes of administration were chosen accordingly to the literature.[29,34-38]

2.3 | Self-administration of high levels of ethanol in dependent rats

In order to facilitate high level of ethanol intake, rats were trained to consume 20% ethanol solution in a two-bottle-choice drinking procedure[28,39,40] followed by several weeks of operant ethanol self-administration.[28,41,42] For detailed description, see the Supporting Information section. After 5 weeks of stable levels of responding in operant ethanol self-administration (FR-3, 30 min), rats were collectively housed (two per cage) in pressured and ventilated chambers and exposed to chronic and intermittent ethanol vapor inhalation in order to induce dependence, as previously described.[41,43-45] For detailed description, see the Supporting Information section. Animals were maintained under these conditions for 12 weeks until the start of any pharmacological treatment.

During the inhalation protocol, blood ethanol concentrations were regularly determined to adjust ethanol concentration in inhalation chambers. Blood ethanol samples were collected from sublingual vein, and BECs were determined using ANALOX AM1 Instrument analyzer (IMLAB, Lille, France).

2.4 | Drug injections

After operant responding stabilized in the procedures described above, rats received i.p. injections (volume: 1 mL/kg of body weight) of either the vehicle (saline) or the drug treatment, 30 minutes before the start of the operant session. Each rat received all the different treatments at the different doses tested in a Latin square counterbalanced design with, at least, a 1-day washout period between each dose.

2.4.1 | Experiment 1: Dose-response curves for RS(±)-Baclofen and its enantiomers

The doses tested for R(+)-Baclofen, S(−)-Baclofen, and RS(±)-Baclofen were 0.5, 1, and 2 mg/kg. All rats received drug treatments twice a week (every Tuesday and Thursday), taking 5 weeks to complete the dose-effect curves. On the other days of the week, operant ethanol self-administration sessions were conducted in the presence of saline injections.

2.4.2 | Experiment 2: 1.5mg/kg R(+)-Baclofen and homemade RS(±)-Baclofen

When Experiment 1 was finished, we decided to test, within the same group of rats, the 1.5 mg/kg dose of R(+)-Baclofen and also our homemade RS(±)-Baclofen (2 mg/kg) to be sure of the enantiomer ratio (1:1).

2.4.3 | Experiment 3: Effect of R(+)-Baclofen and RS(±)-Baclofen on ethanol seeking

Three weeks after, we evaluated the effect of 1.5 mg/kg of R(+)-Baclofen, 2 mg/kg of RS(±)-Baclofen, and vehicle (saline) on ethanol-seeking behavior. A single 30-minute session of extinction responding for ethanol was performed 24 hours after the last session of operant self-administration. During the extinction sessions, rats were exposed to the operant chamber and ethanol-associated cues (light above the lever and in the magazine), but lever-pressing did not result in any ethanol presentation.[35,46-48] All three doses were tested in each rat (n=23), and extinction sessions were conducted once a week for 3 weeks. After each extinction session, ethanol self-administration rapidly recovered to baseline levels.

2.4.4 | Experiment 4: Effect of R(+)-Baclofen and RS(±)-Baclofen on relapse after abstinence

This paradigm consisted of introducing a period of forced abstinence. Here, the animals were not introduced into the operant chambers for 2 weeks, during which rats were housed in the home cage without

ethanol vapor. The animals were divided into three groups (n=8) with equivalent baseline of ethanol self-administration. The baseline corresponded to the average of the last three operant sessions before abstinence. On test day (relapse), 1.5 mg/kg of R(+)-Baclofen, 2 mg/kg of RS(±)-Baclofen, or vehicle were injected 30 minutes before the start of the operant ethanol self-administration session, and ethanol was available during the 30-minute session.

2.4.5 | Experiment 5: Effect of R(+)-Baclofen and RS(±)-Baclofen on 1% sucrose operant self administration

Rats (n=8/group) were exposed to operant sucrose self-administration in 30-minute daily sessions under a fixed-ratio 3 schedule of reinforcement. Each response resulted in delivery of 0.1 mL of sucrose (1% w/v). Following a stable self-administration baseline, we tested the effect of R(+)-Baclofen (1 and 1.5 mg/kg), 2 mg/kg of RS(±)-Baclofen or vehicle, injected (i.p) 30 minutes before the operant session, on sucrose self-administration.

2.5 | Locomotor activity

Sedation is a common cited side-effect and limits tolerability in some patients; thus, we measured locomotor activity in our animals treated with either R(+)-Baclofen or RS(±)-Baclofen.[9] The effect of high doses of R(+)-Baclofen (1.5 and 2mg/kg) and RS(±)-Baclofen (2mg/kg) were tested at the end of the study on rats that have already been tested for the three drugs. For a detailed description of the protocol, see the Supporting Information section.

2.6 | Baclofen quantification in plasma and correlation with its efficacy to reduce ethanol intake

Plasma samples collected from sublingual and retro-orbital sinus were used to determine the concentrations of baclofen by liquid chromatography-tandem mass spectrometry (LC-MS/MS) after protein precipitation, as previously described.[49] Plasma samples were collected 30, 60, and 180 minutes after RS(±)-Baclofen (2mg/kg) injection in dependent rats. Correlation was tested between Baclofen efficacy and plasma Baclofen concentrations. Efficacy to reduce ethanol intake represents the percentage of change in ethanol intake before and after treatment. For a detailed description of the protocol, see the Supporting Information section.

2.7 | Statistical analyses

The SigmaStat 4.0 (Systat Software, Inc) and Prism 8 (GraphPad) softwares were used for all statistical analyses. Data were analyzed using one- or two-way analyses of variance (ANOVA), with or without repeated measures (RM) when normality and variance equality were confirmed; otherwise, a Kruskal-Wallis test was used. After confirming the significance of the primary findings using ANOVA or Kruskal-Wallis, a significance level of $P<.05$ was applied to all remaining post hoc analyses. The design was a within subject design for Experiments 1, 2, 3, and 5 and the dosage of plasmatic Baclofen. A between subject design was used for the Experiment 4 (Relapse). The repeated factors were the doses and the treatments for all the within subjects studies. The analysis of the distribution of the population in Experiment 1 was performed using a Chi square test.

For single comparisons, we used a Student's *t* test (two-tailed); for multiple comparisons, we used a Tukey test after the ANOVA and the Dunn's test after the Kruskal-Wallis test. Data were expressed as mean ± SEM (standard error of the mean) throughout the text and figures. A Pearson test was used for the correlation studies.

3 | RESULTS

3.1 | Effect of drugs on ethanol intake

Dependent rats were daily submitted to operant alcohol self-administration sessions for 30 minutes. We tested the effects of four different doses (0, 0.5, 1, and 2 mg/kg) of the two enantiomers of Baclofen (R(+)-Baclofen and S(−)-Baclofen) separately and the commercial racemic formula (RS(±)-Baclofen) on ethanol consumption (Figure 1). We found that S(−)-Baclofen did not reduce ethanol

