Effect of N-acetylcysteine on motivation, seeking and relapse to ethanol self-administration

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ABSTRACT

Alcohol use disorder is a chronic and highly relapsing disorder, characterized by a loss of control over alcohol consumption and craving. Several studies suggest a key role of glutamate in this disorder. In recent years, the modulation of cystine/glutamate exchange via the xc system has emerged as a new therapeutic alternative for reducing the excitatory glutamatergic transmission observed after ethanol self-administration in both rats and humans. The objective of this study was to determine whether a treatment with N-acetylcysteine (NAC), a cystine prodrug, could reduce ethanol self-administration, ethanol-seeking behavior and reacquisition of ethanol self-administration. Male Long Evans rats were trained to self-administer 20 percent ethanol in operant cages for several weeks. Once the consumption surpassed 1 g of ethanol/kg body weight/15 minutes, the effect of an acute intraperitoneal injection of NAC (0, 25, 50 or 100 mg/kg) 1 hour before the beginning of each test was evaluated on different aspects of the operant self-administration behavior. We demonstrated antimotivational properties of NAC (100 mg/kg), as ethanol-reinforced responding was reduced in a fixed ratio (−35 percent) and in a progressive ratio schedule (−81 percent). NAC also reduced ethanol-seeking behavior (−77 percent) evaluated as extinction responding in a single extinction session. NAC was able to reduce reacquisition in rats that were abstinent for 17 days, while NAC had no effect on ethanol relapse in rats previously exposed to six extinction sessions. Overall, our results demonstrate that NAC limits motivation, seeking behavior and reacquisition in rats, making it a potential new treatment for the maintenance of abstinence.

Keywords ethanol self-administration, N-acetylcysteine, rat, relapse, seeking.

INTRODUCTION

Alcohol use disorder (AUD) is a chronic disorder characterized by the compulsive use of alcohol, loss of control over intake and development of a negative emotional state during withdrawal (Koob 2013, 2015). Because AUD can be defined as a chronically relapsing disorder, there is a pressing need for effective treatments to reduce alcohol intake or relapse rate. For most theories of addiction, craving is considered as a central hallmark contributing to development, perseveration and relapse, and craving is now a diagnostic criterion for addiction (Skinner & Aubin 2010; Sinha, Shaham, & Heilig 2011). Because N-acetylcysteine (NAC) decreases craving for cocaine in both rats and humans (Baker et al. 2003; Madayag et al. 2007; Amen et al. 2011; Kupchik et al. 2012; Murray, Everitt, & Belin 2012), it is emerging as a useful agent in the treatment of addiction (Kalivas & Volkow 2011; Asevedo et al. 2014).

Disruption of glutamate homeostasis is associated with many addictive disorders, including AUD (Gass & Olive 2008; Kalivas 2009; Burnett, Chandler, & Trantham-Davidson 2016). NAC is a cystine prodrug that enhances cystine/glutamate exchange via the xc system, the system that imports a molecule of cystine inside glial cells while exporting a molecule of glutamate in the extracellular compartment (Kalivas 2009). The modulation of cystine/glutamate exchange via the xc system recently has emerged as a new therapeutic alternative for reducing the excitatory glutamatergic transmission observed in addiction (Kau et al. 2008).
In rats, NAC treatment reduces nicotine self-administration and cue-induced reinstatement for nicotine (Gipson et al. 2013, Ramirez-Niño, D’Souza, & Markou 2013, Moro et al. 2016) and relapse to cocaine self-administration (Amen et al. 2011; Kupchik et al. 2012). Furthermore, NAC treatment blocks the development of cocaine-induced and ethanol-induced behavioral sensitization (Madayag et al. 2007; Morais-Silva, Alves, & Marin 2016), which is noteworthy because behavioral sensitization constitutes a paradigm highly relevant to studying drug-induced plasticity changes following repeated drug exposure (Legastelois et al. 2014).

