Received: 18 October 2019 | Revised: 7 September 2020 | Accepted: 1 October 2020

DOI: 10.1002/jnr.24736

RESEARCH ARTICLE

Efficacy of N-acetylcysteine in the prevention of alcohol relapse-like drinking: Study in long-term ethanol-experienced male rats

María José Cano-Cebrián | Sandra Fernández-Rodríguez | Lucia Hipólito | Luis Granero | Ana Polache | Teodoro Zornoza

Departament de Farmàcia i Tecnologia Farmacèutica i Parasitologia, Universitat de València, Burjassot, Spain

Correspondence
Teodoro Zornoza, Departament de Farmàcia i Tecnologia Farmacèutica i Parasitologia, Universitat de València, Avda Vicente Andrés Estellés s/n, 46100, Burjassot, Spain.
Email: teodoro.zornoza@uv.es

Funding information
Generalitat Valenciana, Grant/Award Number: GVA2016-096

Abstract

Alcohol use disorders are chronic and highly relapsing disorders, thus alcoholic patients have a high rate of recidivism for drug use even after long periods of abstinence. The literature points to the potential usefulness of N-acetylcysteine (NAC) in the management of several substance use disorders probably due to its capacity to restore brain homeostasis of the glutamate system disrupted in addiction. However, there is little evidence in the case of alcohol. The aim of this study was to explore the potential anti-relapse efficacy of NAC using the alcohol deprivation effect (ADE) model in long-term experienced rats. Two experiments were performed in male Wistar rats to: (a) test the efficacy of NAC to prevent relapse and (b) discriminate the best administration schedule (intermittent vs. continuous) for NAC. In the first experiment, animals were implanted with mini-osmotic pumps delivering 0 or 1 mg/hr NAC during 14 days. In a second experiment, rats received 0, 60, or 100 mg/kg once daily by subcutaneous injection. The efficacy to prevent ADE was evaluated in both experiments. NAC subcutaneously administered, either by continuous infusion or by intermittent injections regimen, is able to block the ADE. The best results were obtained after using 60 mg/kg NAC dose. Our findings support the hypothesis that NAC may represent a valuable therapy in the management of alcohol relapse.

KEYWORDS

alcohol deprivation effect, alcohol use disorders, cue-induced relapse, ethanol relapse prevention, glutamate neurotransmission, N-acetylcysteine, pharmacotherapy

1 | INTRODUCTION

Alcohol use disorders (AUDs) are among the most prevalent and undertreated psychopathologies in developed countries (Rehm et al., 2015), causing around 3.3 million deaths/year—which represents 5.9% of all deaths worldwide—and having an enormous health and socioeconomic impact (WHO, 2014). Notwithstanding the prevalence of AUDs, few drugs have been marketed for the treatment of this neuropsychiatric disease along the last decades. Acamprosate and naltrexone, which are the latest medications for alcohol relapse prevention approved by the FDA, reduced the risk of heavy drinking only to 83% and 86% with respect to the placebo group (Rösner et al., 2010a, 2010b). Jonas et al. (2014), after a systematic review and meta-analysis, reported that no significant differences were found between both drugs. Unfortunately, these

Edited by Alex Marshall. Reviewed by Cassandra D. Gipson and Kate Reissner.

María José Cano-Cebrián and Sandra Fernández-Rodríguez contributed equally to this work.

pharmacotherapies also show low compliance rates and adverse side effects that leads to a limited efficacy (Ch'Ng & Lawrence, 2018).

AUDs can be defined as chronically relapsing disorders, in which patients have a high rate of recidivism for drug use even after long periods of abstinence have been attained. Sixty to 80% of abstinent alcoholics will relapse during their lifetime (Barrick & Connors, 2002; Weiss et al., 2001). Hence, from a clinical point of view, there is a pressing need for effective treatment to reduce the relapse rate or, to a lesser extent, reduce alcohol intake (Reilly et al., 2014; Spanagel & Vengeliene, 2012). The rationale to explore N-acetylcysteine (NAC) for AUD is due to its potential mechanism of action underlying the process of drug relapse. According to the literature, contextual stimuli associated previously with the consumption of the drug trigger the excessive release of neurotransmitters (cue-induced firing), inevitably activating the circuit that puts the relapse process in motion (Kalivas, 2009). This process is related to the glutamate neurotransmission within the mesocorticolimbic system (Gipson et al., 2013) and could be modulated through NAC (Brown et al., 2013; Olive et al., 2012). Although preclinical and clinical research has increasingly explored the potential efficacy of NAC to regulate/modify drug-related behaviors (Brown et al., 2013; McClure et al., 2014), the literature is mixed on its efficacy, particularly depending on the drug of abuse considered. For instance, with respect to cocaine, NAC has been found to reduce cocaine-seeking behavior in rats (Amen et al., 2011; Baker et al., 2003; Kupchik et al., 2012; Murray et al., 2012), to prevent the escalation of drug intake (Madayag et al., 2007), and to facilitate self-imposed abstinence after cocaine escalation (Ducret et al., 2016). Likewise, preliminary clinical data indicate that repeated administration of NAC to cocaine-dependent human subjects produced a significant reduction in craving (Amen et al., 2011). The clinical trial conducted by LaRowe et al. (2013) failed to demonstrate that NAC reduces cocaine use in actively using cocaine-dependent individuals, but they demonstrated its anti-relapse effect in patients who had already achieved abstinence from this drug. Nicotine self-administration, extinction response and cue-induced reinstatement have also been reduced in rats treated with NAC (Moro et al., 2018; Powell et al., 2019; Quintanilla et al., 2018; Ramirez-Niño et al., 2013). Human smokers treated with NAC reported a reduction in cigarette smoking but without any effect on the measurements of withdrawal and craving symptoms (Knackstedt et al., 2009). Nevertheless Froeliger et al. (2015) reported that NAC was able to prevent nicotine craving. In a general sense, meta-analyses of NAC trials support the use of NAC in ameliorating drug craving, although in some trials NAC failed to reduce craving (Deepmala et al., 2015; Duailibi et al., 2017). In a preclinical setting, effects of NAC on heroin-seeking behavior have also been identified (Hodebourg et al., 2018; Zhou & Kalivas, 2008), and a potential role in the management of methamphetamine dependence has also been claimed (McKetin et al., 2017). Thus, there is currently sufficient evidence that points to the potential usefulness of NAC in the management of several substance use disorders probably due to its capacity to restore drug-induced alterations in glutamate homeostasis within the mesocorticolimbic system (for review see McClure et al., 2014 and Morley et al., 2018).

Significance

Alcohol addiction is among the most prevalent and undertreated psychopathologies in developed countries causing around 3.3 million deaths/year worldwide. Notwithstanding these data, few drugs have been marketed for the treatment of this disease. N-acetylcysteine (NAC) has been used in therapeutic practices for several decades (as mucolytic agent, for treatment of paracetamol intoxication, etc). Literature studies suggest the potential usefulness of NAC in the management of several substance use disorders such as cocaine, nicotine, or heroine. We have gathered more evidence to lend support to the hypothesis that NAC may represent a valuable therapy in the management of alcohol relapse.

Studies with ethanol evaluating the preclinical effectiveness of NAC are scarce and show controversial results as well. For example, Weiland et al. (2015) reported that NAC did not attenuate the cue-primed reinstatement of alcohol seeking in male Sprague–Dawley rats. Quintanilla et al. (2016) demonstrated that NAC (30 or 60 mg/kg, ip) was able to reduce voluntary drug intake during the maintenance phase, but not along the acquisition phase. Later, the same group also reported that oral NAC administration during 14 days reduced ethanol binge-like behavior (Quintanilla et al., 2018). Naassila and Vilpoux's group also showed that NAC, under different alcohol exposure protocols, was able to reduce ethanol-motivational properties, ethanol-seeking behavior, and re-acquisition after abstinence in rats but it failed to limit re-acquisition after extinction (Lebourgeois et al., 2018, 2019). Curiously, in all these latter experiments, only a single dose of the drug was intraperitoneally injected 60 min before the session. Thus, it seems difficult to attribute the restoration of glutamate systems disrupted in addiction to NAC, but rather to the acute effects of NAC. Finally, Garcia-Keller et al. (2019) also demonstrated that subchronic NAC treatment, using several timing variations, is able to reduce conditioned stress reinstatement after cocaine or alcohol self-administration, expanding the potential use of this drug in the treatment of substance use disorders, given that, until now NAC had principally explored in preventing cue-induced or context-induced reinstatement.

