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N-acetylcysteine for treating cocaine addiction – A systematic review

Marco Antonio Nocito Echevarria*, Tassio Andrade Reis, Giulianno Ruffo Capatti, Victor Siciliano Soares, Dartiu Xavier da Silveira, Thiago Marques Fidalgo

Addiction Unit (PROAD), Department of Psychiatry, Federal University of São Paulo - Avenida Professor Ascendino Reis, 763 – Vila Clementino – São Paulo, SP, Brazil. – CEP: 04027-040

*Corresponding author: Marco Antonio Nocito Echevarria, MD, Email: marco_eche@hotmail.com

Abstract

The aim of this paper is to extensively review the current literature available on N-acetylcysteine (NAC) treatment for cocaine dependence (clinical and experimental studies). We screened all articles published before February 2016 reporting on the use of NAC as a pharmacological intervention for cocaine dependence or discussed its potential as a therapeutic approach for cocaine dependence. We described our results qualitatively. 21 studies matched our search criteria: 6 clinical trials and 15 animal studies. Four clinical studies showed NAC's capacity to reduce craving, desire to use cocaine, cocaine-cue viewing-time and cocaine-related spending. Studies in animal models also support this reinstatement prevention application of NAC. NAC reverses the disruption of glutamate homeostasis caused by long-term cocaine use restoring function of the cystine-glutamate exchanger in glial cells and reversing the downregulated GLT-1 receptor. Current data suggest promising potential for NAC as an anti-relapse agent, as a double-blind placebo trial was mainly negative, except in the subgroup of patients who were already abstinent. An optimal dose for relapse prevention may be one that restores extrasynaptic glutamate to physiological levels and predominantly activates mGluR2 and 3, but not mGluR5 receptors, which are linked to relapse. NAC may be better suited for avoiding relapse in already abstinent subjects.

1. Introduction

Cocaine addiction represents one of the most challenging diseases worldwide. The World Drug Report, compiled by the United Nations Office for Drugs and Crime (UNODC) in 2013, indicated that 17 million people used cocaine at least once during the past year (UNODC, 2013). However, effective treatment options for this condition are lacking (Karila et al., 2008). As with other drugs of abuse, maladaptive brain responses are responsible for the inability of cocaine addicts to cease seeking the drug despite the negative consequences associated with its use (Volkow et al., 2012). Cocaine's ability to impair the brain's natural reward system accounts for the user's difficulty with drug-seeking cessation (Volkow et al., 2012). Impaired communication between the prefrontal cortex and the *nucleus accumbens (NAcc)* has been implicated in the inability of cocaine-dependent patients to control drug-seeking behaviours (Kalivas, 2009).

Cocaine's rewarding effects primarily involve the dopaminergic circuitry of the brain, and all drugs of abuse are well recognized for artificially increasing dopamine within the *NAcc* (mesolimbic pathway) (Volkow et al., 2012). The *NAcc* receives projections from the ventral tegmental area (VTA), which are associated with the rewarding effects of drugs. These projections are known as the mesocortical (projections from the VTA into the frontal cortex) and mesostriatal (projections from the *substantia nigra* into the dorsal *striatum*) dopamine pathways (Volkow et al., 2012). Glutamatergic mechanisms of the reinstatement of the behaviour of substance use have been studied in the past decade (Kalivas, 2009). These studies have introduced new possibilities for developing pharmacological approaches in treating cocaine dependence.