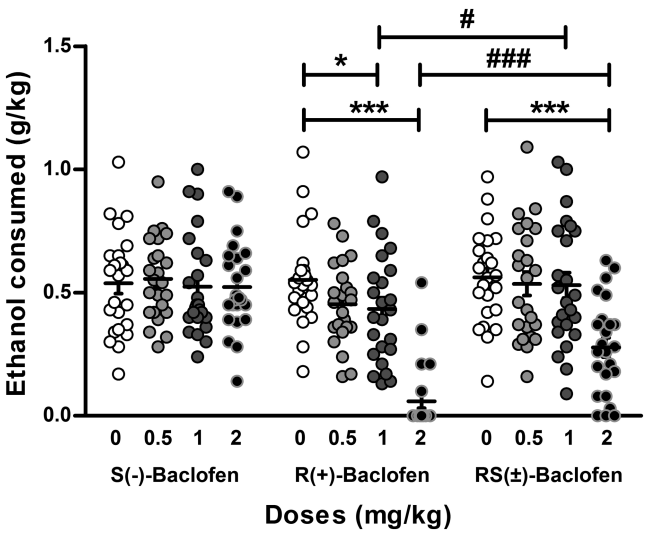

FIGURE 1 Dose-response effect of the different enantiomers of Baclofen on ethanol operant self-administration in dependent rats. n = 24. The same animal received all the different treatments but as a single injection per week. Results are expressed as mean of g of pure ethanol consumed per kg of bodyweight (g/kg). *P <.05, ***P <.001 vs. dose of 0 mg/kg same solution. #P <,05, ###P <,001 vs. same dose of RS-Baclofen. For clarity purpose, we did not represent the following differences between the doses of S-Baclofen with the other treatments. S-Baclofen vs. R-Baclofen: 0.5 mg/kg: P <.05; 2 mg/kg: P <.001. S-Baclofen vs. RS-Baclofen: 2 mg/kg: P <.001

consumption at any doses. On the contrary, R(+)-Baclofen and RS(±)-Baclofen dose dependently reduced ethanol consumption with a maximum decrease from 0.55 to 0.059 g/kg of pure ethanol consumed for the R(+)-Baclofen at the dose of 2 mg/kg. The analysis of the data using a 2-way RM-ANOVA indicated a main effect of the factor treatment ($F_{(2,138)}$ = 38.8, P <.001), of the factor doses ($F_{(3,138)}$ = 48.2, P <.001), and revealed an interaction between both factors ($F_{(6,138)}$ = 13.4, P <.001). A Tukey test was performed for multiple comparisons between treatments and doses. This post hoc test did not show any difference between the doses within the S(−)-Baclofen treatment (P >.05). Within the R(+)-Baclofen treatment, only the doses of 1 and 2 mg/kg were significantly different from the dose of 0 mg/kg (P <.05 and P <.001 respectively). Significant differences were also observed between the groups 0.5 and 1 mg/kg vs. the 2 mg/kg dose (P <.001 for both). Concerning the RS(±)-Baclofen treatment, only the dose of 2 mg/kg differs significantly from the 0, 0.5 and 1 mg/kg doses (P <.001). Within the dose of 0 mg/kg, no differences were observed between the treatments (P >.9 for all comparisons). For the dose of 0.5, a significant difference between the treatments S(−)-Baclofen and R(+)-Baclofen (P <.05) was observed, whereas for the dose of 1 mg/kg, a significant difference was obtained between the RS(±)-Baclofen) and R(+)-Baclofen treatments (P <.05). A marginal effect was nonetheless observed between the treatments S(−)-Baclofen and R(+)-Baclofen (P =.059) for this latter dose. Within the dose of 2 mg/kg, all treatments differ from the others (P <.001 for all comparisons). The effect of R(+)- and RS(±)-Baclofen is short lasting since the levels of ethanol self-administration are back to baseline the day after the injection (Figures S1A-S1C). For a detailed statistical analysis, see the Supporting information section.

The data analysis at the individual level revealed different profiles of response (Figure S2). Indeed, some animals did not respond to the drug, some others exhibited a decrease in ethanol consumption, but surprisingly, some individuals showed an increase in ethanol consumption after treatment. To estimate the proportion of responder vs. nonresponder rats, we tested two different thresholds of change as compared with the dose of 0 mg/kg, namely, 10 and 15% change. Data are presented only for the 10% change threshold because the one of 15% does not bring more information. As shown in Figure 2, we evaluated from the same set of data than in Figure 1, the proportion of each category of response for the doses of 1 (Figure 2A) and 2 mg/kg (Figure 2B) of the three treatments and for the 10% change threshold. The striking finding in this analysis is the fact that for the dose of 1 mg/kg in both the S(−)-Baclofen and RS(±)-Baclofen treatments, 29 and 25% of the rats respectively showed an increase in ethanol consumption. The Chi square analysis revealed a significant difference (P <.05) between the S(−)- and R(+)-Baclofen (Figure 2A). For the dose of 2mg/kg, only the S(−)-Baclofen induced an increase in ethanol consumption in a large proportion of rats (45.8%). Only one of the rats receiving the R(+)-Baclofen treatment showed an increase in ethanol intake (Figure 2B). The Chi square analysis revealed a significant difference in the proportions of responders vs. nonresponders between both the R(+)-Baclofen and RS(±)-Baclofen vs. the S(−)-Baclofen treatment (P <.001 and P <.01 respectively). Interestingly,

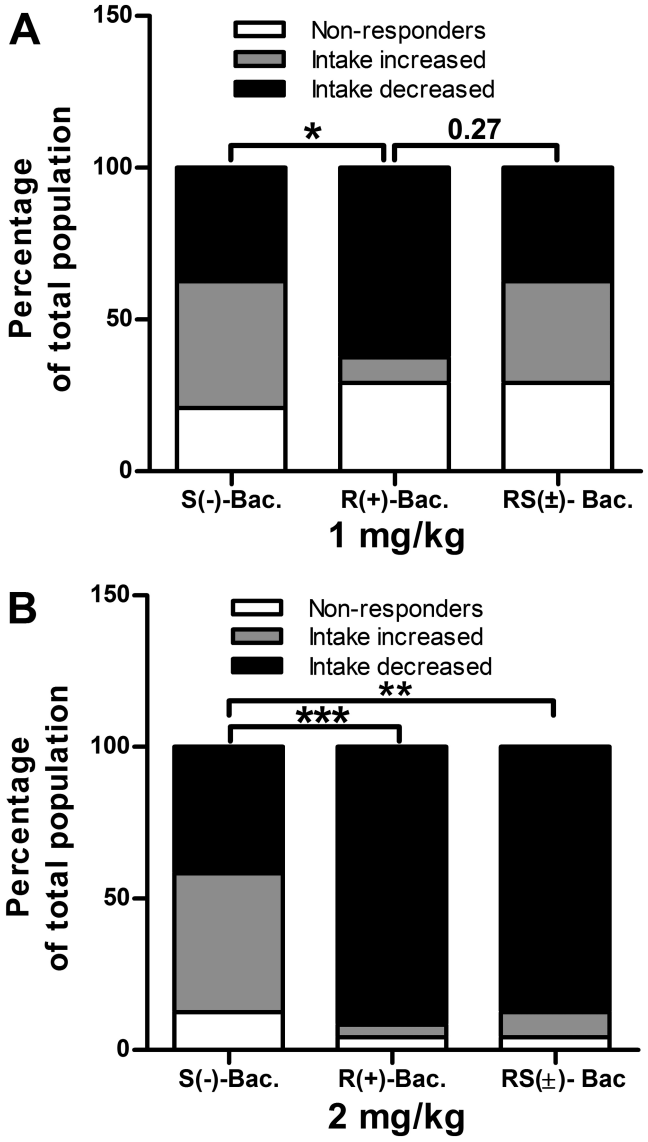

FIGURE 2 The S(−)-Baclofen enantiomere bidirectionnaly modifies ethanol self-administration. Rats were divided in three groups depending on the modification in ethanol self-administration induced by the Baclofen treatments: Nonresponders, responders displaying either an increase or a decrease in ethanol intake with a threshold of 10% change compared with Vehicle. Results are expressed as percentage of total population (n = 24) for the three different treatments at the doses of 1 mg/kg (A) and 2 mg/kg (B). *P <.05, **P <.01, and ***P <.001

the proportion of rats showing an increase in consumption after the RS(±)-Baclofen treatment dropped to 4.2 after the dose of 2 mg/kg (Figure 2B). The Chi square analysis revealed a significant difference in the proportions of responders vs. nonresponders between both the R(+)-Baclofen and RS(±)-Baclofen vs. the S(−)-Baclofen treatment (P < .01 for both comparisons).