In humans, NAC has been found to prevent craving for cocaine and nicotine (Amen et al. 2011; Froeliger et al. 2015), reduce relapse for cocaine in patients initially abstinent before treatment (LaRowe et al. 2013), maintain cannabis and nicotine abstinence (Gray et al. 2012; Froeliger et al. 2013) and reduce cigarette smoking (Knackstedt et al. 2009) (for a review, see Deepmala et al. 2015). NAC also reduces non-drug compulsive behavior such as pathological gambling (Grant et al. 2014) and trichotillomania (Grant, Odlaug, & Kim 2009). With respect to alcohol consumption in humans, NAC has been shown to reduce alcohol drinking in adolescents using marijuana (Squeglia et al. 2016).

Taken together, the literature shows that NAC possesses anticraving and antirelapse properties, which suggest its utility as a treatment for addiction; however, few studies have evaluated the effect of NAC on ethanol addiction. One study did report that NAC decreased ethanol withdrawal-induced anxiety and elevated corticosterone levels in rats (Schneider et al. 2015), and another study found that NAC inhibited ethanol intake in rats chronically exposed to ethanol in a two-bottle choice paradigm (Quintanilla et al. 2016). Thus, NAC may decrease ethanol intake and other related behaviors, but the effect of NAC on the motivation to self-administer ethanol has not yet been investigated.

Thus, here, we sought to evaluate whether NAC is effective in reducing ethanol consumption, ethanol seeking (a behavioral correlates of craving), reacquisition after extinction and relapse after abstinence in rats chronically trained to self-administer ethanol. We tested the efficacy of NAC on both extinction and abstinence paradigms because it has been demonstrated that different neural substrates likely mediate drug seeking depending on the paradigm.

MATERIALS AND METHODS

Animals

Male Long Evans rats, weighing 280–300 g at the beginning of the experiment, were purchased from Janvier labs (Le Genest Saint Isle, France). Animals were individually housed in a controlled environment under a 12-hour light/dark cycle (lights on at 8 AM) with food and water available ad libitum. All experiments were performed in conformity with the European Community guidelines for the care and use of animals (2010/63/UE, CE Off. J., 20 October 2010), the French decree no. 2013-118 (French Republic Off. J., 2013), and approved by the local ethics committee (Comité Régional d’Éthique en Matière d’Expérimentation Animale de Picardie, University of Picardie Jules Verne).

Drugs

For self-administration experiments, ethanol (VWR, Strasbourg, France) was diluted in tap water to a final concentration of 20 percent (v/v). For intraperitoneal (i.p) injections, NAC (Sigma-Aldrich, Saint Quentin Fallavier, France) was dissolved in sterile saline (0.9 percent NaCl) and administered at the doses of 25, 50 and 100 mg/kg (1 ml/kg), which were chosen based on previous findings (Kau et al. 2008; Reichel et al. 2011). 60 minutes before the start of self-administration session.

Behavioral acquisition of the self-administration task

The self-administration behavior was induced by a two-step paradigm: first, rats were exposed to intermittent access to 20 percent ethanol for 3 weeks to facilitate the acquisition of a high level of ethanol intake (Wise 1973). Thereafter, rats were trained to self-administer a 20 percent ethanol solution during short-session operant access periods (15 minutes) until stable levels of intake were reached. This procedure was used to test the effect of NAC in a model of chronic ethanol self-administration, as rats were submitted to daily self-administration sessions for several weeks, five sessions per week.

Intermittent access to 20 percent ethanol

After 1 week of acclimation, intermittent access to 20 percent ethanol was used to achieve high levels of ethanol intake (> 5 g/kg/24 hours) (Simms et al. 2008). Briefly, rats were given access to two bottles, one bottle containing tap water and the other containing 20 percent ethanol, for 24-hour sessions on Mondays, Wednesdays and Fridays for 3 weeks (a total of nine drinking sessions). At the end of each session, bottles were weighed to assess both ethanol consumption (g ethanol/kg body weight) and preference (the ratio of ethanol consumed to total fluid intake). The bottle placement in the cage (left or right) was alternated between each session to avoid side preferences.
Operant self-administration apparatus and training methods