Cocaine-induced chronic adaptations to glutamatergic homeostasis within the *NAcc* include downregulation of the cystine-glutamate exchanger (also named System xc) on glial cells and of the glial glutamate transporter 1 (GLT-1) (Kalivas, 2009). The cystine-glutamate exchanger is an amino acid antiporter located in various cell types inside and outside the CNS (central nervous system). In the CNS, the exchanger is mainly expressed on the membranes of glial cells and mediates a 1:1 exchange of intracellular glutamate for extracellular cystine (Bridges et al., 2012). A well-recognized function of this exchanger is to provide sufficient cystine, which is thereafter reduced to cysteine, a precursor of the oxidative stress protector glutathione (GSH). Because mature neurons express low levels of the cystine-glutamate exchanger, glial cells (mainly astrocytes)

presumably play an important role in providing mature neurons with sufficient cysteine for GSH production and protection against oxidative cell damage (Bridges et al., 2012). The lower levels of extrasynaptic glutamate cause reduced tone on the metabotropic glutamate receptor 2 and 3 (mGluR2/3) responsible for inhibiting synaptic glutamatergic release (Kalivas, 2009). GLT-1 also plays an important role as a buffer that prevents excess glutamate from being released into the synaptic cleft, leaving the synaptic space and entering the extrasynaptic space, which activates extrasynaptic receptors such as mGluR5 and NMDA (Kalivas, 2009).

In animal models of drug addiction, cocaine-seeking behaviours that occur during the reinstatement of the behavior, after a withdrawal period, and those induced by a cocaine prime or stress are associated with an increase in synaptic glutamate from prefrontal projections onto the *NAcc* (Uys and LaLumiere, 2008). The excess glutamate in the synaptic cleft is caused by reduced tone on mGluR2/3, which occurs as a consequence of chronically low extrasynaptic glutamate levels (Brown et al., 2013). This increase in glutamate levels does not occur in cocaine-naïve animal models (Uys and LaLumiere, 2008).

1.1. N-acetylcysteine

Although *N*-acetylcysteine (NAC) has been used for many years, mainly as a mucolytic, it may offer some therapeutic benefits for treating addiction (Asevedo et al., 2014). As a derivative of cysteine, it is oxidized into cystine within the brain, increasing the availability of cystine for the glial cystine-glutamate exchanger. This action increases the amount of glutamate exchanged by glial cells, elevating the concentration of glutamate within the extrasynaptic space and effectively restoring downregulated GLT-1 expression (Berk et al., 2013; Brown et al., 2013). Cystine-glutamate activation may prevent relapse by restoring extrasynaptic glutamate and mGluR2/3 tone, thereby decreasing the excess synaptic glutamate, which halts the signalling associated with relapse and the probability of activating mGluR5 and NMDA. Concurrently, any excess glutamate overflows into the synaptic cleft will be reduced by the restored GLT-1 receptor (Brown et al., 2013). These processes represent the mechanisms underlying the normalization of glutamate homeostasis, which may be impaired by long-term drug use. Other investigations have explored NAC's potential as a novel treatment in psychiatry

and neurology. Because NAC acts through mechanisms (oxidative stress control, excitatory neurotransmission and neuroplasticity) that are presumably involved in multiple psychiatric and neurologic conditions (e.g., schizophrenia, attention deficit hyperactivity disorder, amyotrophic lateral sclerosis, autism and bipolar disorder), it has drawn substantial attention, although most studies are limited and offer mixed results (Deepmala et al., 2015). Particularly interesting are the results regarding the reduction in symptom severity in both depressive and manic/hypomanic states, although the frequency of these mood states may be unaltered (Deepmala et al., 2015). In substance use disorders, limited clinical trials exist, often with mixed or negative results, although most of such studies investigated NAC as an abstinence-promoting treatment and not as an anti-relapse agent, as subsequently discussed in the present review (McClure et al., 2014).

1.2. Aims of the study

In this study, we review NAC's role in the treatment of cocaine addiction, including pre-clinical and clinical studies. We intend to extensively review the neurobiological mechanisms involving NAC, the glutamatergic system and their interrelationship for treating cocaine addiction and to summarize the findings of the current literature available on this subject.

2. Materials and methods

We screened all articles that studied NAC as a pharmacological intervention for cocaine dependence or that discussed its potential as a therapeutic approach for cocaine dependence. We searched for articles published before February 2016 using the following databases: MEDLINE, Web of Science, Cochrane, and SCIELO. We also examined reference lists in systematic reviews and retrieved articles that fulfilled our criteria. Our search strategy included "N-acetylcysteine AND cocaine" to be as broad as possible.