In order to demonstrate if the doses of baclofen used here may induce locomotor side effects, we tested the effect of the doses of 1.5 and 2 mg/kg of R(+)-Baclofen vs. the effect 2 mg/kg of RS(±)-Baclofen on locomotor activity in an open field (Figure 3). We

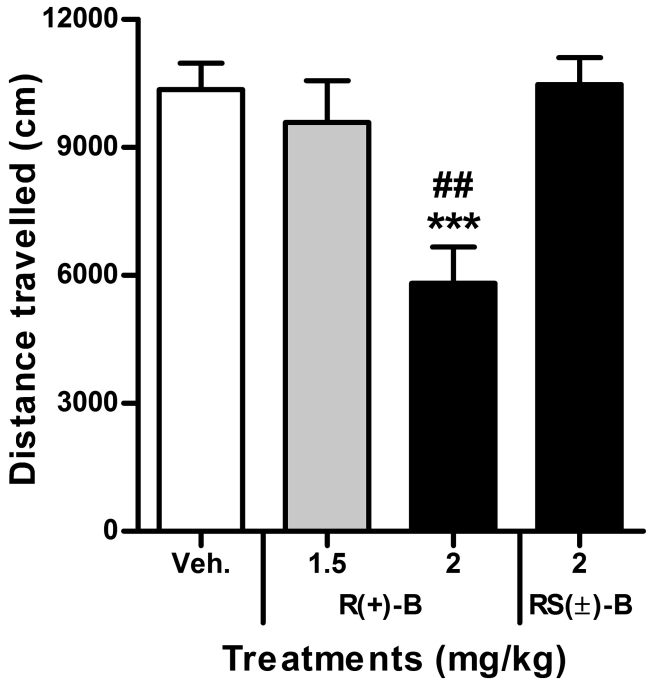

FIGURE 3 Locomotor activity is decreased with the highest dose of R(+)-Baclofen but not with the RS(±)-Baclofen. Rats were placed in the open field 30 min after the injection of saline, R(+)-Baclofen (1.5 and 2 mg/kg) and RS(±)-Baclofen (2 mg/kg) for 30-min sessions. Results are expressed as mean ± SEM of the total travelled distance in cm. ***P <.001 vs. Vehicle, ## P <.01 vs. RS(±)-Baclofen (2 mg/kg). Vehicle n = 24, 1.5 mg/kg R(+)-Baclofen n = 19, 2 mg/kg R (+)-Baclofen n = 18, 2 mg/kg RS(±)-Baclofen n = 24

found that only the dose of 2 mg/kg of R(+)-Baclofen induced a decrease in the total distance travelled. The normality test failed; thus, a Kruskal-Wallis test was performed and indicated a main effect of the factor treatment (H = 19.19.46, P <.001). The Dunn's multiple comparison test revealed significant differences between the dose of 2 mg/kg of R(+)-Baclofen vs. the vehicle group (P <.001) and vs. the treatment of RS(±)-Baclofen at 2m/kg. We thus tested this new dose of R(+)-Baclofen (1.5 mg/kg) on ethanol self-administration in comparison with the dose of 2 mg/kg of the homemade mixture 1:1 of R(+)-Baclofen/S(−)-Baclofen. We found that both treatments decreased ethanol consumption (Figure S3 and Supporting information) and that the effect is transient with a total recovery the day after the injection. A significant rebound in the amount of ethanol consumed was nevertheless noticed for the dose of 1.5 mg/kg of R(+)-Baclofen (Figure S4).

In Table 1, we depicted the percentage of rats in each category of response to the treatments determined from Experiment 1 and the corresponding variation in ethanol consumption. We found that the increase in ethanol consumption induced by the S(−)-Baclofen treatment (1 or 2 mg/kg) is of about 47%, whereas the decrease is of about 33%. The amplitudes of these increase and decrease are similar to the ones observed with the RS(±)-Baclofen treatment at 1 mg/kg (48% increase and 40% decrease) for the same order of proportions already observed (one third of the total population). When the dose was increased to 2 mg/kg, only 1 rat continued to show an increased in ethanol consumption (+21.4%), whereas 87.5% of the rats exhibited a decrease of 56.1% in their ethanol consumption. In contrast, the dose of 1 mg/kg of R(+)-Baclofen induced an increase in ethanol consumption in only 8.3% of the rats for an increase of 34.5%. The majority of the rats (54.2%) exhibited a decrease of 41.8% in ethanol consumption. For the dose of 2 mg/kg, the effect is even larger with a decrease in ethanol consumption of 95.2% for 91.7% of the rats. Only one rat showed a limited increase of 16.7% in ethanol consumed. For the intermediate dose of R (-)-Baclofen (1.5 mg/kg, which has no locomotor effect), it has a similar global effect as for the RS(±)-Baclofen dose of 2 mg/kg, and it is noteworthy that 75% of the rats showed a decrease of more than

TABLE 1 Proportion of responders (increase, decrease, and no response) after treatment with each enantiomer or the racemic compound at different doses

	S(−)-Baclofen		R(+)-Baclofen		RS(±)-Baclofen	
1 mg/kg	**% of Rats**	**% of Change**	**% of Rats**	**% of Change**	**% of Rats**	**% of Change**
Nonresponders	41.7	+0.1	35.7	−3.9	37.5	+1.6
Intake increased	29.2	*+47.9*	8.3	*+34.5*	25.0	*+48.6*
Intake decreased	29.2	−34.6	54.2	−41.8	37.5	−40.1
	S(−)-Baclofen		R(+)-Baclofen		RS(±)-Baclofen	
2 mg/kg	% of Rats	% of Change	% of Rats	% of Change	% of Rats	% of Change
Nonresponders	25.0	+6.5	4.2	0	8.3	+6.1
Intake increased	33.3	+46.9	4.2	16.7	4.2	+21.4
Intake decreased	41.7	−31.5	91.7	*−95.2*	87.5	*−56.1*
			R(+)-Baclofen			
1.5 mg/kg			% of Rats			% of Change
Nonresponders			16.7			−7.9
Intake increased			8.3			*+30.9*
Intake decreased			75			−89.3

89% of their ethanol consumption, whereas only 8.3% showed an increase of 30.9%.

3.2 | Effect of drugs on ethanol seeking

The effect of the R(+)-Baclofen at 1.5 mg/kg and the RS(±)-Baclofen at 2 mg/kg on seeking was evaluated during a single session in which no delivery of ethanol occurred even after the three consecutive presses on the active lever and the appearance of the cues (light and sound). We found that both treatments decreased the seeking (number of the active lever presses) as compared with the vehicle administration (Figure 4A). The one-way RM-ANOVA revealed a main effect of the factor treatment ($F_{(2,44)}$ = 50.03, P <.001). The post hoc test indicated a significant difference between the groups R(+)-Baclofen (1.5 mg/kg) and RS(±)-Baclofen (2 mg/kg) vs. the vehicle group (P <.001). The analysis also revealed a marginal difference between the R(+)-Baclofen (1.5 mg/kg) and RS(±)-Baclofen (2 mg/kg) treatment (P = .056).

3.3 | Effect of drugs on relapse after abstinence

One major challenge for the treatment of AD is to prevent the relapse phenomenon after abstinence. After 10 days of abstinence, rats were submitted to new sessions of self-administration with regular access to the 20% ethanol solution (FR3 – 30 min) and treated with either the vehicle, R(+)-Baclofen (1.5 mg/kg) or RS(±)-Baclofen (2 mg/kg). We found that both treatments blocked the relapse as compared with the vehicle (Figure 4B). A two-way RM-ANOVA revealed a main effect of the factor time point ($F_{(1,21)}$ = 21.96, P <.001) and of the factor treatment $F_{(2,21)}$ = 10.57, P <.001) and an interaction between both factors ($F_{(2,21)}$ = 7.94, P <.01). The post hoc analysis indicated a significant difference between the Baseline and the relapse for both