The drug shortage to relieve abstinence syndrome symptoms and prevent relapse in alcohol-addicted individuals together with the potential mechanism of action of NAC underlying the process of drug relapse prompted us to carry out the present research. Particularly, the main objective of this study was the evaluation of the efficacy of prolonged NAC treatment in the prevention of alcohol relapse using a high face, predictive and ecological validity model in the preclinical setting such as the alcohol deprivation effect (ADE) model. Our results could provide preclinical data supporting the possible/potential

use of NAC as an adjuvant in the therapy to attenuate drug seeking and alcohol relapse.

2 | MATERIAL AND METHODS

2.1 | Animals

Two different cohorts of male Wistar rats were used for the ADE experiments: 16 rats from our own breeding colony at Faculty of Pharmacy of University of Valencia (Experiment 1) and 30 animals purchased from ENVIGO (Barcelona, Spain) for Experiment 2. All animals, weighing 275 ± 15 g at the beginning of the experiments, were housed in individual cages in a temperature- and humidity-controlled room with a 12-hr inverted light/dark cycle (on 22:00, off 10:00. All the procedures were carried out in strict accordance with Directive 2010763/EU, Spanish laws (RD 53/2013) and animal protection policies. All experiments were approved by the Animal Care Committee of University of Valencia and authorized by the Regional Government (Conselleria de Agricultura, Medio Ambiente y Cambio Climático).

2.2 | Long-term voluntary alcohol drinking with repeated deprivation phases

Rats were subjected to a long-term voluntary ethanol drinking procedure with repeated deprivation phases, which lasted for a total of 38–40 weeks. This procedure was previously validated under our experimental conditions (Orrico et al., 2013, 2014). During the procedure, animals were given continuous access to tap water and to 5%, 10%, and 20% (v/v) ethanol solutions in their home cages. Every time bottles were removed to determine the intake, the position of the four bottles was changed to avoid location preferences. Bottles were weighed from 9:00 to 10:00 a.m., just before the active phase began, in order to avoid intromissions during this period. Rats were subjected to four random drinking (6 ± 2 weeks) and deprivation (2 ± 1 weeks) periods. Hence, the duration of these periods was irregular in order to prevent behavioral adaptations (Vengeliene et al., 2005). The progression of the body weight of animals chronically exposed to alcohol followed the same pattern than that previously observed (Orrico et al., 2013, 2014). All the pharmacological studies were initiated at the end of the fourth alcohol deprivation period.

2.3 | Drugs

Alcohol drinking solutions were prepared from 96% v/v (Scharlau S.A., Spain) and then diluted with tap water to the different concentrations. NAC was purchased from Sigma-Aldrich Quimica, S.A. (Spain) and was freshly dissolved before use in phosphate buffer 0.4 M (pH was adjusted to 7.2 with NaOH), at a concentration of 100 mg/ml in all the experiments.

2.4 | Experimental design

The pharmacological study was performed when animals had approximately 9 months of ethanol experience. Daily ethanol (expressed as g/kg/day) and total fluid intake (expressed as ml/day), were calculated individually from daily weighing of the bottles before abstinence and following the reintroduction of the ethanol bottles. Basal ethanol intake was considered the average of the 4-day measurement of ethanol intake displayed before the forced abstinence period. Rats were randomly distributed to the different experimental groups ensuring no group differences in basal alcohol intake, which was then assessed by one-way analysis of variance (ANOVA). Two experiments were developed.

2.4.1 | Experiment 1: Effect of continuous NAC administration (1 mg/hr) on the ADE

The experimental design of Experiment 1 (continuous infusion of NAC 1 mg/h) was based on previous research developed in our laboratory in which D-penicillamine was administered using a similar approach. The NAC dose was selected in virtue of its maximum solubility and the repertoire of ALZET pumps (mod. 2ML1) that provides the highest flux (10 μl/hr) along a week. According to the average weight of the animals (0.55 ± 0.05 kg), the NAC dose assayed in this experiment was around 40–45 mg/kg. Hence, the assayed dose of the drug is in line with the preclinical literature, in which the most common NAC doses tested to regulate/modify drug-related behaviors range from 15 to 100 mg/kg (Lebourgeois et al., 2019; Murray et al., 2012). The pharmacological treatment was initiated during the fourth abstinence period, 9 days before the reintroduction of the ethanol bottles. The duration of the treatment, but not the administration routine, was similar to that experienced by other authors (Quintanilla et al., 2016, 2018). All the animals were briefly anesthetized with isoflurane (1.5 MAC) and a mini-osmotic Alzet® pump was subcutaneously (SC) implanted delivering either vehicle or 1 mg/hr of NAC (n = 8 rats/group). Seven days after the surgery, when the pump reservoir was almost empty, rats were, again, briefly anesthetized and a second Alzet pump was implanted in order to continue with the same treatment. Forty-eight hours after this procedure, animals were re-exposed (post-abstinence days), in their respective home cages, to the ethanol solutions. After a 21-day withdrawal period, the daily weighing routine was restored in order to assess the possible appearance of the ADE phenomenon during the four post-abstinence days (see Figure 1a). The NAC infusion-rate (1 mg/hr) assayed fits with the maximum solubility of NAC and maximal flow-rate available, as previously discussed.

2.4.2 | Experiment 2: Effects of 60 or 100 mg/kg subcutaneous NAC administration on the ADE

In a second experiment, the effect of NAC for relapse prevention on alcohol intake was evaluated using a different cohort of

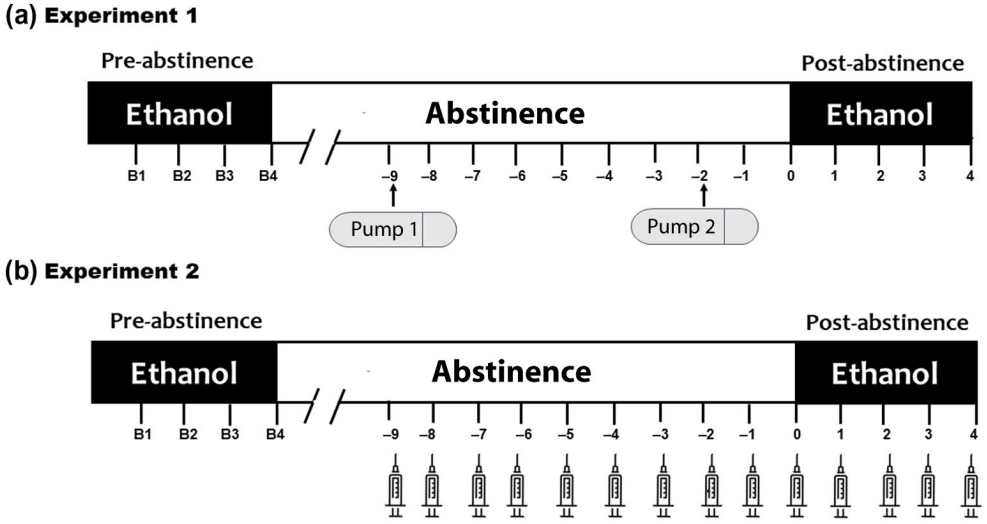

FIGURE 1 Schematic representation of the experimental procedure used in this study. (a) Experimental protocol used in Experiment 1. (b) Experimental protocol used in Experiment 2. B1, B2, B3, and B4 represent the baseline measurements taken throughout 4 days prior to the abstinence period. Hatch bars correspond to a 12-day period in both experiments, so that the total duration of the abstinence period was 21 days

rats (from ENVIGO), administration modus and doses than those used in Experiment 1. In this experiment, rats received vehicle, NAC 60 mg/kg or NAC 100 mg/kg ($n = 10$ rats/group), once a day by subcutaneous injection for 14 consecutive days (before lights were turned on in the facilities). NAC doses and schedule were selected on the basis of previous studies (Corbit et al., 2014; Ducret et al., 2016; Quintanilla et al., 2016). Nine days after the initiation of the treatment, ethanol bottles were reintroduced in all the experimental groups, and the daily ethanol intake was measured in order to evaluate whether the ADE phenomenon was expressed (see Figure 1b). As in Experiment 1, the duration of the last withdrawal period was 21 days.