Ethanol self-administration training was conducted in standard operant chambers connected to PACKWIN software (Bioseb, Vitrolles, France), as previously described (Alaux-Cantin et al. 2013; Jeanblanc et al. 2014a, 2014b). Briefly, chambers were equipped with two opposite levers located below a light cue and next to a delivery magazine. A press on the active lever triggered the associated light cue for 2 seconds and produced the delivery of 0.1 ml of 20 percent ethanol solution. The activation of the light cue during 2 seconds corresponded to a time-out period during which each press was recorded, but not reinforced. Responses on the other lever were recorded, but produced no consequence (i.e. neither light cue nor delivery). After two overnight sessions (16 hours), three 1-hour sessions and three 30-minute sessions under a fixed ratio (FR) 1 schedule, rats were trained on an FR3 schedule of reinforcement, with the session duration progressively decreased to 15 minutes. During each session, the number of presses emitted on each lever and the number of ethanol deliveries were recorded, and these sessions continued until stable ethanol-reinforced responding was achieved (i.e. three consecutive sessions with <20 percent variation in responses). Stable responding was achieved after 23 sessions (five sessions per week), at which time rats reached an intake of 1.22 ± 0.09 g/kg of body weight/15 minutes.

Behavioral testing: effect of acute NAC on ethanol self-administration

The 20 percent ethanol self-administration test

Once ethanol consumption was high and stable, the effect of an acute injection of NAC (0, 25, 50 or 100 mg/kg; i.p. 60 minutes before the test) was tested on the level of ethanol 20 percent self-administration. The doses of NAC and the delay between injection and self-administration sessions were chosen according to previous work (Baker et al. 2003; Moussawi et al. 2009; Amen et al. 2011; Reichel et al. 2011). Injections were performed according to a Latin-square counterbalanced design with 1 day of washout between each injection.

Ethanol-seeking test

After several weeks of ethanol self-administration, we evaluated the effect of NAC on ethanol-seeking behavior. A single 15-minute session of extinction responding, during which the light cue remained off and no ethanol was available, was performed 24 hours after the last session of ethanol self-administration. NAC (0, 25, 50 and 100 mg/kg) was injected i.p. 60 minutes before this extinction responding test, and the persistence of drug-seeking behavior in the absence of the drug (i.e. a behavior modeling craving) was assessed by recording the number of active lever presses.

Progressive ratio test

A progressive ratio (PR) schedule test was performed to evaluate the effect of NAC (0 or 100 mg/kg) on the motivation to consume ethanol. In this test, the effort necessary to obtain one reward (i.e. the number of presses on the active lever) was continuously increased after each reward delivery (3, 4, 5, 7, 9, 12, 15, 18, 20, 23, 25, 28, 30, 33 and 35). During the 15-minute session, the maximum ratio value (breaking point) completed to receive a single reward of ethanol was measured and considered as an index of motivation.

Reacquisition test after extinction

Rats were exposed to six daily extinction sessions in which the light cue stayed off and no ethanol was available. The extinction criterion was two consecutive sessions with the number of presses less than 20 percent of the baseline (i.e. the mean of the two last sessions before the beginning of extinction). When the extinction criterion was reached, we used the reacquisition model of relapse (Alaux-Cantin et al. 2013; Jeanblanc et al. 2014a, 2015; Simon-O’Brien et al. 2015), which consists of the reintroduction of ethanol in the operant self-administration chambers. Briefly, a priming delivery of ethanol (0.1 ml of a 20 percent ethanol solution) was given non-contingently at the beginning of the reacquisition session to provide an olfactory and gustatory cue. Ethanol was then available on an FR3 schedule for the duration of the 15-minute session. During this test, NAC (0 or 100 mg/kg) was injected i.p. 60 minutes before the 15-minute session.

Reacquisition test after abstinence

During this test, we used the same relapse paradigm previously described (i.e. the reacquisition test), but daily extinction sessions were replaced with abstinence for 17 days, during which rats were housed in the home cage and had no access to the ethanol self-administration chambers. Again, in this reacquisition test, NAC (0 or 100 mg/kg) was injected i.p. 60 minutes before the 15-minute session.

Locomotor activity

In a control experiment, we demonstrated that the effect of NAC cannot be attributed to a locomotor effect. Both
locomotor activity in the open field and the reinstatement after extinction were not changed by the NAC treatment, which is consistent with previous studies showing that NAC does not alter locomotor activity (see Supporting Information) (Madayag et al. 2007; Murray et al. 2012).