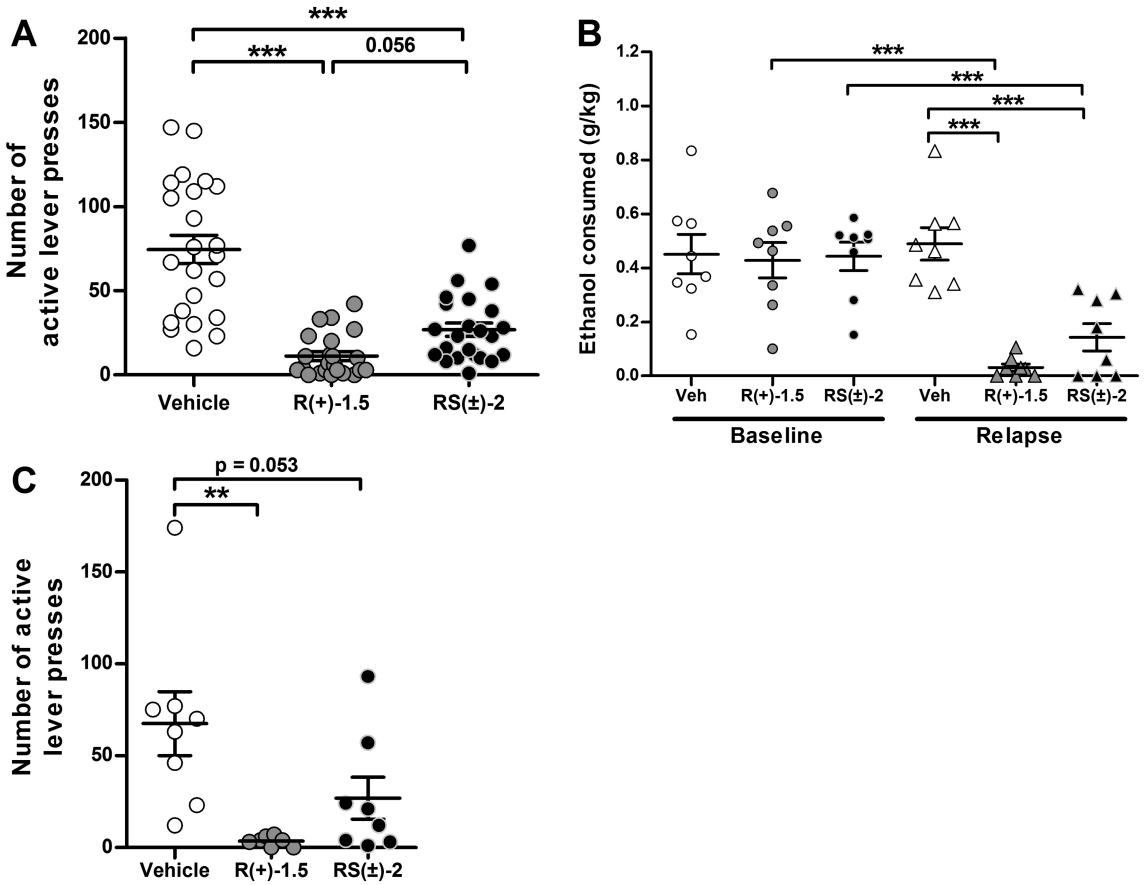

FIGURE 4 A, Both R(+) and RS(±)-Baclofen reduce ethanol seeking. Results are expressed as mean ± SEM of the number of active lever presses during the session in which no alcohol was delivered after the active lever presses. The rats received the three injections: vehicle, R(+)-Baclofen at 1.5 mg/kg, RS(±)-Baclofen at 2 mg/kg. n = 23, ***P <.001. B, Both R(+) and RS(±)-Baclofen reduce ethanol relapse. After 10 days of abstinence, rats underwent a regular self-administration session with ethanol delivered after three consecutive active lever presses. Rats were divided into three equivalent groups (n = 8) in regard with their baseline level of ethanol consumed and then received either vehicle or R(+)-Baclofen at 1.5 mg/kg or RS(±)-Baclofen at 2 mg/kg. *** P <.001. C, Baclofen treatments reduce sucrose self-administration. Alcohol-dependent rats were submitted to sucrose self-administration sessions (30-min daily sessions, 5 days a week). Once stable baseline of responding is observed, rats were divided in three groups to receive an injection of either vehicle, R(+)-Baclofen (1.5 mg/kg) or RS(±)-Baclofen (2 mg/kg). n = 8 per group, **P <.01

treatments (P <.001) but not for the vehicle group (P >.05). Within the relapse time point, the analysis revealed a significant difference between the vehicle group and both treatment groups (P <.001).

3.4 | Effect of drugs on 1% sucrose intake

The latter treatments were tested on operant self-administration of a 1% sucrose solution. We found that both treatments decreased sucrose self-administration (Figure 4C). The one-way ANOVA performed revealed a significant main effect of the factor treatment ($F_{(2,21)}$ = 7.2, P <.01). The post hoc analysis indicated a significant difference between the vehicle group and the R(+)-Baclofen group (1.5 mg/kg) (P <.01) and a marginal effect between the vehicle group and the RS(±)-Baclofen (2 mg/kg) (P =.053).

3.5 | Correlation between Baclofen plasma concentrations and its efficacy to reduce ethanol self-administration

As expected, we found a peak concentration at 30 minutes and then a rapid decrease at 60 and 180 minutes (Figure 5A). The analysis of the data indicated a main effect of the factor time ($F_{(2,44)}$ = 250.12, P <.001). The Tukey test revealed a significant difference between each of all the three time points (P <.001). It is striking to note that at 30 minutes, there was a significant correlation between the plasma Baclofen concentrations and the efficacy to reduce ethanol self-administration at both doses (R^2=0.35 and P <.01 at 1mg/kg and R^2=0.27 and P <.05 at 2mg/kg) (Figures 5B and 5C).

4 | DISCUSSION

Market authorization of RS(±)-Baclofen has been decided in France by the National Agency for the Safety of Medicines (ANSM) in October 2018 with the maximum dose requirement of 80 mg per day and use after failure of other treatments. However, the international debate about its benefit/risk balance is still open as well as some questions about the great variability in the clinical response and the lack of a dose response.[50] Further studies are thus required in order to better understand the reason of the observed variability.[16]

It is established that both RS(±)-Baclofen and R(+)-Baclofen reduce ethanol intake in rodents, while S(−)-Baclofen has no effect or increases ethanol intake.[36,51,52] Studies in high-alcohol-preferring mice have showed that RS(±)-Baclofen has stereospecific effect with the more active enantiomer, R(+)-Baclofen, suppressing alcohol intake and the less active enantiomer, S(−)-Baclofen, stimulating alcohol intake.[51,52] Furthermore, RS(±)-Baclofen (3mgkg) and R(+)-Baclofen (0.75-3mg/kg), but not S(−)-Baclofen (6-24mg/kg), reduced 15% alcohol operant self-administration in selectively bred Sardinian alcohol-preferring rats.[36] We confirmed this assumption in outbred Long Evans rats with our results showing that one administration of both