2.5 | Statistics

A power analysis was performed that revealed a sample size of $N = 8$–10/group was determined necessary to detect differences in the key variables at an α level of $p < 0.05$ and 80% power. The use of a mixed two-way ANOVA to analyze the data was discarded since the observed covariance matrices of the dependent variable between groups were different (Box–Pierce test; $p = 0.00172$). Hence, ethanol intake along 8 days (4 days before and after the abstinence period) was analyzed both, by a one-way repeated-measures ANOVA, time being the targeted factor, and by a paired Student's *t* test. In this latter comparison, the daily alcohol intake obtained on the 4 days before and after the abstinence period was collapsed. When the sphericity assumption was violated, the repeated-measures ANOVA was appropriately corrected according to the Huynh–Feldt approximation (Tabachnick and Fidell, 2007). In order to analyze treatment effect, one-way ANOVA between groups were performed during the post-abstinence period alcohol intake. When significant differences were detected, multiple comparison Tukey test was applied. To ensure no group differences in basal alcohol intake among groups, a one-way ANOVA analysis was made. Total fluids intake and the weight of the animals before and after receiving the NAC treatment were analyzed by a paired Student's *t* test. All data are presented as mean ± standard deviation (*SD*) and all the analyses were carried out using IBM SPSS Statistics 24 and R.

3 | RESULTS

3.1 | Experiment 1: Constant subcutaneous infusion of N-acetylcysteine blocks the ADE expression

First of all, we confirmed that the profile and the magnitude of the ADE after the fourth deprivation period were very similar to those observed in the third period, in which no surgery was applied to the animals (data not shown), suggesting that animals did not alter their alcohol intake to alleviate post-surgical pain in Experiment 1. These results are in accordance with previous results reported by our group (Orrico et al., 2014).

Figure 2a,b show the daily time course (left panel) and the 4-day average intake of ethanol (right panel) before (basal) and after the abstinence period (post-abstinence), that lasted 21 days, in vehicle- and NAC-treated rats. One-way ANOVA confirmed that average basal alcohol intake did not show statistical differences between both experimental groups [$F(1, 62) = 0.314$; $n = 16$; $p = 0.575$]. As can be seen in Figure 2a left panel, one-way ANOVA for repeated measures showed a statistically significant effect on daily alcohol intake over time [$F(3.25, 22.76) = 3.605$; $n = 8$; $p = 0.026$] in vehicle-treated rats. Moreover, as can be observed in Figure 2a right panel, the Student's *t* test for paired measures confirmed that, after

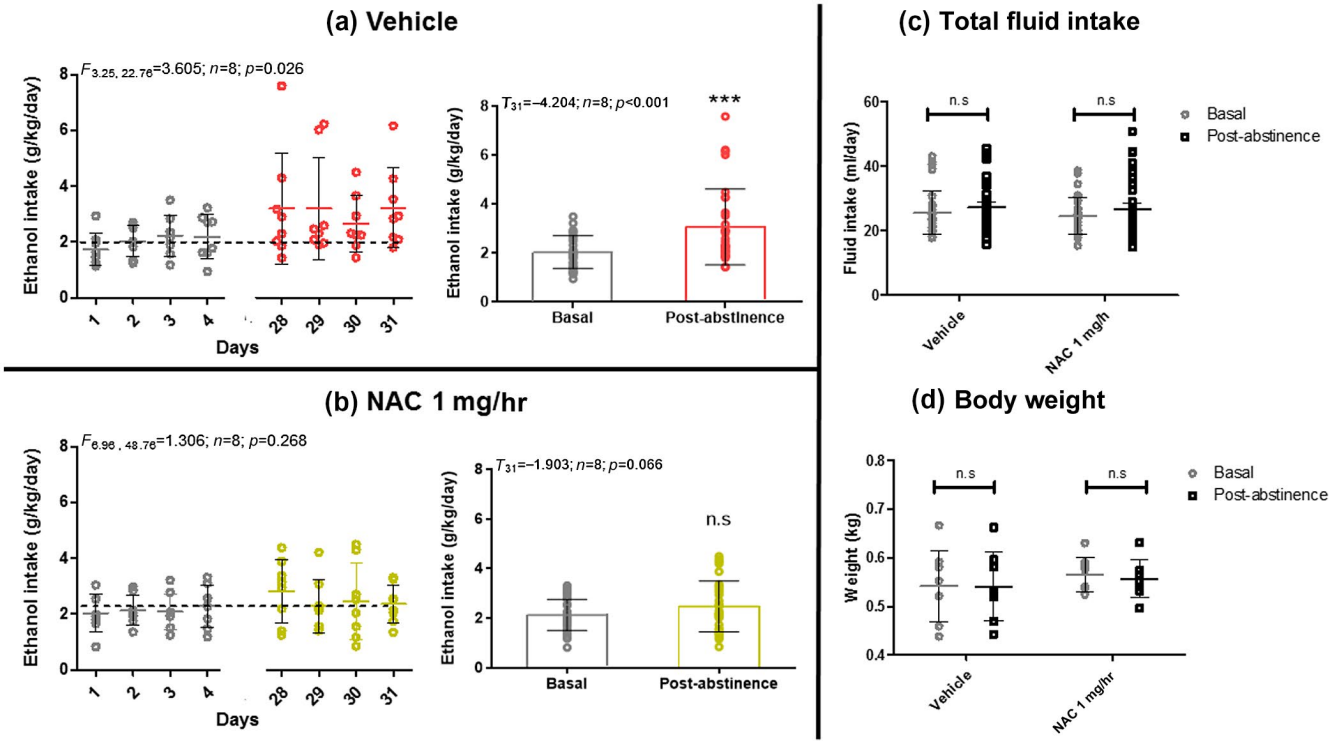

FIGURE 2 Effect of continuous administration of N-acetylcysteine (NAC) on alcohol deprivation effect (ADE) measurements. On the left part of panels (a) and (b), the mean daily ethanol intake, determined during the 4 days before (baseline) and after (post-abstinence) the imposed withdrawal period, is shown; results of the one-way repeated-measures ANOVA for ethanol intake are represented in this part of the panel. The dashed line represents the mean baseline value. B1, B2, B3, and B4 represent the baseline measurements taken throughout 4 days prior to the abstinence period. On the right part of these two panels, the collapsed values of alcohol intake determined during the 4 days of baseline and the post-abstinence period are represented as bars; the results of the Student's *t* test for the ethanol intake are included. Ethanol intake determined before and after the continuous administration of vehicle or NAC (1 mg/hr) is depicted in panel (a) and (b), respectively ($n = 8$/group). Effect of the constant subcutaneous infusion of vehicle or NAC on total fluid intake per day and body weight in rats used in Experiment 1 is shown in panel (c) and (d), respectively. *Asterisks* denote statistically significant differences between groups ($^{*}p < 0.05$, $^{***}p < 0.001$). Data are presented as mean ± *SD* [Color figure can be viewed at wileyonlinelibrary.com]

the 3-week abstinence period, average ethanol intake increased significantly, with respect to basal value, up to 50.4% [$T(31) = -4.204$; $n = 8$; $p < 0.001$] in vehicle-treated rats, thus confirming the expression of the ADE in this group of animals. On the other hand, the continuous subcutaneous administration of NAC (1 mg/hr) blocked the increase in ethanol intake on post-abstinence days, (Figure 2b). Again, statistical analysis confirmed this observation, since neither ANOVA for repeated measures [$F(6.96, 48.76) = 1.306$; $n = 8$; $p = 0.268$] (left panel) nor the paired Student's *t* test [$T(31) = -1.903$; $n = 8$; $p = 0.066$] (right panel) detected any significant difference, suggesting the potential anti-relapse effect of NAC under our experimental conditions. One-way ANOVA also confirmed the existence of a NAC treatment effect between groups during the post-abstinence period [$F(1, 62) = 6.607$; $n = 16$; $p = 0.013$)]. The total fluid intake expressed as milliliters per day, and animal body weight before and after NAC treatment is shown in Figure 2c,d, respectively. As can be observed, the total fluid intake was not modified during the post-abstinence days with respect to its respective baseline value neither in the vehicle- [$T(31) = -1.693$; $n = 8$; $p = 0.111$] nor the NAC-treated group [$T(31) = -1.550$; $n = 8$; $p = 0.131$]. Hence, the changes in ethanol intake determined in the absence or presence of NAC are not conditioned by any change or reduction in total fluid intake, suggesting that the effect of NAC on ethanol consumption is apparently selective. On the other hand, continuous infusion of NAC during 14 days did not cause any significant alteration in the body weight of the animals [$T(7) = -1.350$; $n = 8$; $p = 0.219$] evidencing no appreciable changes in food consumption patterns.