Statistical analysis

All results were expressed as mean ± standard errors of the mean. Statistical analyses were made using one-way or two-way ANOVAs with or without repeated measures (RMs) followed by a Tukey’s post hoc test. For simple comparisons, data were analyzed with a Student’s t-test. Statistical analyses were performed using SIGMAPLOT software (version 11; Systat software, Inc., San Jose, CA, USA), and a \( P < 0.05 \) was considered significant.

RESULTS

N-acetylcysteine reduces ethanol self-administration

The effect of an i.p. injection of NAC (0, 25, 50 or 100 mg/kg; 1 hour before the test) on ethanol self-administration was evaluated. The mean numbers of active lever presses were analyzed using a one-way RM ANOVA, which revealed a significant main effect of treatment \( (F(3,42) = 7.045, P < 0.001) \). Subsequent post hoc Tukey tests showed that total active lever presses were significantly lower for the NAC 100 mg/kg group compared with saline group (−35 percent) (Fig. 1a). Similarly, total active lever presses were significantly lower for the NAC 100 mg/kg group compared with NAC 25 or 50 mg/kg groups. The total number of inactive lever presses did not significantly differ between groups: \( F(4,32) = 2.685, P > 0.05 \). NAC also reduced ethanol intake (Fig. 1b). We observed a significant main effect of NAC pretreatment on ethanol intake, \( F(3,42) = 6.752, P < 0.001 \), whereby 100 mg/kg NAC significantly reduced ethanol intake compared with saline-treated rats (−35 percent). Together, these data indicate that an acute injection of NAC at the dose of 100 mg/kg, but not at the dose of 25 or 50 mg/kg, was able to reduce ethanol consumption. This dose effect was consistent with a previous observation that NAC reduced nicotine-seeking behavior in rats at 100, but not 30 or 60 mg/kg i.p. (Moro et al. 2016).

N-acetylcysteine reduces ethanol seeking

Next, we assessed the effect of an acute injection of NAC (0, 25, 50 or 100 mg/kg; i.p. 1 hour before the test) on ethanol seeking, during one single session of extinction responding after several weeks of ethanol self-administration. In this test, rats were placed in ethanol self-administration chambers, and each press on the active lever was counted, but had no consequences (neither ethanol nor light cue). Extinction responding, (i.e. total active lever presses) was analyzed using a one-way ANOVA, which revealed a significant effect of treatment \( (F(3,47) = 7.449, P < 0.001) \). Subsequent pairwise analyses (Tukey’s test) showed that the number of active lever presses was significantly lower for the NAC (100 mg)-treated rats compared with those treated with saline, revealing that NAC 100 mg/kg was able to strongly reduce the persistence of drug-seeking behavior in the absence of ethanol by 77 percent (Fig. 2). However, the total number of inactive lever presses was not significantly different between groups, \( F(3,47) = 0.335, P > 0.05 \). Given this result, the NAC dose of 100 mg/kg was considered the effective dose and was used for the rest of our experimentation.

Figure 1  N-acetylcysteine (NAC) reduces ethanol self-administration: effect of an i.p. injection of NAC (0, 25, 50 or 100 mg/kg; 1 hour before the test; \( n = 15/group \)) on ethanol self-administration. (a) Total active lever presses were significantly lower for the NAC 100 mg/kg group compared with saline group, \( P < 0.001 \), and there was no effect on inactive lever presses. (b) Rats treated with 100 mg/kg NAC consumed significantly less ethanol than saline-treated rats. *** \( P < 0.001 \)
N-acetylcysteine decreases the motivation to consume ethanol

To further assess the effect of NAC (0 or 100 mg/kg) on the motivation to consume ethanol, we used a progressive ratio schedule. The number of active lever presses and the breaking point values during the 15-minute session are depicted in Fig. 3. Statistical analyses using Student’s t-test revealed that NAC-treated rats emitted significantly fewer presses during the PR session (−88 percent; \( P < 0.001 \)) and displayed a significantly lower breaking point compared with saline-treated rats (−81 percent; \( P < 0.001 \)). Total number of inactive lever presses did not significantly differ between groups (\( P > 0.05 \)).