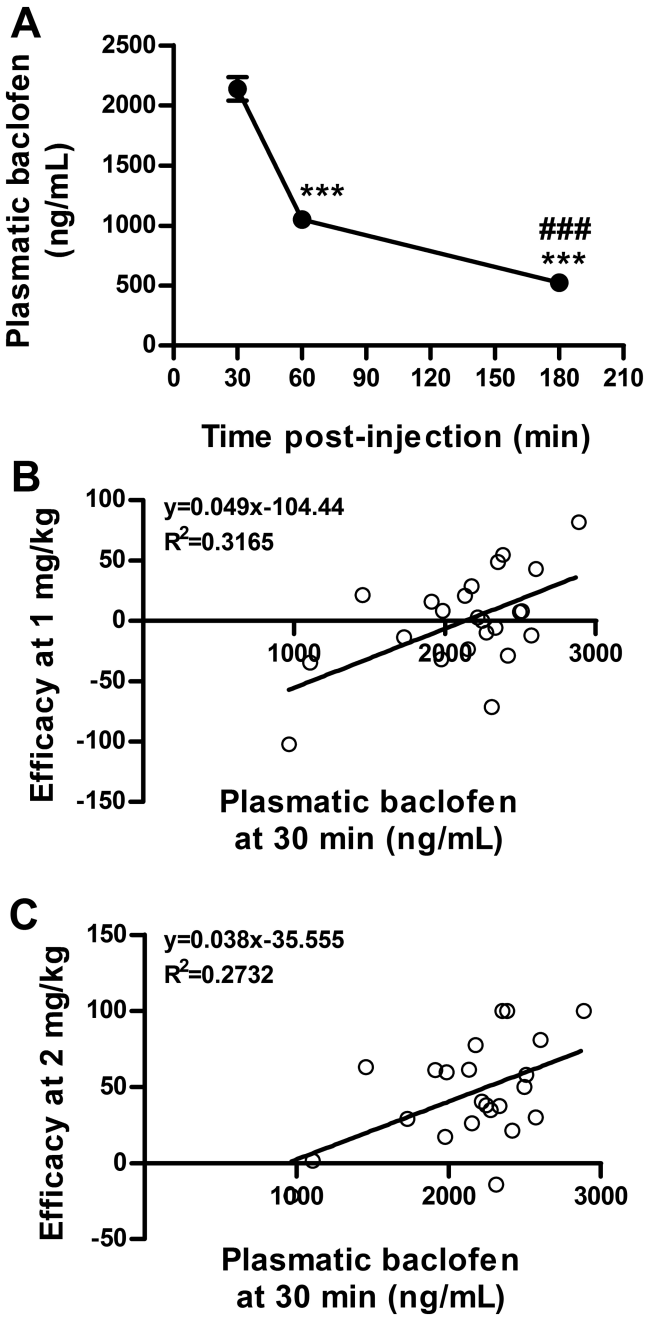

FIGURE 5 Correlation of plasma concentrations of Baclofen (1-2mg/kg) with its efficacy to reduce ethanol self-administration. A, Plasmatic Baclofen concentration 30, 60, and 180 min after injection of RS(±)-Baclofen at the 1mg/kg dose (***P <.001 compared with the 30-min time point; ###P <.001 compared with the 60-min time point). B, C, Correlation of plasmatic Baclofen concentration 30 min after injection of RS(±)-Baclofen at the 1 (A) or 2 (B) mg/kg dose

RS(±)-Baclofen and R(+)-Baclofen reduce ethanol intake, while S(−)--Baclofen can either increase or have no effect in the alcohol post-dependent state model of AD. In addition, we found that S(−)-Baclofen can also decrease ethanol intake, and to our knowledge, this effect has not been reported in the literature. Our results showed that both RS(±)-Baclofen and R(+)-Baclofen reduced 1% sucrose intake. We observed that R(+)-Baclofen is more potent to reduce

ethanol intake than RS(±)-Baclofen at both doses 1 and 2mg/kg. At 1.5mg/kg, R(+)-Baclofen induces a strong decrease of ethanol intake without any sedative side effects. R(+)-Baclofen may also be more potent on sedation because alteration of locomotor activity is observed at 2mg/kg, whereas RS(±)-Baclofen has no effect at the same dose. At 1.5 mg/kg, R(+)-Baclofen was more effective in reducing seeking behavior and relapse than 2mg/kg RS(±)-Baclofen.

As observed in humans, our study demonstrated a large variability in the response to the different enantiomers. We identified subgroups of responders, and the most striking finding shows that the efficacy of RS(±)-Baclofen used in humans may be limited by the effects of S(−)-Baclofen that can induce an increase in ethanol intake (~50% increase) in about 30% of the population.

4.1 | Efficacy and variability in the response to the different enantiomers

As found in humans,[13] we found a difference of response to RS(±)-Baclofen that is associated with a different exposure despite a similar dosage. Thus, our results confirm that the efficacy to reduce ethanol self-administration is linked to pharmacokinetic factors. We found that R(+)-Baclofen is more effective than RS(±)-Baclofen at both 1 and 2mg/kg. Interestingly, we observed a rebound effect for the 1.5mg/kg dose of R(+)-Baclofen thus further suggesting that the R(+) enantiomer may display better efficacy highlighted by the "deprivation-like" effect. Because we observed a reduction in locomotion at the 2mg/kg dose of R(+)-Baclofen, we then tested the 1.5 mg/kg dose. The latter dose induces a strong decrease in ethanol intake without having any motor side effect. Also, when looking at the mean of the population, the S(−)-Baclofen has no effect on ethanol intake. However, the analysis of individual differences revealed unexpected results with both responder and nonresponder rats. S(−)-Baclofen, at the two doses, induced an increase of ethanol intake (~50%) in about 30% of the population and a decrease of about 30% in 30 to 40% of the population. At the 1mg/kg dose, the distribution of responders to RS(±)-Baclofen is similar to that of S(−)-Baclofen, whereas at the 2mg/kg dose, the distribution of responders to (±)-Baclofen is similar to that of R(+)-Baclofen. The number of responders showing a reduction in ethanol intake is largely increased by doubling the dose of RS(±)-Baclofen and R(+)-Baclofen. Interestingly, at 1mg/kg, the proportion of rats displaying a decrease in ethanol intake is higher in the R(+)-Baclofen groups compared with the one of RS(±)-Baclofen and S(−)-Baclofen in which the proportion of rats displaying an increase in ethanol intake is still important (25 to 30%). At 1mg/kg, 29% of the population treated by S(−)-Baclofen and 30% of the population treated by RS(±)-Baclofen increased their consumption by about 50%, while a smaller increase (about 35%) is observed in less than 10% of rats treated with R(+)-Baclofen. Thus, the limited efficacy of RS(±)-Baclofen may be linked to the increasing effect of S(−)-Baclofen on ethanol intake and this opposite effects of S(−)-Baclofen and R(+)-Baclofen may explain, at least in part, the variability of the response to RS(±)-Baclofen and the reduced efficacy of RS(±)-Baclofen (-56% for RS(±)-Baclofen versus −95% for R(+)-Baclofen at the 2mg/kg dose).

Comparing our preclinical data to those obtained in patients is of interest. First, at 2.0mg/kg of RS(±)-Baclofen, we observed a decrease of about 56% (in about 80% of the population) in ethanol drinking and it is striking to note that the same level of decrease (61.8%) is observed in humans on the drinks per drinking day criterion after 30mg/day Baclofen in a 12-week trial.[53] Interestingly, in the above cited study, when looking at the individual changes in the drinks per drinking day of the 12 patients, three subgroups are observed: nonresponders (n=3), responders with decrease in drinking (n=6), and responders with an increase in drinking (n=3). Thus, the different subgroups identified in animals are also observed in patients and these results confirm that the efficacy of the RS(±)-Baclofen compound may be limited by the fact that patients are not responding but also by the fact that some patients display an increase in ethanol drinking. The same study also showed that the three female participants were among the best responders and thus suggests that looking for subgroups of responders is of great interest regarding the efficacy of RS(±)-Baclofen.[53] It is also important to point out that when looking at the outcome of drink per drinking days at the end of treatment, a recent meta-analysis showed that Baclofen has an increasing effect (mean difference 1.55, 95% CI, 1.32 to 1.77; two studies 72 participants) compared with the placebo group, but this result displays a low level of certainty of evidence.[8] Finally, our results demonstrate that the efficacy to reduce ethanol intake is correlated to plasma Baclofen concentrations. We show that a similar dosage can lead to an exposure varying from simple to triple and could explain treatment outcome as suggested in human studies.[13]

4.2 | Efficacy depending on baseline ethanol intake

Given the only moderate effect sizes of AD drugs and because in general patients with AD are very heterogeneous populations, more recent research tried to identify "target population" and predictive factors such as genetic polymorphisms. Thus, we also investigated if the effectiveness of Baclofen enantiomers 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 AD by the EMA in its 2010 guideline.[54] Some studies have suggested that AD 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.[55] 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 World Health Organization ("target population").[56] Identification of "target population" has led us to analyze our data on drug efficacy depending on the basal level of intake. Our results do not show a better efficacy in rats displaying higher ethanol intake since the efficacy of 1.5mg/kg R(+)-Baclofen is inversely correlated with ethanol intake before treatment (data not shown). Nevertheless, AD is a heterogeneous

phenotype and acquired operant alcohol intake in rats could be considered a good model of behavioral dependence, while the post-dependent state resulting from ethanol vapor inhalation could be regarded as a model of AD with physical withdrawal symptoms. It is noteworthy that the effectiveness of RS(±)-Baclofen to reduce alcohol intake in dependent animals may also be explained by its ability to decrease alcohol withdrawal-induced anxiety.[37]