3.2 | Experiment 2: ADE expression can be inhibited by subcutaneous injections of N-acetylcysteine

Before Experiment 2 was completed, some aspects in relation to the alcohol intake profile displayed by each cohort of rats were compared. Concretely, before the pharmaceutical experiment was performed, Wistar rats from our own colony weighed 0.55 ± 0.05 kg versus 0.61 ± 0.05 kg of the purchased rats [$T(44) = -3.79$; $n = 48$; $p = 0.0004$]. Moreover, in-house bred animals showed lower total fluid intake than purchased rats [$T(182) = -2.63$; $n = 48$; $p = 0.0094$] yet, alcohol intake did not show differences between them [$T(182) = -1.81$; $n = 48$; $p = 0.0720$]. Therefore, while there was sufficient evidence to suggest that fluid consumption was different

across the cohorts, there was insufficient evidence to suggest that alcohol consumption differed.

Results of Experiment 2 are illustrated in Figure 3. As previously, Figure 3a (vehicle), 3B (NAC 60 mg/kg), and 3C (NAC 100 mg/kg) show the daily time course (left panel) and the 4-day collapsed ethanol intake (right panel) before (baseline) and after the abstinence period (post-abstinence), that lasted 21 days, obtained by each experimental group. There were no statistically significant differences in average alcohol intake among groups [$F(2, 117)$ 2.016; $n = 30$; $p = 0.138$]. As expected, after the abstinence period, vehicle-treated animals showed an increase in alcohol intake in comparison with that observed in the baseline period (Figure 3a). One-way ANOVA for repeated measures detected a statistically significant effect on daily alcohol intake over time [$F(7, 63) = 2.362$; $n = 10$; $p = 0.033$]. Student's t test for paired measures determined that average ethanol intake increased significantly after the deprivation period by up to 25% [$T(39) = -2.960$; $n = 10$; $p = 0.005$], confirming that vehicle-treated rats experienced the ADE phenomenon. As can be seen in Figure 3b,c, a 14-day treatment based on one daily subcutaneous NAC injection of 60 or 100 mg/kg, was able to block the expression of the ADE phenomenon. Statistical analysis did not reveal significant differences in ethanol intake in the post-abstinence period when rats were treated with the highest dose assayed (Figure 3c) since neither ANOVA for repeated measures detected a statistically significant effect on daily alcohol consumption over time [$F(7, 63) = 1,166$; $n = 10$; $p = 0.335$] nor did the Student's t test determine differences between mean ethanol intake values [$T(39) = -0.980$; $n = 10$; $p = 0.333$]. However, animals treated with the 60 mg/kg dose displayed a significant decrease (around −20%) in ethanol intake after the forced abstinence period, (the effect on daily alcohol intake over time was statistically significant [$F(7, 63) = 2.155$; $n = 10$; $p = 0.05$], as confirmed by ANOVA for repeated measures, and also the paired t test for collapsed values [$T(39) = 3.110$; $n = 10$; $p = 0.003$]).

One-way ANOVA also detected the existence of a NAC treatment effect between groups during the post-abstinence period [$F(2, 117) = 4.845$; $n = 30$; $p = 0.01$], identifying significant statistical differences between NAC60- and vehicle-treated animals ($p = 0.007$).Total fluid intake and animal body weight before and after NAC treatment, are illustrated in Figure 3d,e, respectively. It can be seen that the total fluid intake (Figure 3d) did not

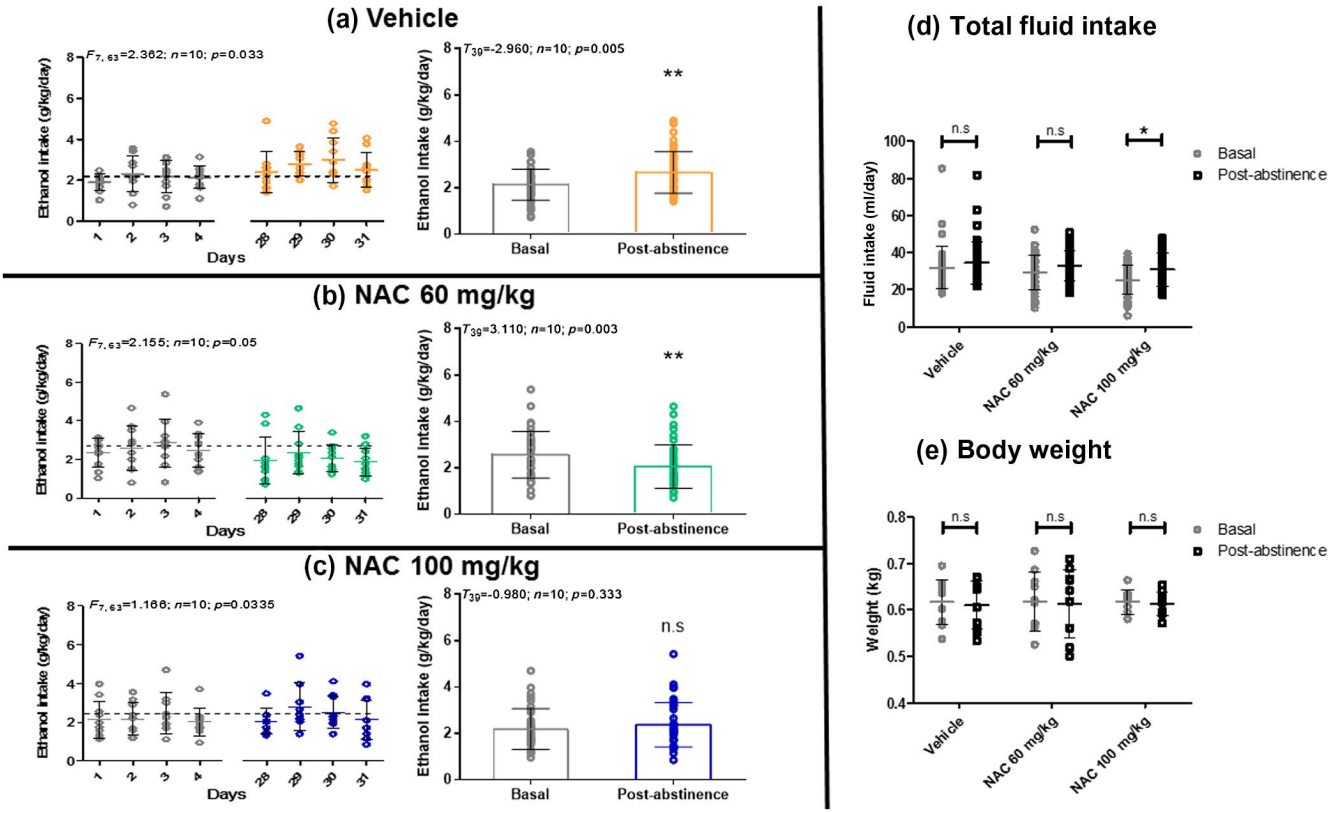

FIGURE 3 Effect of subcutaneous injections of N-acetylcysteine (NAC) on alcohol deprivation effect (ADE) measurements. Effect of the subcutaneous injections once a day for 12 days of vehicle (a), NAC 60 mg/kg (b) or NAC 100 mg/kg (c) on ethanol intake, total fluid intake (d) and body weight (e) ($n = 10$/group). On the left part of a, b, and c panels, the mean daily ethanol intake determined during the 4 days before (baseline) and after (post-abstinence) the imposed withdrawal period is shown; results of the one-way repeated-measures ANOVA for ethanol intake are represented in this part of the panel. The dashed line represents the mean baseline value. B1, B2, B3, and B4 represent the baseline measurements taken throughout 4 days prior to the abstinence period. On the right part of panels a, b, and c, the collapsed values of alcohol intake determined during the 4 days of baseline and the post-abstinence period are represented as bars; the results of the Student's t test for the ethanol intake are included. *Asterisks* denote statistically significant differences between groups ($^*p < 0.05$, $^{**}p < 0.01$). Data are presented as mean ± *SD* [Color figure can be viewed at wileyonlinelibrary.com]

decrease in any of the experimental groups during the post-abstinence period after NAC treatment. Neither in the vehicle- [$T(39, 0.05) = -1.562$; $n = 10$; $p = 0.126$] nor in the 60 mg/kg NAC-treated group [$T(39) = -1.487$; $n = 10$; $p = 0.131$] did the total fluid intake change. Curiously, the 100 mg/kg NAC-treated group showed a significant total fluid increase [$T(39) = -3.71$; $n = 10$; $p = 0.006$] in the post-abstinence period. Finally, as can be seen in Figure 3e, no significant alteration in the body weight of the animals was detected.