2.1. Search criteria

The following inclusion criteria were used: (i) articles written in English, (ii) studies using NAC as a pharmacological intervention for cocaine dependence or discussing its potential as a therapeutic approach for cocaine dependence, (iii) studies conducted in humans and animals, and (iv) studies published before February 2016.

2.2. Positive versus negative findings

For our study, we defined a positive finding as a significant observed clinical improvement and a negative finding as clinical results that were not significant.

2.3. Data extraction

The data were independently extracted by the authors (MANE, VSS, GRC and TAR) using a structured format. For animal studies, the following variables were extracted: (1) author and year of publication and (2) main findings. For clinical trials, the following variables were extracted: (1) author and year of publication, (2) study type, (3) sample size, (4) what the study evaluated, (5) details about what was evaluated, (6) results and (7) study limitations. Discrepancies were resolved by consensus, and the senior author of this paper (TMF) was consulted when necessary.

2.4. Data analysis

We qualitatively described our results because no quantitative information was available to perform a meta-analysis.

3. Results

Fifty-four results were initially retrieved. We identified 21 studies that matched our search criteria: six of these were clinical trials and 15 were animal studies. Some studies were excluded from this review because they were poorly or only indirectly related to our investigation (e.g., related to other drugs or not directly investigating NAC and cocaine).

3.1. Animal studies

A study conducted in 2003 by Baker et al. tested the hypothesis that the basal low levels of extrasynaptic glutamate following cocaine withdrawal in rats were caused by a reduction of the cystine-glutamate exchanger activity. In the same study, the investigators tested whether the pharmacological restoration of the exchanger's activity would block the reinstatement of cocaine use behaviour after a withdrawal period. Their results showed that cocaine-treated rats displayed lower levels of basal extrasynaptic glutamate in the *NAcc.* These lower levels may be mimicked by the administration of a cystine-glutamate exchanger blocker. Activation of the exchanger by direct administration of cystine into the *NAcc* or systemically administered NAC reversed low levels of extrasynaptic glutamate in cocaine-treated rats; however, it did not elevate glutamate levels in drug-naïve rats. This finding supports the theory that only drug-dependent neuroadaptations may be corrected by NAC. The correction of extrasynaptic levels of

glutamate by the administration of NAC or (-)-2-oxothiazolidine-4-carboxylic acid (another cysteine pro-drug) also produced a dose-dependent reduction in reinstatement. The mechanisms by which the activity of the exchanger is reduced are not known; however, the levels of mRNA encoding the xCT protein of the exchanger were not altered. The authors proposed a possible mechanism by which phosphorylation of the exchanger could be involved in its reduced activity (Baker et al., 2003a).

A study of rodents demonstrated that NAC increased the levels of extracellular glutamate by activating the cystine-glutamate exchanger (Moran et al., 2005). The stimulation of presynaptic receptors mGluR2/3 by nonsynaptic glutamate derived from the cystine-glutamate exchanger inhibits synaptic glutamate release and, consequently, the excitatory synaptic activity, contributing to the ability of NAC to inhibit cocaine-primed reinstatement. This study is consistent with previous theories regarding the mechanism of NAC (Baker et al., 2003b).

A 2007 article demonstrated that NAC administered before cocaine use prevented the establishment of plasticity-dependent cocaine seeking, even when this behaviour was assayed 21 days after the last injection of N-acetylcysteine. This article also indicated that pretreatment with NAC prevented changes in the levels of glutamate in the *NAcc* and plasticity involving the cystine-glutamate exchanger and that these changes might be induced by cocaine (Madayag et al., 2007).