4.3 | Efficacy on ethanol seeking and on relapse after abstinence

Ethanol-associated cues are powerful mediators of craving in ethanol dependent patients. Cue reactivity and cue-induced craving predict, to some degree, relapse in ethanol-dependent patients.[57] Baclofen has been shown to reduce craving in ethanol-dependent patients and possibly through a decrease in brain reactivity to ethanol-associated cues.[5,53,58] In animals, ethanol seeking could reflect ethanol craving observed in humans as ethanol-associated cues are still present while lever-responding still unreinforced. In absence of ethanol, we found that both R(+)-Baclofen and RS(±)-Baclofen reduced ethanol seeking behavior and our results are in line with those obtained in Sardinian alcohol-preferring rats with RS(±)-Baclofen (1-3mg/kg).[35] We found that both 1.5mg/kg R(+)-Baclofen and 2.0mg/kg RS(±)-Baclofen were very effective in reducing reacquisition of ethanol intake after abstinence. This result is in line with our previous findings in an animal model of chronic binge drinking showing that 1.0mg/kg R(+)-Baclofen is also very effective in reducing reacquisition of ethanol intake after abstinence.[29] Other studies in ethanol-preferring sP rats and outbred Wistar rats, which used operant self-administration procedure, have shown that RS(±)-Baclofen (1-3mg/kg) was very effective in reducing ethanol seeking induced by ethanol-associated cue after a period of extinction of lever- or nose-poke responding for ethanol.[59,60] Altogether, preclinical data show that both R(+)-Baclofen and RS(±)-Baclofen are effective to reduce ethanol seeking and reacquisition. Interestingly, when comparing the results obtained on seeking with 1.5mg/kg R(+)-Baclofen to those with 2.0mg/kg RS(±)-Baclofen, we observed that some rats are less responding to the treatment in the RS(±)-Baclofen group, while a majority of good responders was seen in the R(+)-Baclofen group; thus, the difference between the two treatments approached the level of significance (P=.056). These results are in line with those obtained in humans showing that two subpopulations of patients have been described based on their speed of response to the decreasing effect of Baclofen on craving.[58]

4.4 | Effects on sucrose intake

We found that 1.5mg/kg R(+)-Baclofen completely abolished 1% sucrose responding and this result is in line with those of another study in mice showing that R(+)-Baclofen reduced saccharin intake.[52] At 2mg/kg, RS(±)-Baclofen did not significantly reduce 1% sucrose intake and this is in contrast with other studies that showed for example that 1 to 5.6mg/kg RS(±)-Baclofen reduced sucrose responding.[19,21,26,61] Altogether, the studies show that both R(+)-Baclofen and RS(±)-Baclofen display lack of selectivity with the reduction of ethanol intake but also of alternative and nondrug reinforcers such as sucrose, saccharin, and food pellets.

4.5 | Mechanism of action of Baclofen of the different enantiomers

S(−)-Baclofen is less potent than R(+)-Baclofen.[62,63] At the pharmacological level, R(+)-Baclofen and RS(±)-Baclofen may have different behavioral outcome because S(−)-Baclofen without being itself active, especially at low dose, may increase or decrease the response to R(+)-Baclofen.[27,64] Furthermore, one human study reported a stereoselective metabolic difference between R(+)- and S(−)-Baclofen with no metabolites observed after oral administration of the R-enantiomer, whereas an oxidative deamination metabolite was observed after the administration of the R(+)- and S(−)- mixture.[65] The effect of the two enantiomers cannot be explained by a pharmacokinetic basis since the R(+)-Baclofen dose-dependently decreased ethanol consumption, whereas the high S(−)-Baclofen dose increased ethanol consumption when injected directly into the nucleus accumbens shell of C57Bl/6 J mice.[52] It would be very interesting to test the effect of the different enantiomers on striatal dopamine release after chronic alcohol intake. Nowadays, no clear explanation has been proposed to understand the bidirectional effect and the difference of efficacy of Baclofen depending on its enantiomers. One factor could be a difference in the binding affinity of the two enantiomers and the existence of low- and high-affinity GABAB receptors with low-affinity sites more selective for S(−)-Baclofen.[52,66] Another possibility is that the different enantiomers may have different neurotransmitter targets. R(+)-Baclofen may interact with nongabaergic ones,[67] while S(−)-Baclofen may interact with norepinephrine.[68]

4.6 | Limitations

Our study has some limitations that have to be pointed out. It should be noted that the level of ethanol intake of postdependent rats in the present study is lower than the one seen in other studies using Wistar rats; thus, rats of the present study may not display a severe phenotype of postdependent state. However, this low level of ethanol intake (below the 1.0g/kg threshold) has been reported in other studies that used the Long Evans strain.[69-71] We used only male rats that received multiple acute treatments. Multiple treatments may not have impacted our results because of the short half-life of Baclofen and because we show that the recovery is complete and very quick. Since we tested only the effect of single injection, the potential development of tolerance or sensitization after repeated drug injections remains unknown but seems unlikely because we used low doses and other study has already showed no tolerance after repeated injections.[69] Testing only acute treatments is a clear limitation of most of

the studies in the psychiatry field.[72] It is noteworthy that because of its short half-life (3-4 h), Baclofen treatment needs to be repeated every day, and importantly, since it can be used in a reduction goal (not abstinence), some clinicians recommends to take it as an "as needed" treatment 1 to 2 hours before the usual peak of craving depending on the time of day and on each patient. Thus, it is likely that some patients take Baclofen as needed and not every day. In France, Baclofen has a marketing authorization approval for doses up to 80 mg daily to reduce ethanol intake (not abstinence) to low drinking risk level in patients with high drinking risk level (>60 and >40g ethanol/day in men and women, respectively). We also did not carry out full dose-response curves of the different enantiomers and thus are not able to directly compare the responses at all doses, partly because locomotor side effects appeared at lower dose of the R(+) enantiomer.

4.7 | Conclusion

In summary, we provide here new and original data demonstrating that a single administration of R(+)-Baclofen is more effective than RS(±)-Baclofen and is also more effective in nondependent (heavy drinker) animals compared with dependent animals. These results are encouraging and reinforce the idea of testing R(+)-Baclofen in a clinical trial to explore if low doses may be efficient to reduce alcohol craving and intake and may also limit the occurrence of side effects as seen in patients with the racemic form of Baclofen, thus increasing the benefit/risk balance of the drug. This strategy could also avoid the lack of response or the increase of alcohol intake seen with the S(−) enantiomer. Recently, the STX209 drug comprising the single, active R-enantiomer of Baclofen and the novel prodrug of the R(+)-Baclofen, arBaclofen placarbil, have been developed and are currently under clinical study in other diseases.[73]