4 | DISCUSSION

In the present work, the potential anti-relapse efficacy of NAC in long-term ethanol-experienced rats has been explored. The most significant finding of the current study is that NAC treatment suppresses the ADE in long-term ethanol-experienced rats, an animal model with high face and predictive validity (Bell et al., 2017; Rodd et al., 2004; Sanchis-Segura & Spanagel, 2006; Spanagel, 2017). According to our results, NAC (either by SC continuous infusion or by intermittent SC injections) was able to block the ADE phenomenon. Specifically, the best results were obtained after the administration of NAC 60 mg/kg once per day through SC injection.

4.1 | The ADE model in preclinical research

Modeling alcoholism, which is a human mental disorder, in rodents is a challenge given its complexity. As widely reported, models of alcoholism in rats constitute an excellent platform for screening and developing pharmacological treatments for this disorder (Belin-Rauscent et al., 2016; Bell et al., 2012). Concretely, the ADE model is probably the most commonly used preclinical approach to ethanol relapse-drinking behavior. The rationale behind the selection of the ADE model for our research is based on the literature, supporting this model as an excellent model in its face and predictive validity (Bell et al., 2012; Spanagel, 2017). This model has been used broadly in the literature with rats from early works of Sinclair and Senter in 1967 to the latest works of different laboratories studying alcohol relapse such us Dr. Spanagel's lab, Dr. Kreek's lab, and others (Spanagel & Kiefer, 2008; Zhou & Kreek, 2019). The ADE phenomenon is a marked and transient increase in the alcohol intake over basal values following a period of deprivation, which is correlated with the alcohol relapse-like drinking behavior. This animal model encompassed the entire range of the addiction cycle, including acquisition and maintenance of drug taking, withdrawal and craving during periods of drug abstinence and ultimately relapse; processes that were repeated several times in this experimental model (Leong et al., 2018). Hitherto, preclinical studies in which NAC has been suggested to prevent alcohol relapse have relied upon a single period of abstinence (Lebourgeois et al., 2018; Quintanilla et al., 2018), which does not parallel the clinical condition as most individuals seeking treatment have experienced multiple cycles of abstinence and relapse. Hence, the use of the ADE model could overcome this limitation. Moreover, the predictive value of this experimental approach is best illustrated by the fact that the latest approved medications for alcohol relapse prevention—acamprosate and naltrexone—were developed by means of adequate animal models, more concretely this particular ADE model, and then translated into a clinical setting (Spanagel, 2017). Our laboratory has a great deal of experience in this model, and we have used it to test the ability of acetaldehyde sequestering agents (D-penicillamine) in blocking the ADE occurrence (Orrico et al., 2013, 2014).

4.2 | Anti-relapse effect of NAC: Influence of the experimental schedule

Several studies indicate that NAC may be useful in preventing relapse to drug use (Duailibi et al., 2017; Ducret et al., 2016), however, preclinical and clinical reported outcomes are not clear on its efficacy, especially when the duration of the therapy is short or the patient is not abstinent (LaRowe et al., 2013; Powell et al., 2019).

The reduction in relapse vulnerability in preclinical studies has been attributed, at least in part, to the ability of prolonged NAC treatment to reverse drug-induced plasticity (Ducret et al., 2016; Madayag et al., 2007; Reichel et al., 2011; Zhou & Kalivas, 2008). Along this line, one of the most extended hypotheses suggests that NAC is able to restore the cystine-glutamate exchanger (xCT) and/or glial Na+gradient-dependent GLT-1 expression, contributing to the normalization of the glutamate function in several key brain areas (McClure et al., 2014; Reissner et al., 2015).

At present, it is not clear if the continuous presence of NAC in the brain is required to exercise its effects on the xCT and GLT-1 function and expression. According to the principles of pharmacokinetics and therapeutics, an adequate dosing interval to be used in a multiple dosing pharmacological treatment is the elimination half-life of the drug to be dosed (Jambheckar & Breen, 2009). Hence, since the elimination half-life of NAC in plasma is extremely short (around 34 min in mice (Zhou et al., 2015) and 46 min in cats (Buur et al., 2013)) it would lead to an impracticable dosage regimen and that would not assure the constant presence of the drug in the brain. Therefore, we employed in Experiment 1, the continuous subcutaneous infusion of NAC with the aid of ALZET minipumps. This procedure, which we already used in previous studies with other anti-relapse compounds (Orrico et al., 2014), is able to assure the stable presence of the administered drug in plasma and the brain when the drug is able to cross the blood–brain barrier (Orrico et al., 2013). Using this strategy to administer NAC along 14 days, we demonstrated that this drug is able to block the ADE expression in male Wistar rats.

In order to gather more evidence and evaluate if the continuous presence of NAC is necessary to exercise its effects on ADE prevention, the second experiment was carried out using a more conventional mode of administration of NAC: the intermittent (once a day) subcutaneous injection protocol, previously used by

other authors (Corbit et al., 2014; Ducret et al., 2016; Quintanilla et al., 2016). Our results demonstrate that this administration protocol was also effective to prevent the ADE in our animal model, suggesting that, in spite of the short half-life of NAC, the use of a once a day subcutaneous injection does not hamper the efficacy of the treatment. In fact, in most studies reporting positive anti-relapse effects of NAC, the drug was administered only once a day (Ducret et al., 2016; Murray et al., 2012; Ramirez-Niño et al., 2013; Zhou & Kalivas, 2008). Moreover, Quintanilla et al. (2018) observed NAC effects after 72 hr of the last administered dose, thus indicating that chronic NAC administration generates a neurochemical effect that extends beyond the molecule's presence in the brain. NAC restores glutamate homeostasis disrupted in drug addiction when administered daily for 5 or more days (Amen et al., 2011; Knackstedt et al., 2010; McKetin et al., 2017), but an acute effect could additionally be attributed as NAC might serve as an exogenous source of cysteine. Hence, our results are in agreement with those reported by Powell et al., 2019 who observed that NAC administration (100 mg/kg/day) for 4 days failed in inhibiting nicotine seeking or accumbens synaptic plasticity but, after the administration of chronic NAC treatment (along 15 days), detected a significantly decreased extinction responding and reduced reinstatement of nicotine seeking, evidencing that the duration and the dosage regimen might be crucial in the therapeutic efficacy of NAC.

In order to rule out that a possible loss of interest in the consumption of liquids could have caused the observed reduction in ethanol intake displayed by the animals treated with NAC, total fluid intake was evaluated. As depicted in Figures 2c and 3d, total fluid intake was not significantly reduced during the post-abstinence days in any of the experimental groups, suggesting that NAC might have a specific effect on the mechanisms that mediate relapse. Curiously, total fluid intake remained unaltered in all the experimental groups except for the animals assigned to the 100 mg/kg NAC group in Experiment 2, in which a significant increase was detected. This result stood out, and it was, therefore, analyzed in depth. After our analysis, we thought that the heterogeneity of population could explain this result. Indeed, after animals were randomly assigned to different experimental groups, we determined no group differences in baseline alcohol intake but not in total fluid intake. That is to say, once the pharmacological experiment had been performed, we applied one-way ANOVA and it detected differences between groups ($F(2, 117) = 5.556$; $p = 0.005$) when total fluid intake was analyzed before the abstinence period took place. According to this result, we think that animals coincidentally assigned to 100 mg/kg NAC showed a significant lower total liquid consumption than other groups, providing a plausible explanation for the obtained result.