The decrease in the cystine-glutamate exchanger activity, which decreases the release of glutamate, appears to be required for cocaine-primed reinstatement in rats. This antiporter has been implicated as a potential target of the stabilizing effect of NAC in glutamate release, particularly in the *NAcc*. NAC inhibits the decrease in the function of the cystine-glutamate exchanger that results from cocaine use. Kau et al. (2008) demonstrated that NAC increases the activity of the cystine-glutamate exchanger, reducing cocaine-primed reinstatement in rodents. These findings are consistent with recent results that indicate a reduction in the use of cocaine with the use of NAC (Kau et al., 2008).

In a 2012 study, Kupchik and colleagues investigated the role of presynaptic mGluR2/3 and postsynaptic mGluR5 glutamate receptors. They showed that the administration of an mGluR2/3 antagonist (LY341495) blocked the inhibition of

reinstatement mediated by NAC in animals trained to self-administer cocaine. They also showed that excitatory postsynaptic currents (EPSCs) increased in the *NAcc* responded in a biphasic manner, whereby low-concentration doses of NAC (0.5 µmol/l) reduced EPSC amplitudes and high-concentration doses of NAC (500 µmol/L) increased the EPSC amplitude. NAC's ability to alter EPSCs in the *NAcc* was shown to be dependent on the cystine-glutamate exchanger, as both NAC effects, regardless of the dose, were non-existent when the cystine-glutamate exchanger blocker (s)-4-carboxyphenylglycine (CPG) was coadministered. To demonstrate that the EPSC reductions on amplitude induced by low-dose NAC depended on mGluR2/3 receptors, the authors coadministered an mGluR2/3 blocker (LY341495) with NAC. Indeed, these blockers prevented EPSC reductions with NAC (0.5 µmol/l) but not an increase in EPSC amplitude with high-dose NAC (500 µmol/L). The increase in the EPSC amplitude by high-dose NAC could be blocked by an mGluR-5 negative allosteric modulator (MTEP). Finally, they concluded that NAC increases extrasynaptic glutamate, which then activates both mGluR2/3 and mGluR5 receptors. Because these receptors have opposite effects on synaptic glutamate transmission, the final effect may reflect a balance between the activation of mGluR2/3 and mGluR5. High concentrations of NAC could promote drug-seeking reinstatement by activating mGluR5 and, thereby, the glutamate synaptic transmission that occurs with reinstatement. They also suggested future clinical studies to evaluate the combination of an mGluR5 inhibitor with NAC, which might improve the efficacy of NAC (Kupchik et al., 2012).

Drug addiction is characterized by an inability to cease or reduce drug-seeking behaviours, despite environmental information indicating the maladaptiveness of the behaviour. This environmental information is integrated into the prefrontal cortex (PFC) and is capable of modifying striatal habit behaviour via glutamatergic cortico-striatal projections from the PFC to the *NAcc*. Chronic cocaine administration in animal models induces metaplasticity, which is characterized by an absence of long-term potentiation (LTP) or long-term depression (LTD) of these synapses from the PFC to the *NAcc* (Moussawi et al., 2009). Moussawi et al. conducted an animal experiment in which the authors showed that NAC treatment restored LTP and LTD in these synapses. LTP restoration is possible via a mechanism that depends on elevating extrasynaptic glutamate

levels with subsequent activation of mGluR2/3 receptors because the cotreatment with an mGluR2/3 antagonist abolished the ability of NAC to restore LTP. Contrarily, LTD is possibly mediated through the same elevated glutamate levels, activating postsynaptic mGluR5 receptors. The activation of mGluR5 by NAC may indeed promote the reinstatement of the behaviour of cocaine use, considering that the stimulation of these receptors by the positive allosteric modulator CDPPB has been shown to induce the reinstatement of the behaviour of cocaine use, and the mGluR5 antagonist MPEP reduced cocaine-seeking behaviour (Moussawi et al., 2009).

Another study conducted by Moussawi et al. showed that NAC reduction of cocaine seeking in rats continued for at least 2 weeks after the last NAC administration. They demonstrated that NAC normalization of the synaptic strength from the prefrontal cortex to the *NAcc* depended on an mGluR2/3 mechanism by injecting an mGluR2/3 antagonist in the *NAcc* core and showing that this substance blocked the effects of NAC (Moussawi et al., 2011).