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CONFLICT OF INTEREST

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AUTHOR'S CONTRIBUTION

MN, VAE, and JJ designed the experiments. VEA, PS, and JJ contributed to the acquisition of animal data. LL and MS conducted experiments to measure plasmatic levels of Baclofen. MN, VAE, and JJ assisted with data analysis and interpretation of findings. MN, VAE, and JJ wrote the paper. All authors critically reviewed content and approved final version for publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Rehm J, Anderson P, Barry J, et al. Prevalence of and potential influencing factors for alcohol dependence in Europe. *Eur Addict Res*. 2015;21(1):6-18.
2. Paille F, Reynaud M. L'alcool, une des toutes premières causes d'hospitalisation en France. *BEH*. 2015;440-449.
3. Kohn R, Saxena S, Levav I, Saraceno B. The treatment gap in mental health care. *Bull World Health Organ*. 2004;82(11):858-866.
4. Jonas DE, Amick HR, Feltner C, et al. Pharmacotherapy for adults with alcohol use disorders in outpatient settings. *JAMA*. 2014;311(18):1889-1900.
5. Logge WB, Morris RW, Baillie AJ, Haber PS, Morley KC. Baclofen attenuates fMRI alcohol cue reactivity in treatment-seeking alcohol dependent individuals. *Psychopharmacology (Berl)*. 2019. https://doi.org/10.1007/s00213-019-05192-5
6. de Beaurepaire R, Benyamina A, Granger B, Hill C, Sicard D, Jaury P. Sécurité du baclofène: l'étrange appréciation de l'Agence française du médicament. *PSN*. 2018;16:37-53.
7. Rose AK, Jones A. Baclofen: its effectiveness in reducing harmful drinking, craving, and negative mood. A meta-analysis. *Addiction*. 2018;113(8):1396-1406.
8. Minozzi S, Saulle R, Rösner S. Baclofen for alcohol use disorder. *Cochrane Database Syst Rev*. 2018;11:CD012557. https://doi.org/10.1002/14651858.CD012557.pub2
9. Rolland B, Labreuche J, Duhamel A, et al. Baclofen for alcohol dependence: Relationships between baclofen and alcohol dosing and the occurrence of major sedation. *Eur Neuropsychopharmacol*. 2015;25(10):1631-1636.
10. Rolland B, Paille F, Fleury B, Cottencin O, Benyamina A, Aubin H-J. Off-label baclofen prescribing practices among French alcohol

specialists: results of a national online survey. *PLoS One*. 2014;9(6): e98062. https://doi.org/10.1371/journal.pone.0098062
11. Agabio R, Sinclair JM, Addolorato G, et al. Baclofen for the treatment of alcohol use disorder: the Cagliari Statement. *Lancet Psychiatry*. 2018;5(12):957-960.
12. Morley KC, Baillie A, Leung S, Addolorato G, Leggio L, Haber PS. Baclofen for the treatment of alcohol dependence and possible role of comorbid anxiety. *Alcohol Alcohol*. 2014;49(6):654-660.
13. Marsot A, Imbert B, Alvarez J-C, et al. High variability in the exposure of baclofen in alcohol-dependent patients. *Alcohol Clin Exp Res*. 2014; 38(2):316-321.
14. Durant CF, Paterson LM, Turton S, et al. Using Baclofen to explore GABA-B receptor function in alcohol dependence: insights from pharmacokinetic and pharmacodynamic measures. *Front Psych*. 2018;9: 664. https://doi.org/10.3389/fpsyt.2018.00664
15. Farokhnia M, Deschaine SL, Sadighi A, et al. A deeper insight into how GABA-B receptor agonism via baclofen may affect alcohol seeking and consumption: lessons learned from a human laboratory investigation. *Mol Psychiatry*. 2018;31. https://doi.org/10.1038/s41380-018-0287-y
16. Simon N, Franchitto N, Rolland B. Pharmacokinetic studies of Baclofen are not sufficient to establish an optimized dosage for management of alcohol disorder. *Front Psych*. 2018;9:485.
17. Chevillard L, Sabo N, Tod M, et al. Population pharmacokinetics of oral baclofen at steady-state in alcoholic-dependent adult patients. *Fundam Clin Pharmacol*. 2018;32(2):239-248.
18. Daoust M, Saligaut C, Lhuintre JP, Moore N, Flipo JL, Boismare F. GABA transmission, but not benzodiazepine receptor stimulation, modulates ethanol intake by rats. *Alcohol*. 1987;4: 469-472.
19. Anstrom KK, Cromwell HC, Markowski T, Woodward DJ. Effect of baclofen on alcohol and sucrose self-administration in rats. *Alcohol Clin Exp Res*. 2003;27:900-908.
20. Stromberg MF. The effect of baclofen alone and in combination with naltrexone on ethanol consumption in the rat. *Pharmacol Biochem Behav*. 2004;78:743-750.
21. Janak PH, Michael GT. Comparison of the effects of allopregnanolone with direct GABAergic agonists on ethanol self-administration with and without concurrently available sucrose. *Alcohol*. 2003;30(1):1-7.
22. Colombo G, Agabio R, Carai MAM, et al. Ability of Baclofen in reducing alcohol intake and withdrawal severity: I-Preclinical Evidence. *Alcohol Clin Exp Res*. 2000;24:58-66.
23. Colombo G, Serra S, Vacca G, Carai MAM, Gessa GL. Effect of the combination of naltrexone and baclofen, on acquisition of alcohol drinking behavior in alcohol-preferring rats. *Drug Alcohol Depend*. 2005;77(1):87-91.
24. Smith BR, Boyle AE, Amit Z. The effects of the GABA(B) agonist baclofen on the temporal and structural characteristics of ethanol intake. *Alcohol*. 1999;17(3):231-240.
25. Smith BR, Robidoux J, Amit Z. GABAergic involvement in the acquisition of voluntary ethanol intake in laboratory rats. *Alcohol Alcohol*. 1992;27(3):227-231.
26. Petry NM. Benzodiazepine-GABA modulation of concurrent ethanol and sucrose reinforcement in the rat. *Exp Clin Psychopharmacol*. 1997; 5(3):183-194.
27. Olpe H-R, Demiéville H, Baltzer V, et al. The biological activity of d-baclofen (Lipresal®). *Eur J Pharmacol*. 1978;52(1):133-136.
28. Jeanblanc J, Sauton P, Jeanblanc V, et al. Face validity of a pre-clinical model of operant binge drinking: just a question of speed. *Addict Biol*. 2018;23:643-652.
29. González-Marín MC, Lebourgeois S, Jeanblanc J, Diouf M, Naassila M. Evaluation of alcohol use disorders pharmacotherapies in a new preclinical model of binge drinking. *Neuropharmacology*. 2018; 140:14-24.
30. Jeanblanc J, Rolland B, Gierski F, Martinetti MP, Naassila M. Animal models of binge drinking, current challenges to improve face validity. *Neurosci Biobehav Rev*. 2019;106:112-121.
31. Meinhardt MW, Sommer WH. Postdependent state in rats as a model for medication development in alcoholism. *Addict Biol*. 2015;20(1): 1-21.
32. Barbier E, Johnstone AL, Khomtchouk BB, et al. Dependence-induced increase of alcohol self-administration and compulsive drinking mediated by the histone methyltransferase PRDM2. *Mol Psychiatry*. 2017; 22(12):1746-1758.
33. Reynaud M, Aubin H-J, Trinquet F, et al. A randomized, placebo-controlled study of high-dose baclofen in alcohol-dependent patients—the ALPADIR study. *Alcohol Alcohol*. 2017;52(4):439-446.
34. Colombo G, Carai MAM, Gessa GL. Suppression by γ-Hydroxybutyric Acid of "Alcohol deprivation effect" in rats: preclinical evidence of its anti-relapse properties. *Front Psych*. 2012;3:95.
35. Colombo G, Vacca G, Serra S, Brunetti G, Carai MAM, Gessa GL. Baclofen suppresses motivation to consume alcohol in rats. *Psychopharmacology (Berl)*. 2003;167(3):221-224.
36. Lorrai I, Maccioni P, Gessa GL, Colombo G. R(+)-Baclofen, but not S (−)-Baclofen, alters alcohol self-administration in alcohol-preferring rats. *Front Psych*. 2016;7(68).
37. Walker BM, Koob GF. The gamma-aminobutyric acid-B receptor agonist baclofen attenuates responding for ethanol in ethanol-dependent rats. *Alcohol Clin Exp Res*. 2007;31(1):11-18.
38. Fattore L. Baclofen antagonizes intravenous self-administration of nicotine in mice and rats. *Alcohol Alcohol*. 2002;37(5):495-498.
39. Wise RA. Voluntary ethanol intake in rats following exposure to ethanol on various schedules. *Psychopharmacologia*. 1973;29(3):203-210.
40. Simms JA, Steensland P, Medina B, et al. Intermittent Access to 20% Ethanol Induces High Ethanol Consumption in Long-Evans and Wistar Rats. *Alcohol Clin Exp Res*. 2008;32(10):1816-1823.
41. Simon O'Brien E, Legastelois R, Houchi H, et al. Fluoxetine, desipramine, and the dual antidepressant milnacipran reduce alcohol self-administration and/or relapse in dependent rats. *Neuropsychopharmacology*. 2011;36(7):1518-1530.
42. Alaux-Cantin S, Warnault V, Legastelois R, et al. Alcohol intoxications during adolescence increase motivation for alcohol in adult rats and induce neuroadaptations in the nucleus accumbens. *Neuropharmacology*. 2013;67:521-531.
43. Simon-O'Brien E, Alaux-Cantin S, Warnault V, Buttolo R, Naassila M, Vilpoux C. The histone deacetylase inhibitor sodium butyrate decreases excessive ethanol intake in dependent animals. *Addict Biol*. 2015;20(4):676-689.
44. Alaux-Cantin S, Buttolo R, Houchi H, Jeanblanc J, Naassila M. Memantine reduces alcohol drinking but not relapse in alcohol-dependent rats. *Addict Biol*. 2015;20(5):890-901.
45. Houchi H, Persyn W, Legastelois R, Naassila M. The adenosine A2A receptor agonist CGS 21680 decreases ethanol self-administration in both non-dependent and dependent animals. *Addict Biol*. 2013;18: 812-825.
46. Lebourgeois S, Vilpoux C, Jeanblanc J, et al. Pharmacological activation of mGlu4 and mGlu7 receptors, by LSP2-9166, reduces ethanol consumption and relapse in rat. *Neuropharmacology*. 2018;133: 163-170.
47. Lebourgeois S, González-Marín MC, Jeanblanc J, Naassila M, Vilpoux C. Effect of N -acetylcysteine on motivation, seeking and relapse to ethanol self-administration. *Addict Biol*. 2018;23(2): 643-652.
48. Jeanblanc J, Balguerie K, Coune F, Legastelois R, Jeanblanc V, Naassila M. Light alcohol intake during adolescence induces alcohol addiction in a neurodevelopmental model of schizophrenia. *Addict Biol*. 2015;20(3):490-499.
49. Labat L, Goncalves A, Cleophax C, Megarbane B, Decleves X. Dosage du baclofène dans le plasma en chromatographie phase liquide