Hence, although this result was noteworthy, it did not invalidate the obtained outcomes. On the other hand, NAC has an established record of being safe and well tolerated (Whyte et al., 2007). As can be observed under our experimental conditions, chronic NAC treatment did not affect animal body weight. All in all, the obtained results make the effect of NAC in preventing ethanol relapse-like drinking evident, probably by counteracting neuroadaptations that occur during ethanol withdrawal without affecting total fluid consumption and body weight.

As indicated above, only a few authors have explored the potential preclinical efficacy of NAC to prevent alcohol relapse. Lebourgeois et al. showed that a single NAC dose was able to inhibit ethanol relapse-like behavior in an operant model and under two different experimental procedures: with or without forced ethanol vapor exposure. They reported that a minimum effective dose of NAC (25 –100 mg/kg), i.p. administered 1 hr before the beginning of the test, changed by virtue of the paradigm and experimental model assayed (Lebourgeois et al., 2018, 2019). In their entire research, animals had ethanol limited access (15 min) in the operant chamber on the day of the test, hence somewhat limiting the ecological validity of this model. Moreover, these results could be attributed to the acute effects of NAC, but not to its capacity to restore homeostasis to the brain glutamate system, as treatment was administered, only once, and 60 min before the operant-cued relapse testing. On the other hand, Quintanilla et al. (2018) reported that oral NAC reduced ethanol relapse binge-like drinking, in selectively bred female rats (UChB rats) treated with oral NAC (100 mg/kg/day) for 14 days during abstinence. These results were obtained after a limited re-access period lasting for 60 min. Overall, our results are in agreement with previous outcomes reported by other groups.

4.3 | Anti-relapse effect of NAC: Importance of the administered dose

Under our experimental conditions, the anti-relapse effect of NAC was higher with 60 mg/kg/day NAC than 100 mg/kg/day. This observation might fit with an inverted U-shaped dose-effect curve. From a mechanistic point of view, this result could be in agreement with data reported by Kupchik et al in 2012 using electrophysiological techniques (*in vitro* whole cell recording) that demonstrated that low concentrations of NAC inhibit glutamate transmission onto Nucleus Accumbens core spiny cells via activation of presynaptic mGluR2/3, but higher concentrations of the drug counteract this effect by stimulating mGluR5 at the postsynaptic level. Along this line, they concluded that the effect of NAC on relapse to cocaine seeking depends on the balance between stimulating mGluR2/3 and mGluR5 in this brain region of the mesocorticolimbic system. We are aware that our preclinical conditions are far from those used *in vitro* by Kupchik and colleagues, but, generally, the results obtained point in the same direction and underline the importance of selecting the appropriate dose of NAC.

4.4 | Conclusion remarks and future direction

The results obtained show that NAC effectively inhibits alcohol relapse in the ADE model. Moreover, our results also demonstrate

that, under our experimental conditions, the administration of NAC 60 mg/kg once per day through SC injection provided the best preclinical outcome. We should point out that basal alcohol intake in our rats was about 2 g/kg/day. This value is lower than that displayed by selectively bred high alcohol-consuming rat lines, although similar to that reported in previous studies using heterogeneous randomly selected Wistar rats (Juárez & Eliana, 2007; Orrico et al., 2013, 2014; Vengeliene et al., 2013). Nevertheless, our animals showed steady voluntary consumption and exhibited, repeatedly, the ADE phenomenon throughout the study (38–40 weeks), giving experimental validity to the results obtained. We are aware of the low magnitude of the reported effects, but preclinical data reproducibility, using different animal models and paradigms is, at present, a great challenge for neuroscience, which is backed up by numerous institutions (Stecker, 2015). Hence, our results, obtained in rats under a very prolonged model of ethanol experience, gather more evidence to lend support to previously reported data that suggest that NAC may represent a valuable therapy in the management of alcohol relapse.

Last but not least, the mechanism by which NAC, or another derivate, exerts its diverse effects are complex and still remain unclear. In fact, at present, it is unknown which the molecular fraction of the drug is, that correlates with its pharmacological effects (Samuni et al., 2013). Moreover, several papers have recently reported sex differences in the psycho-pharmacological effects of NAC under different indications and experimental paradigms (Goenaga et al., 2020; Monte et al., 2020). Hence, following the pipeline to the clinical evaluation of its alcohol anti-relapse effect, it would be highly recommended to previously elucidate its molecular mechanism of action, to determine plasma NAC levels that correlate with their pharmacological response as well as to evaluate different NAC treatment regimens and doses focusing on the sex and gender differences previously reported for this drug. Future experiments will address this issue.

DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the *Journal of Neuroscience Research*, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

ACKNOWLEDGMENTS

The authors are indebted to Jose A. Latorre and Raquel Montón for taking care of the animals and for helping us in the measurement of alcohol intake. The authors are grateful to F. Montes and M.C. Fuentes who kindly assisted us in the statistical analysis and, also to Preesha Gajparia for her help in typing data into the spreadsheets.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed substantially to this research, revised and approved the final version of this manuscript. *Conceptualization*, L.G., A.P., and T.Z.; *Methodology*, L.G., A.P., and T.Z.; *Acquisition of data*, M.J.C.-C., S.F.-R., and L.H.; *Formal analysis*, M.J.C.-C. and L.H.; *Writing – Original Draft*, T.Z.; *Writing – Review & Editing*, L.G., A.P., and T.Z.; *Funding Acquisition*, T.Z.; *Supervision*, A.P and T.Z.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/jnr.24736.

DATA AVAILABILITY STATEMENT

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

ORCID

María José Cano-Cebrián https://orcid.org/0000-0003-2950-6737
Sandra Fernández-Rodríguez https://orcid.org/0000-0001-5290-0826
Lucia Hipólito https://orcid.org/0000-0002-6834-5748
Teodoro Zornoza https://orcid.org/0000-0001-6362-8223

REFERENCES

Amen, S. L., Piacentine, L. B., Ahmad, M. E., Li, S. J., Mantsch, J. R., Risinger, R. C., & Baker, D. A. (2011). Repeated N-acetyl cysteine reduces cocaine seeking in rodents and craving in cocaine-dependent humans. *Neuropsychopharmacology*, *36*, 871–878.

Baker, D. A., McFarland, K., Lake, R. W., Shen, H. U. I., Toda, S., & Kalivas, P. W. (2003). N-acetyl cysteine-induced blockade of cocaine-induced reinstatement. *Annals of the New York Academy of Sciences*, *1003*, 349–351.

Barrick, C., & Connors, G. J. (2002). Relapse prevention and maintaining abstinence in older adults with alcohol-use disorders. *Drugs and Aging*, *19*, 583–594.

Belin-Rauscent, A., Fouyssac, M., Bonci, A., & Belin, D. (2016). How preclinical models evolved to resemble the diagnostic criteria of drug addiction. *Biological Psychiatry*, *79*, 39–46.

Bell, R. L., Hauser, S. R., Liang, T., Sari, Y., Maldonado-Devincci, A., & Rodd, Z. A. (2017). Rat animal models for screening medications to treat alcohol use disorders. *Neuropharmacology*, *122*, 201–243.

Bell, R. L., Sable, H. J., Colombo, G., Hyytia, P., Rodd, Z. A., & Lumeng, L. (2012). Animal models for medications development targeting alcohol abuse using selectively bred rat lines: Neurobiological and pharmacological validity. *Pharmacology, Biochemistry and Behavior*, *103*, 119–155.

Brown, R. M., Kupchik, Y. M., & Kalivas, P. W. (2013). The story of glutamate in drug addiction and of N-acetylcysteine as a potential pharmacotherapy. *JAMA Psychiatry*, *70*, 895–897.

Buur, J. L., Diniz, P. P., Roderick, K. V., KuKanich, B., & Tegzes, J. H. (2013). Pharmacokinetics of N-acetylcysteine after oral and intravenous administration to healthy cats. *American Journal of Veterinary Research*, *74*, 290–293.