Along with NAC, the antibiotic Ceftriaxone has been shown to alter the homeostasis of extrasynaptic glutamate in the *NAcc* by restoring GLT-1 levels. In an animal study, rats underwent a 2-week course of cocaine self-administration, which was followed by 3-week extinction training. Subgroups received NAC, Ceftriaxone or daily saline injections for the last 7 days of extinction training and were then euthanized. The *NAcc* regions were dissected and the levels of GLT-1 and the catalytic subunit of the cystine-glutamate exchanger were measured. Both the NAC and Ceftriaxone groups had restored levels of GLT-1 and of the cystine-glutamate exchanger. This restoration was not observed in drug-naïve rats (Knackstedt et al., 2010).

In a study with 93 rats trained for 12 days to seek cocaine, NAC reduced cocaine-seeking behaviour both after extinction training and after abstinence without prior extinction training. The reduction occurred under various reinstatement protocols (cue or cue + cocaine-primed relapse tests) (Reichel et al., 2011). This study supported the use of NAC as an anti-reinstatement drug.

Murray et al. conducted an animal study in 2011 showing NAC's ability to reduce cocaine-seeking behaviour both in the early and late stages of the acquisition and during maintenance of the behaviour. NAC's ability to reduce lever presses to obtain cocaine

was dose-dependent. NAC had no effect on the reinforcement properties of cocaine and on general locomotor activity (Murray et al., 2012).

In a clinical setting, NAC is chronically administered. In another study with rats, LaRowe and Kalivas investigated the efficacy of NAC in animals trained to self-administer cocaine, starting NAC or saline injections on the first day of the extinction training. Each animal underwent 7 daily extinction sessions. The NAC group was associated with a reduced number of lever presses during the extinction sessions (LaRowe and Kalivas, 2010).

Cocaine and other psychostimulant exposure accelerate the loss of initial goal-directed behaviours and volitional control over reward seeking in exposed animals. Habit learning becomes stronger and animals become insensitive to devalued outcomes, such as continually pressing the lever in a chamber without earning food. These changes are believed to be mediated by impairment in the balance between the dorsomedial striatum (linked to goal-directed behaviour) and dorsolateral striatum (linked to habit learning) (Corbit et al., 2014). In an animal study, Corbit et al. conducted three experiments with rats. In the first experiment, they demonstrated that cocaine-exposed animals became insensitive to devaluation outcomes, showing more rapid habit acquisition compared to controls. In the second experiment, the rats were exposed to cocaine (30 mg/kg) or saline for 6 days. Rat brain slices of the dorsomedial striatum and dorsolateral striatum were subjected to electrophysiological recording. Spontaneous and miniature excitatory postsynaptic currents were increased in frequency in the dorsomedial striatum of rats exposed to cocaine. This change was not observed in the dorsolateral striatum, providing evidence of alterations to the brain region linked to goal-directed learning, thereby resulting in early control of the habit system over the goal-directed learning system. Interestingly, the third experiment showed that NAC cotreatment normalized the EPSCs frequency alterations in the dorsomedial striatum of rats exposed to cocaine and reversed sensitivity to the devalued outcome, thus restoring goal-directed behaviour over habitual control (Corbit et al., 2014).

NAC's ability to inhibit cue-induced reinstatement after extinction training in animals is believed to be mediated by two main mechanisms related to extracellular glutamate homeostasis in the *NAcc*: enhancement of the cystine-glutamate exchanger and