couplée à de la spectrométrie de masse en tandem: à propos d'un cas de surdosage. *Toxicol Anal Clin*. 2016;28:211-217.
50. Agabio R, Leggio L. Baclofen in the treatment of patients with alcohol use disorder and other mental health disorders. *Front Psych*. 2018; 9:464.
51. Kasten CR, Blasingame SN, Boehm SL. Bidirectional enantioselective effects of the GABAB receptor agonist baclofen in two mouse models of excessive ethanol consumption. *Alcohol*. 2015;49 (1):37-46.
52. Kasten CR, Boehm SL. Intra-nucleus accumbens shell injections of R (+)- and S(−)-baclofen bidirectionally alter binge-like ethanol, but not saccharin, intake in C57Bl/6J mice. *Behav Brain Res*. 2014;272: 238-247.
53. Flannery BA, Garbutt JC, Cody MW, et al. Baclofen for alcohol dependence: a preliminary open-label study. *Alcohol Clin Exp Res*. 2004;28(10):1517-1523.
54. Rolland B, Paille F, Mann K, Aubin H-J. The 2015 French guidelines on alcohol misuse, issued in partnership with the European Federation of Addiction Societies: a focus on children and adolescents. *Eur Child Adolesc Psychiatry*. 2016;25:1145-1148.
55. Leggio L, Garbutt J, Addolorato G. Effectiveness and safety of Baclofen in the treatment of alcohol dependent patients. *CNS Neurol Disord Drug Targets*. 2010;9:33-44.
56. Mann K, Torup L, Sørensen P, et al. Nalmefene for the management of alcohol dependence: review on its pharmacology, mechanism of action and meta-analysis on its clinical efficacy. *Eur Neuropsychopharmacol*. 2016;26(12):1941-1949.
57. Grüsser SM, Wrase J, Klein S, et al. Cue-induced activation of the striatum and medial prefrontal cortex is associated with subsequent relapse in abstinent alcoholics. *Psychopharmacology (Berl)*. 2004;175 (3):296-302.
58. Imbert B, Alvarez J-C, Simon N. Anticraving effect of Baclofen in alcohol-dependent patients. *Alcohol Clin Exp Res*. 2015;39(9):1602-1608.
59. Maccioni P, Pes D, Fantini N, Carai MAM, Gessa GL, Colombo G. $γ $-Hydroxybutyric acid (GHB) suppresses alcohol's motivational properties in alcohol-preferring rats. *Alcohol*. 2008;42(2):107-113.
60. Vengeliene V, Takahashi TT, Dravolina OA, et al. Efficacy and side effects of baclofen and the novel GABAB receptor positive allosteric modulator CMPPE in animal models for alcohol and cocaine addiction. *Psychopharmacology (Berl)*. 2018;235(7):1955-1965.
61. Czachowski CL, Legg BH, Stansfield KH. Ethanol and sucrose seeking and consumption following repeated administration of the GABA(B) agonist baclofen in rats. *Alcohol Clin Exp Res*. 2006;30: 812-818.
62. Falch E, Hedegaard A, Nielsen L, Jensen BR, Hjeds H, Krogsgaard-Larsen P. Comparative stereostructure-activity studies on GABAA and GABAB receptor sites and GABA uptake using rat brain membrane preparations. *J Neurochem*. 2006;47(3):898-903.
63. Witczuk B, Khaunina RA, Kupryszewski G. 3-(p-Chlorophenyl)-4-aminobutanoic acid--resolution into enantiomers and pharmacological activity. *Pol J Pharmacol Pharm*. 1980;32:187-196.
64. Fromm GH, Shibuya T, Nakata M, Terrence CF. Effects of d-baclofen and l-baclofen on the trigeminal nucleus. *Neuropharmacology*. 1990; 29(3):249-254.
65. Sanchez-Ponce R, Wang L-Q, Lu W, von Hehn J, Cherubini M, Rush R. Metabolic and pharmacokinetic differentiation of STX209 and racemic Baclofen in humans. *Metabolites*. 2012;2(3):596-613.
66. Bowery NG, Hill DR, Hudson AL. Characteristics of GABAB receptor binding sites on rat whole brain synaptic membranes. *Br J Pharmacol*. 1983;78(1):191-206.
67. Waddington JL, Cross AJ. Gaba-ergic properties of baclofen in vivo and in vitro. *Brain Res Bull*. 1980;5:503-505.
68. Karbon EW, Duman RS, Enna SJ. GABAB receptors and norepinephrine-stimulated cAMP production in rat brain cortex. *Brain Res*. 1984;306(1-2):327-332.
69. Aufrère G, Le Bourhis B, Beaugé F. Ethanol intake after chronic intoxication by inhalation of ethanol vapour in rats: behavioural dependence. *Alcohol* Published Online First. 1997;14(3):247-253.
70. Priddy BM, Carmack SA, Thomas LC, Vendruscolo JCM, Koob GF, Vendruscolo LF. Sex, strain, and estrous cycle influences on alcohol drinking in rats. *Pharmacol Biochem Behav*. 2017;152:61-67.
71. Funk D, Coen K, Tamadon S, Lê AD. Effect of chronic alcohol vapor exposure on reinstatement of alcohol seeking induced by U50,488. *Neuropharmacology*. 2019;148:210-219.
72. Bespalov A, Müller R, Relo AL, Hudzik T. Drug tolerance: a known unknown in translational neuroscience. *Trends Pharmacol Sci*. 2016;37 (5):364-378.
73. Lal R, Sukbuntherng J, Tai EHL, et al. Arbaclofen Placarbil, a novel R-Baclofen prodrug: improved absorption, distribution, metabolism, and elimination properties compared with R-Baclofen. *J Pharmacol Exp Ther*. 2009;330(3):911-921.

SUPPORTING INFORMATION

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

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