Ch'Ng, S. S., & Lawrence, A. J. (2018). Investigational drugs for alcohol use disorders: A review of preclinical data. *Expert Opinion on Investigational Drugs*, *27*, 459–474.

Corbit, L. H., Chieng, B. C., & Balleine, B. W. (2014). Effects of repeated cocaine exposure on habit learning and reversal by N-acetylcysteine. *Neuropsychopharmacology*, *39*, 1893–1901.

Deepmala D., Slattery, J., Kumar, N., Delhey, L., Berk, M., Dean, O., Spielholz, C., & Frye, R. (2015). Clinical trials of N-acetylcysteine in psychiatry and neurology: A systematic review. *Neuroscience and Biobehavioral Reviews*, *55*, 294–321.

Duailibi, M. S., Cordeiro, Q., Brietzke, E., Ribeiro, M., LaRowe, S., Berk, M., & Trevizol, A. P. (2017). N-acetylcysteine in the treatment of craving in substance use disorders: Systematic review and meta-analysis. *American Journal on Addictions*, *26*, 660–666.

Ducret, E., Puaud, M., Lacoste, J., Belin-Rauscent, A., Fouyssac, M., Dugast, E., Murray, J. E., Everitt, B. J., Houeto, J., & Belin, D. (2016). N-acetylcysteine facilitates self-imposed abstinence after escalation of cocaine intake. *Biological Psychiatry*, *80*, 226–234.

Froeliger, B., McConnell, P. A., Stankeviciute, N., McClure, E. A., Kalivas, P. W., & Gray, K. M. (2015). The effects of N-acetylcysteine on frontostriatal resting-state functional connectivity, withdrawal symptoms and smoking abstinence: A double-blind, placebo-controlled fMRI pilot study. *Drug and Alcohol Dependence*, *156*, 234–242.

Garcia-Keller, C., Smiley, C., Monforton, C., Melton, S., Kalivas, P. W., & Gass, J. (2019). *N*-Acetylcysteine treatment during acute stress prevents stress-induced augmentation of addictive drug use and relapse. *Addiction Biology*, *25*, e12798.

Gipson, C. D., Reissner, K. J., Kupchik, Y. M., Smith, A. C., Stankeviciute, N., Hensley-Simon, M. E., & Kalivas, P. W. (2013). Reinstatement of nicotine seeking is mediated by glutamatergic plasticity. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 9124–9129.

Goenaga, J., Powell, G. L., Leyrer-Jackson, J. M., Piña, J., Phan, S., Prakapenka, A. V., Koebele, S. V., Namba, M. D., McClure, E. A., Bimonte-Nelson, H. A., & Gipson, C. D. (2020). N-acetylcysteine yiels sex-specific efficacy for cue induced reinstatement of nicotine seeking. *Addiction Biology*, *25*, e12711.

Hodebourg, R., Murray, J. E., Fouyssac, M., Puaud, M., Everitt, B. J., & Belin, D. (2018). Heroin seeking becomes dependent on dorsal striatal dopaminergic mechanisms and can be decreased by N-acetylcysteine. *European Journal of Neuroscience*, *50*(3), 2036–2044.

Jambheckar, S. S., & Breen, P. J. (2009). *Basic pharmacokinetics*. London, United Kingdom: Pharmaceutical Press.

Jonas, D. E., Amick, H. R., Feltner, C., Bobashev, G., Thomas, K., Wines, R., Kim, M. M., Shanahan, E., Gass, C. E., Rowe, C. J., & Garbutt, J. C. (2014). Pharmacotherapy for adults with alcohol use disorders in outpatient settings: A systematic review and meta-analysis. *JAMA*, *4*(311), 1889–1900.

Juárez, J., & Eliana, B. D. T. (2007). Alcohol consumption is enhanced after naltrexone treatment. *Alcoholism, Clinical and Experimental Research*, *31*, 260–264.

Kalivas, P. W. (2009). The glutamate homeostasis hypothesis of addiction. *Nature Reviews Neuroscience*, *10*, 561–572.

Knackstedt, L. A., LaRowe, S., Mardikian, P., Malcolm, R., Upadhyaya, H., Hedden, S., Markou, A., & Kalivas, P. W. (2009). The role of cystine-glutamate exchange in nicotine dependence in rats and humans. *Biological Psychiatry*, *65*, 841–845.

Knackstedt, L. A., Melendez, R. I., & Kalivas, P. W. (2010). Ceftriaxone restores glutamate homeostasis and prevents relapse to cocaine seeking. *Biological Psychiatry*, *67*, 81–84.

Kupchik, Y. M., Moussawi, K., Tang, X. C., Wang, X., Kalivas, B. C., Kolokithas, R., Ogburn, K. B., & Kalivas, P. W. (2012). The effect of N-acetylcysteine in the nucleus accumbens on neurotransmission and relapse to cocaine. *Biological Psychiatry*, *71*, 978–986.

LaRowe, S. D., Kalivas, P. W., Nicholas, J. S., Randall, P. K., Mardikian, P. N., & Malcolm, R. J. (2013). A double-blind placebo-controlled trial of N-acetylcysteine in the treatment of cocaine dependence. *American Journal on Addictions*, *22*, 443–452.

Lebourgeois, S., González-Marín, M. C., Antol, J., Naassila, M., & Vilpoux, C. (2019). Evaluation of N-acetylcysteine on ethanol self-administration in ethanol-dependent rats. *Neuropharmacology*, *150*, 112–120.

Lebourgeois, S., González-Marín, M. C., Jeanblanc, J., Naassila, M., & Vilpoux, C. (2018). Effect of N-acetylcysteine on motivation, seeking and relapse to ethanol self-administration. *Addiction Biology*, *23*, 643–652.

Leong, K. C., Cox, S., King, C., Becker, H., & Reichel, C. M. (2018). Oxytocin and rodent models of addiction. *International Review of Neurobiology*, *140*, 201–247.

Madayag, A., Lobner, D., Kau, K. S., Mantsch, J. R., Abdulhameed, O., Hearing, M., Grier, M. D., & Baker, D. A. (2007). Repeated N-acetylcysteine administration alters plasticity-dependent effects of cocaine. *Journal of Neuroscience*, *27*, 13968–13976.

McClure, E. A., Gipson, C. D., Malcolm, R. J., Kalivas, P. W., & Gray, K. M. (2014). Potential role of N-acetylcysteine in the management of substance use disorders. *CNS Drugs*, *28*, 95–106.

McKetin, R., Dean, O. M., Baker, A. L., Carter, G., Turner, A., Kelly, P. J., & Berk, M. (2017). A potential role for N-acetylcysteine in the management of methamphetamine dependence. *Drug and Alcohol Review*, *36*, 153–159.

Monte, A. S., da Silva, F. E. R., Lima, C. N. C., Vasconcelos, G. S., Gomes, N. S., Miyajima, F., Vasconcelos, S. M. M., Gama, C. S., Seeman, M. V., de Lucena, D. F., & Macedo, D. S. (2020). Sex influences in the preventive effects of N-acetylcysteine in a two-hit animal model of schizophrenia. *Journal of Psychopharmacology*, *34*, 125–136.

Morley, K. C., Baillie, A., Van Den Brink, W., Chitty, K. E., Brady, K., Back, S. E., Seth, D., Sutherland, G., Leggio, L., & Haber, P. S. (2018). N-acetyl cysteine in the treatment of alcohol use disorder in patients with liver disease: Rationale for further research. *Expert Opinion on Investigational Drugs*, *27*, 667–675.

Moro, F., Orrù, A., Marzo, C. M., Di Clemente, A., & Cervo, L. (2018). mGluR2/3 mediates short-term control of nicotine-seeking by acute systemic N-acetylcysteine. *Addiction Biology*, *23*, 28–40.

Murray, J. E., Everitt, B. J., & Belin, D. (2012). N-Acetylcysteine reduces early-and late-stage cocaine seeking without affecting cocaine taking in rats. *Addiction Biology*, *17*, 437–440.

Olive, M. F., Cleva, R. M., Kalivas, P. W., & Malcolm, R. J. (2012). Glutamatergic medications for the treatment of drug and behavioral addictions. *Pharmacology, Biochemistry and Behavior*, *100*, 801–810.