reversal of the cocaine-induced downregulation of GLT-1. When these two effects occurred in a recent animal study, Reissner et al. employed an antisense strategy to suppress the cystine-glutamate exchanger or GLT-1 protein levels as well as a control sequence. In this study, NAC reduced reinstatement with the impaired cystine-glutamate exchanger but not when GLT-1 was suppressed, showing that NAC's ability to restore GLT-1 levels is critically important for NAC to inhibit reinstatement (Reissner et al., 2014). Moreover, NAC treatment with impaired GLT-1 augmented reinstatement, which might be explained by high extracellular glutamate levels, which activate mGluR5 receptors (GLT-1 receptors remove excess glutamate from the synaptic cleft such that when these receptors are impaired, more glutamate leaves the synaptic cleft and enters the extrasynaptic space, which could activate mGluR5 when extrasynaptic concentrations of glutamate are very high). Stimulation of this receptor potentiates reinstatement. Only moderate increases in the extracellular glutamate levels, as provided by NAC treatment alone, activate primarily mGluR2/3 receptors (whose stimulation actually reduces reinstatement) rather than mGluR5 (Reissner et al., 2014).

In rodents, restoring the levels of extrasynaptic glutamate blocks reinstatement of self-administered cocaine after extinction training (Baker et al., 2003a). Bauzo and colleagues investigated whether these findings could be extended to nonhuman primates. They conducted an experiment with twenty adult squirrel monkeys that were trained for cocaine self-administration and later underwent extinction training (Bauzo et al., 2012). They showed using *in vivo* microdialysis that cocaine administration alone (0.3 mg/kg and 1.0 mg/kg intramuscular) increased the extracellular dopamine levels in the caudate nuclei in a dose-dependent manner to 150% and 300%, respectively, whereas saline injection did not alter these levels. When NAC (3.0 or 10 mg/kg intramuscular) was administered 3 hours before cocaine administration, a significant attenuation in the extracellular dopamine increase was observed. This reduction did not occur when NAC was given prior to saline injection, showing that NAC per se does not reduce extracellular basal dopamine levels. Interestingly, these NAC effects on limiting the increase in extracellular dopamine caused by cocaine administration did not translate into any behaviour-, reinforcement- or reinstatement-reducing effect. These authors discussed

whether a more significant attenuation of dopamine levels might be necessary to produce behaviour changes (Bauzo et al., 2012).

Recently, an animal study showed that Sprague Dawley rats exposed to a reinforcement schedule and later to punishment-induced abstinence (footshocks at every fourth or fifth lever press) displayed a lower level of cocaine use if they had been chronically treated with NAC. NAC had no effects on the progression of loss of control over cocaine use nor any effects on drug motivation (assessed under a progressive ratio schedule of reinforcement) (Ducret et al., 2015). Table-1 summarizes the findings of pre-clinical studies.

3.2. Clinical trials

In a placebo-controlled double-blind crossover trial, 15 non-treatment-seeking cocaine-dependent patients were treated in a hospital for three days and given 600 mg of NAC or a placebo every 12 hours (1200 mg/day). The patients were then exposed to slides providing stimuli for cocaine use and slides containing neutral, pleasant and unpleasant stimuli. NAC reduced the desire to use cocaine as well as the interest in and viewing time of the stimulus cues. Although NAC showed no significant effect on cocaine cravings, this finding might be related to the small sample size (LaRowe et al., 2007).

A similar placebo-controlled double-blind crossover study was conducted in a three-day hospital setting with 11 cocaine dependents without known psychiatric comorbidities. The patients were treated with either 600 mg of NAC or a placebo every 12 hours (1200 mg/day). Data regarding the rating of cravings, vital signs, side effects, electrocardiography (EKG) and laboratory exams were collected. No differences in the side effects or EKG results were observed; the statistical analyses showed a clinically non-significant trend towards a decrease in the white blood cell count. No significant differences were observed between the NAC and placebo groups in direct comparisons between craving and withdrawal symptoms. However, in a one-sample t-test, the changes in scores for craving and withdrawal symptoms were significantly different in the NAC group but not in the placebo group. This finding indicates that NAC may reduce craving and withdrawal symptoms. This study also indicated that self-reported use was reduced after an average of 7 days of follow-up (LaRowe et al., 2006).