N-acetylcysteine fails to limit reacquisition after extinction

After six extinction sessions (once a day), rats previously trained to self-administer large amounts of ethanol were exposed to a reacquisition session during which ethanol was again available (Fig. 4). In this test, we found that regardless of the treatment received 1 hour before the reacquisition session (saline or NAC 100 mg/kg), similar behavior was observed during the reacquisition session. Indeed, the RM ANOVA on active lever presses revealed a significant effect of session (\( F(2,26) = 34.64, P < 0.001 \)), but no effect of the treatment (\( F(1,26) = 0.226, P = 0.642 \)) and no treatment × session interaction (\( F(2,26) = 0.0949, P = 0.910 \)). Total number of inactive lever presses was not significantly different between treatment groups: \( F(1,26) = 0.921, P > 0.05 \). Thus, an acute injection of 100 mg/kg NAC failed to limit reacquisition after six extinction sessions.
N-acetylcysteine limits reacquisition after abstinence

We also examined the effect of an acute NAC 100 mg/kg treatment on reacquisition after 17 days of protracted abstinence (Fig. 5). During this reacquisition procedure, we observed that saline-treated rats had similar levels of active lever responding before and after abstinence (Fig. 5a). The RM ANOVA revealed a significant effect of treatment ($F(1,17) = 14.447$, $P < 0.001$), a significant effect of session ($F(1,17) = 9.815$, $P = 0.006$) and a significant treatment $\times$ session interaction [treatment $\times$ session; $F(1,17) = 20.773$, $P < 0.001$]. Post hoc analyses confirmed the absence of reacquisition of operant self-administration behavior in NAC 100 mg/kg-treated rats. Similarly, the Tukey's test revealed a difference between the saline and NAC groups during the reacquisition session, whereas no difference was found during baseline, and total number of inactive lever presses did not significantly differ between treatment groups: $F(1,17) = 1.097$, $P > 0.05$. Thus, an acute injection of 100 mg/kg NAC was able to limit reacquisition after a protracted abstinence. As expected, similar results were found regarding ethanol consumption (Fig. 5b). The RM ANOVA revealed a significant effect of the treatment ($F(1,17) = 13.252$, $P = 0.002$), a significant effect of the session ($F(1,17) = 13.886$, $P = 0.002$) and a significant interaction between factors [treatment $\times$ session; $F(1,17) = 19.076$, $P < 0.001$].

**DISCUSSION**

The current study showed for the first time that NAC effectively reduced ethanol operant self-administration, motivation to self-administer ethanol, ethanol seeking and reacquisition after protracted abstinence in rats. First, we demonstrated that an acute injection of NAC (100 mg/kg, i.p.) decreased ethanol-reinforced responding by 35 percent when administered 1 hour before testing. It is worth noting that these rats had been consuming ethanol for several weeks before the test and prior chronic ethanol consumption seems to be necessary for NAC efficiency. Indeed, Quintanilla et al. (2016) observed that in rats bred for high-ethanol intake and left with free access to an ethanol solution for 3 months, NAC decreased ethanol intake; however, in rats given free access to an ethanol beverage for only 5 days, NAC did not produce reduction in ethanol intake. Taken together, these results and our current findings suggest that NAC acts by counteracting neuroadaptations that occurs during chronic ethanol administration.

Although the current study addressed the effects of NAC on ethanol self-administration, other studies suggest that NAC does not strongly affect self-administration of other drugs of abuse. For example, NAC did not decrease cocaine self-administration in rats during either the early or late stages of progression toward cocaine addiction (Amen et al. 2011; Murray et al. 2012) or in rats accustomed to long-term access to cocaine (Ducret et al. 2016).