Orrico, A., Hipólito, L., Sánchez-Catalán, M. J., Martí-Prats, L., Zornoza, T., Granero, L., & Polache, A. (2013). Efficacy of D-penicillamine, a sequestering acetaldehyde agent, in the prevention of alcohol relapse-like drinking in rats. *Psychopharmacology*, *228*, 563–575.

Orrico, A., Martí-Prats, L., Cano-Cebrián, M. J., Granero, L., Polache, A., & Zornoza, T. (2014). Improved effect of the combination naltrexone/D-penicillamine in the prevention of alcohol relapse-like drinking in rats. *Journal of Psychopharmacology*, *28*, 76–81.

Powell, G. L., Leyrer-Jackson, J. M., Goenaga, J., Namba, M. D., Piña, J., Spencer, S., Stankeviciute, N., Schwartz, D., Allen, N. P., Del Franco, A. P., McClure, E. A., Olive, M. F., & Gipson, C. D. (2019). Chronic treatment with N-acetylcysteine decreases extinction responding and reduces cue-induced nicotine-seeking. *Physiological Reports*, *7*, e13958.

Quintanilla, M. E., Morales, P., Ezquer, F., Ezquer, M., Herrera-Marschitz, M., & Israel, Y. (2018). Commonality of ethanol and nicotine reinforcement and relapse in Wistar-derived UChB rats: Inhibition by N-acetylcysteine. *Alcoholism, Clinical and Experimental Research*, *42*, 1988–1999.

Quintanilla, M. E., Rivera-Meza, M., Berríos-Cárcamo, P., Salinas-Luypaert, C., Herrera-Marschitz, M., & Israel, Y. (2016). Beyond the "first hit": Marked inhibition by N-acetyl cysteine of chronic ethanol intake but not of early ethanol intake. Parallel effects on ethanol-induced saccharin motivation. *Alcoholism, Clinical and Experimental Research*, *40*, 1044–1051.

Ramirez-Niño, A. M., D'Souza, M. S., & Markou, A. (2013). N-acetylcysteine decreased nicotine self-administration and

cue-induced reinstatement of nicotine seeking in rats: Comparison with the effects of N-acetylcysteine on food responding and food seeking. *Psychopharmacology*, *225*, 473–482.

Rehm, J., Anderson, P., Barry, J., Dimitrov, P., Elekes, Z., Feijão, F., Frick, U., Gual, A., Gmel, G., Jr., Kraus, L., Marmet, S., Raninen, J., Rehm, M. X., Scafato, E., Shield, K. D., Trapencieris, M., & Gmel, G. (2015). Prevalence of and potential influencing factors for alcohol dependence in Europe. *European Addiction Research*, *21*, 6–18.

Reichel, C. M., Moussawi, K., Do, P. H., Kalivas, P. W., & See, R. E. (2011). Chronic N-acetylcysteine during abstinence or extinction after cocaine self-administration produces enduring reductions in drug seeking. *Journal of Pharmacology and Experimental Therapeutics*, *337*, 487–493.

Reilly, M. T., Noronha, A., & Warren, K. (2014). Perspectives on the neuroscience of alcohol from the National Institute on Alcohol Abuse and Alcoholism. *Handbook of Clinical Neurology*, *125*, 15–29.

Reissner, K. J., Gipson, C. D., Tran, P. K., Knackstedt, L. A., Scofield, M. D., & Kalivas, P. W. (2015). Glutamate transporter GLT-1 mediates N-acetylcysteine inhibition of cocaine reinstatement. *Addiction Biology*, *20*, 316–323.

Rodd, Z. A., Bell, R. L., Sable, H. J., Murphy, J. M., & McBride, W. J. (2004). Recent advances in animal models of alcohol craving and relapse. *Pharmacology, Biochemistry and Behavior*, *79*, 439–450.

Rösner, S., Hackl-Herrwerth, A., Leucht, S., Lehert, P., Vecchi, S., & Soyka, M. (2010a). Acamprosate for alcohol dependence. *Cochrane Database of Systematic Reviews*, *9*, CD004332.

Rösner, S., Hackl-Herrwerth, A., Leucht, S., Vecchi, S., Srisurapanont, M., & Soyka, M. (2010b). Opioid antagonists for alcohol dependence. *Cochrane Database of Systematic Reviews*, *12*, CD001867.

Samuni, Y., Goldstein, S., Dean, O. M., & Berk, M. (2013). The chemistry and biological activities of N-acetylcysteine. *Biochimica et Biophysica Acta*, *1830*, 4117–4129.

Sanchis-Segura, C., & Spanagel, R. (2006). Behavioural assessment of drug reinforcement and addictive features in rodents: An overview. *Addiction Biology*, *11*, 2–38.

Spanagel, R. (2017). Animal models of addiction. *Dialogues in Clinical Neuroscience*, *19*, 247–258.

Spanagel, R., & Kiefer, F. (2008). Drugs for relapse prevention of alcoholism: Ten years of progress. *Trends in Pharmacological Sciences*, *29*, 109–115.

Spanagel, R., & Vengeliene, V. (2012). New pharmacological treatment strategies for relapse prevention. In W. Sommer & S. Rainner (Eds.), *Behavioral neurobiology of alcohol addiction*, in Current Topics in Behavioural Neurosciences, (pp. 583–609). Springer.

Stecker, T. (2015). Preclinical data reproducibility for R&D- the challenge for neuroscicnece. *Psychopahmacology*, *232*, 317–320.

Tabachnick, B. G., & Fidell, L. S. (2007). *Experimental design using ANOVA*. Duxbury.

Vengeliene, V., Bachteler, D., Danysz, W., & Spanagel, R. (2005). The role of the NMDA receptor in alcohol relapse: A pharmacological mapping study using the alcohol deprivation effect. *Neuropharmacology*, *48*, 822–829.

Vengeliene, V., Noori, H. R., & Spanagel, R. (2013). The use of a novel drinkometer system for assessing pharmacological treatment effects on ethanol consumption in rats. *Alcoholism, Clinical and Experimental Research*, *37*, E322–E328.

Weiland, A., Garcia, S., & Knackstedt, L. A. (2015). Ceftriaxone and cefazolin attenuate the cue-primed reinstatement of alcohol-seeking. *Frontiers in Pharmacology*, *6*, 44.

Weiss, F., Ciccocioppo, R., Parsons, L. H., Katner, S., Liu, X. I. U., Zorrilla, E. P., Valdez, G. R., Ben-Shahar, O., Angeletti, S., & Richter, R. R. (2001). Compulsive drug-seeking behavior and relapse: Neuroadaptation, stress, and conditioning factors. *Annals of the New York Academy of Sciences*, *937*, 1–26.

Whyte, I. M., Francis, B., & Dawson, A. H. (2007). Safety and efficacy of intravenous N-acetylcysteine for acetaminophen overdose: Analysis of the Hunter Area Toxicology Service (HATS) database. *Current Medical Research and Opinion*, *23*, 2359–2368.

World Health Organization. (2014). *Global status report on alcohol and health 2014*. *Report*. Author.

Zhou, J., Coles, L. D., Kartha, R. V., Nash, N., Mishra, U., Lund, T. C., & Cloyd, J. C. (2015). Intravenous administration of stable-labeled N-acetylcysteine demonstrates an indirect mechanism for boosting glutathione and improving redox status. *Journal of Pharmaceutical Sciences*, *104*, 2619–2626.

Zhou, W., & Kalivas, P. W. (2008). N-acetylcysteine reduces extinction responding and induces enduring reductions in cue- and heroin induced drug-seeking. *Biological Psychiatry*, *63*, 338–340.

Zhou, Y., & Kreek, M. J. (2019). Clinically utilized kappa-opioid receptor agonist nalfurafine combined with low-dose naltrexone prevents alcohol relapse-like drinking in male and female mice. *Brain Research*, *1724*, 146410.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Transparent Peer Review Report

Transparent Science Questionnaire for Authors

How to cite this article: Cano-Cebrián MJ, Fernández-Rodríguez S, Hipólito L, Granero L, Polache A, Zornoza T. Efficacy of N-acetylcysteine in the prevention of alcohol relapse-like drinking: Study in long-term ethanol-experienced male rats. *J Neurosci Res*. 2021;99:638–648. https://doi.org/10.1002/jnr.24736