An open-label trial consisting of 23 cocaine-dependent subjects, divided into three groups given different doses of NAC (1200 mg/day, 2400 mg/day and 3600 mg/day) over 4 weeks, assessed the overall tolerability, safety, retention and compliance of NAC along with the self-reported cocaine use and abstinence symptoms of the study participants. This study found no significant differences between the side effects experienced among the different dose groups. The side effects were mild and self-limited, and the three most common were pruritus, headache and elevated blood pressure (possibly unrelated to the NAC treatment). The total number of days of cocaine use during the 4-week period was clinically and significantly reduced compared with those during the 28 days prior to treatment. The mean total dollars spent on cocaine after treatment compared with the pretreatment mean was significantly reduced. The retention rates also favoured higher doses of NAC (88% for 2400 mg/day, 83% for 3600 mg/day and 37.5% for 1200 mg/day) (Mardikian et al., 2007).

In a single-blind crossover trial, six cocaine-dependent subjects were treated with NAC dosed at 1200-2400 mg/day (in four subjects) or with Baclofen dosed at 600 mg/day (in four subjects) during one to two hospital stays. Each stay consisted of four days. Before and after three days of treatment, the participants were given an intravenous cocaine infusion and exposed to a cocaine-stimulating video and a neutral video; subsequently, their craving, rush and high feelings were assessed. Participants had no change in their self-reported feelings of high and rush after a cocaine infusion; however, their craving levels after NAC treatment were significantly decreased, which did not occur after Baclofen treatment (Amen et al., 2011).

In another open-label, randomized crossover study conducted with 10 cocaine dependents and 14 controls, subjects received a single dose of 2400 mg of NAC or no medication. One hour after NAC administration, they underwent proton magnetic resonance spectroscopy of the brain. By this examination, compared with the medication-free groups, the NAC groups exhibited a significant reduction in the glutamate/creatinine ratio within the left dorsal anterior cingulate cortex, where dysfunction is associated with impaired impulse inhibition. This reduction was only observed in cocaine dependents; NAC did not reduce the glutamate/creatinine ratio in the non-cocaine-dependent, healthy controls. This result was expected because NAC may only reverse abnormalities in

glutamate homeostasis (i.e., in cocaine-dependent individuals). In the medication-free group, the glutamate/creatinine ratios were also greater in the cocaine-dependent compared with the non-dependent group. This difference was normalized after a single dose of NAC. This study also found a positive correlation between the glutamate/creatinine ratio and impulsivity (Schmaal et al., 2012).

In 2013, a double-blind placebo controlled trial was conducted in 111 cocaine-addicted treatment-seeking adults (LaRowe et al., 2013). They were randomized to placebo, 1200 mg or 2400 mg of NAC daily for an 8-week medication trial. Benzoylecgonine (a cocaine metabolite) levels were assessed. No significant difference in the levels of this metabolite was observed between the groups, indicating that NAC failed to affect abstinence. The study design may account for the negative result of this trial, considering that most individuals were not abstinent. NAC may be better suited for relapse prevention in already abstinent individuals, as shown in animal studies. In fact, in the subgroup of the sample that was already abstinent, NAC increased time to relapse and reduced craving, particularly in the 2400-mg group (LaRowe et al., 2013).

Table-2 summarizes the findings of the clinical studies.

4. Discussion

This investigation presents a systematic review of the studies regarding the potential benefits of NAC in the treatment of cocaine addiction. A systematic review regarding the potential of NAC for cocaine, cannabis, nicotine and methamphetamine addiction and another concerning pathological gambling conducted by Asevedo et al. were published in 2014 (Asevedo et al., 2014). The present review aimed to focus on cocaine dependence, examining the neurobiological mechanisms underlying NAC effects in greater depth. To achieve this, we included clinical studies and animal studies to probe the potential role of NAC in cocaine addiction treatment. Currently, because effective pharmacological treatments for cocaine addiction are lacking, research on NAC's ability to reverse chronic adaptations of neuroplasticity following long-term cocaine use offers an exciting possibility for treating this population. NAC has been shown to reverse the disruption of glutamate homeostasis caused by long-term cocaine use and is able to restore both the function of the cystine-glutamate exchanger in glial cells and to reverse downregulated GLT-1 receptor function (Brown et al., 2013). One study suggested that