Considering other studies showing that NAC decreases seeking for cocaine in rats (Madayag et al. 2007; Kupchik et al. 2012; Murray et al. 2012; Ducret et al. 2016) and has anticraving properties in humans for cocaine, nicotine, cannabis and pathological gambling (for a review, see Asevedo et al. 2014), we investigated the effect of NAC on ethanol seeking, in an experimental procedure that models some aspects of ethanol craving in humans. The effect of NAC was evaluated in a single 15-minute extinction session during which the light cue stayed off and no ethanol

Figure 5  N-acetylcysteine (NAC) limits reacquisition after abstinence: (a) During the reacquisition procedure, saline-treated rats ($n=10$) have the same level of active lever presses before and after abstinence, while NAC 100 mg/kg-treated rats ($n=9$) showed an absence of reacquisition of operant self-administration behavior. Inactive lever presses were unaffected by either reacquisition procedure or NAC treatment. (b) Saline-treated rats have the same level of ethanol consumption before and after abstinence, while NAC 100 mg/kg-treated rats showed a decrease in ethanol intake during the reacquisition session. ***$P < 0.001$ compared with saline group and ***$P < 0.001$ compared with baseline session

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was available and we performed this extinction test after several weeks of ethanol self-administration. NAC produced a 77 percent decrease in ethanol seeking, which suggests that NAC possess anti-ethanol-seeking properties in rats and potentially anticraving properties in AUD patients.

We also assessed the effect of NAC on motivation by recording the breaking point in a progressive ratio schedule of reinforcement. NAC decreased the breaking point by 80 percent, indicating that NAC decreases the motivation for ethanol. In previous investigations, NAC did not decrease breaking point for cocaine (Ducret et al. 2016) or nicotine (Ramirez-Niño et al. 2013); however, the decrease in motivation for ethanol intake observed here appears to be an important aspect of the efficacy of NAC. Specifically, the antimotivation properties associated with NAC's anticraving effect could interact to produce efficacy in preventing relapse. The ability of NAC to reduce motivation for ethanol is important because motivational dysregulation is a hallmark of addiction. The effect of NAC on motivation appears more specific to ethanol, and this specificity may be explained by the level of negative reinforcement that is known to be more intense for ethanol than for cocaine or nicotine.

Although numerous studies have demonstrated the acute efficacy of NAC to decrease cue-induced cocaine-primed reinstatement following extinction (Baker et al. 2003; Moran et al. 2005; Kupchik et al. 2012; Reissner et al. 2015), we did not observe any effect of NAC on the ethanol reacquisition model of relapse following six sessions of extinction. Cue-primed reinstatement of ethanol self-administration after extinction was investigated by Weiland, Garcia & Knackstedt (2015), who found that NAC was unable to attenuate cue-primed reinstatement of ethanol seeking after extinction training. We observed similar findings here in the reacquisition experiment; however, in the experiment of Weiland et al. (2015), the session of ethanol administration lasted for 45 minutes instead of the 15 minutes used here, suggesting that duration of ethanol availability may not have been related to the absence of this effect of NAC. The absence of an NAC effect reported by Weiland et al. was observed following chronic NAC administration for 8 days, 2 hours before extinction session, a protocol different from ours because we administered one single NAC injection 1 hour before the reacquisition session. However, we also did not observe NAC efficacy following extinction of ethanol self-administration.

Time-dependent increases in cue-induced ethanol craving during abstinence were recently reported in AUD patients (Li et al. 2015), demonstrating an ‘incubation of craving’ in the context of AUD. In rats, the ‘incubation of craving’ phenomenon was also observed for cannabinoid (Kirschmann et al. 2016) and for heroin, nicotine, methamphetamine and ethanol self-administration (for a review, see Pickens et al. 2011). Moreover, extinction training alone can reduce drug-seeking behavior (McNally 2014). Accordingly, we did observe in previous unpublished experiments in our laboratory that rats demonstrated lower ethanol self-administration behavior following extinction training than following abstinence, suggesting that extinction may induce less craving than a period of abstinence when animals are reexposed to the drug and conditioned stimuli associated with drug taking. Another study found that craving was higher in rats exposed to abstinence from cocaine for 1 month than for 1 day (demonstrating an ‘incubation of craving’), while in rats exposed to 1 month of abstinence, a single extinction session reduced craving levels to those observed on the first day of abstinence (Madsen et al. 2016). Together, these data suggest that the lack of efficacy of NAC treatment after extinction could be linked to the fact that craving is lower in this situation than following abstinence.

Because NAC has been proposed to reduce craving following abstinence (LaRowe et al. 2013), we investigated the ability of NAC to decrease reacquisition following abstinence. We replaced daily extinction sessions with an abstinence period, during which rats were housed in their home cage and had no access to ethanol self-administration chambers. In this condition, NAC reduced reacquisition by 78 percent, revealing an antirelapse effect of NAC.

Most ‘treatment-as-usual’ protocols for substance use disorder do not explicitly extinguish drug-related cues (Reichel et al. 2011). Thus, the efficacy of NAC after abstinence may predict its efficacy in addressing drug craving in humans.

Some results indicate differential neuroanatomical substrates or neuroadaptations following extinction and abstinence (Fuchs, Branham, & See 2006; Knackstedt et al. 2010). Extinction of drug seeking includes learning aspects (Cleva et al. 2010) that are not present during withdrawal without extinction (i.e. abstinence). Some specific neuroadaptations of postsynaptic density in the nucleus accumbens were found only in rats that had undergone extinction of cocaine self-administration, but not simple abstinence (Knackstedt et al. 2010). For example, extinction led to a blunted long-term depression in the nucleus accumbens core, together with a reduced surface expression of mGluR5. This adaptation is thought to be responsible for extinction-induced inhibition of cocaine seeking, a hypothesis strengthened by the observation that mGluR5 antagonists have antirelapse properties, including for ethanol (Bäckström et al. 2004; Sinclair et al. 2012; Knackstedt, Tranham-Davidson, & Schwendt 2014).
It is unclear whether this internalization of mGluR5 is present following extinction of ethanol self-administration, but if so, this adaptation could have a specific role in NAC’s effects. If extinction by itself leads to mGluR5 internalization, then the NAC action mediated through a decreased tonus on mGluR5 (by restoring GluT1 expression) (Roberts-Wolfe & Kalivas 2015) would be less potent. Thus, the NAC effect on reacquisition after extinction could be absent, as we observed. In fact, we found that the extinction paradigm alone produced a marginally significant reduction in relapse (−15 percent; \( P = 0.054 \)) in control rats compared with their own self-administration after stabilization.

There is now evidence that the efficacy of NAC for treating addictive behaviors may come from both its ability to restore glutamatergic homeostasis and its anti-oxidative properties. First, NAC has been shown to be responsible for the normalization of the hyperglutamatergic synaptic state observed during withdrawal, a mechanism hypothesized to be common across many drugs (Kalivas & Volkow 2011; Mulholland, Chandler, & Kalivas 2016). Second, NAC has anti-oxidative properties. For example, enhancement of reactive oxygen species in the nucleus accumbens contributes to the reinforcing properties of methamphetamine while treatment with a reactive oxygen species scavenger, such as NAC, attenuates methamphetamine and cocaine self-administration (Jang et al. 2015; Jang et al. 2016).

The antioxidant properties of NAC increase its potentially broad therapeutic application to drug-of-abuse-induced central nervous system damages. It has been shown that ethanol abstinence and NAC administration interact synergistically, improving serum lipids and hepatic antioxidant defenses (Ferreira Seiva et al. 2009). Because our results show that NAC possesses anticraving and antirelapse properties in the context of ethanol reward, NAC may be useful for treating AUD and associated somatic damages.

In conclusion, our preclinical results demonstrated that NAC is able to reduce both motivation for ethanol self-administration and ethanol seeking. NAC also reduces relapse following protracted abstinence, a model that comprises a condition with high craving resembling the ecologic context that abstinent patients encounter. Together, these results contribute to other evidence showing the potential therapeutic interest of NAC as an anticraving substance and add ethanol to those drugs of abuse that could benefit from NAC treatment.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

SL, CV, MN and JJ were responsible for the study concept and design. SL, MCGM and CV contributed to the acquisition of animal data. SL, MCGM and CV assisted with data analysis and interpretation of findings. SL and CV drafted the manuscript. MN provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

References


SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Figure S1 N-acetylcysteine does not impair locomotion: Effect of an acute injection of 100 mg/kg NAC (1 h before the test; i.p.) was monitored by the distance travelled by the rat during the 15-minute session in the open field. NAC at the dose of 100 mg/kg (n = 10) had no locomotor effect compared with saline treated rats (n = 9).