restoring GLT-1 function may be more important than restoring the cystine-glutamate exchanger's function (Moussawi et al., 2011). An optimal dose of NAC may be the dose that restores extrasynaptic glutamate levels to previous physiological levels (and that activates predominantly mGluR2 and 3) but not to very high levels because very high levels may stimulate reinstatement by activating mGluR5 (Kupchik et al., 2012). One interesting study also found an attenuation of cocaine-induced increase in *NAcc* dopamine levels after the coadministration of NAC, which merits further investigation because the mechanisms that involve glutamatergic transmission have been more thoroughly examined in studies involving cocaine and NAC compared to dopamine mechanisms (Bauzo et al., 2012). Clinical trials have employed between 1200-3600 mg/day (Amen et al., 2011; LaRowe et al., 2013, 2007, 2006; Mardikian et al., 2007; Schmaal et al., 2012). This double-edged effect of elevating extrasynaptic levels using NAC may be considered in future clinical trials to identify the optimal dose that inhibits relapse.

Data regarding the side effects of NAC have indicated that acute treatment with NAC may cause only mild side effects (pruritus, headache and elevated blood pressure), which might not be associated with NAC (Mardikian et al., 2007). The side effects of varying doses of NAC (1200-, 2400- and 3600-mg doses) were not significantly different (Mardikian et al., 2007). NAC also showed no effects on vital signs and EKG measurements; however, a non-clinically significant trend towards a reduction in blood cell count was reported (LaRowe et al., 2006). The two studies regarding the general safety of NAC showed only mild or non-existent side effects; moreover, NAC has been used for many years for other purposes (e.g., as a mucolytic). Thus, studies with larger sample sizes and assessments of possible long-term side effects would be of interest.

The available data on NAC also showed a normalization of the glutamate/creatinine ratio in the left dorsal anterior cingulate cortex, as assessed by proton magnetic resonance spectroscopy brain scans following a single dose of NAC. This change only occurred in cocaine-dependent subjects, highlighting the importance of the disruption of glutamate mechanisms in cocaine dependence and showing NAC's ability to reverse this disruption (Schmaal et al., 2012). Four studies also showed NAC's potential to reduce cravings, the desire to use cocaine, cocaine-cue viewing-time and cocaine-related spending (Amen et

al., 2011; LaRowe et al., 2006, 2007; Mardikian et al., 2007). These studies only presented preliminary data because they lacked large sample sizes or were open-label trials; however, their preliminary findings merit further investigation.

Finally, considering animal studies (most of which utilized an anti-reinstatement protocol) and the double-blind placebo trial conducted by LaRowe et al. in 2013, in which positive findings were only reported in the subgroup of patients who were already abstinent, NAC may be better suited for relapse avoidance in already abstinent patients. This observation may offer future clinical trials a different approach for cocaine use disorders, that is, by focussing investigations on NAC as a relapse-prevention agent rather than an abstinence-promoting agent. Notably, for clinical reasons, most clinical studies utilized doses of NAC ranging from 1200 mg/day to 3600 mg/day, although the higher doses (3600 mg/day) appear considerably more efficacious without altering the already low side-effect profile of the treatment. A persistently unanswered question is whether a chronic treatment regimen is necessary in future clinical trials because whereas the first NAC animal studies utilized single NAC doses, most later animal and clinical studies administered NAC in multiple doses (ranging from 2 days to 8 weeks). Both animal and clinical studies have already shown that single-dose treatments promote measurable neurochemical changes; however, whether these changes suffice for measurable behaviour changes remains unclear (Bauzo et al., 2012; Schmaal et al., 2012). Because most clinical evidence suggests a dose-dependent efficacy of NAC and supports chronic NAC administration, we speculate that future clinical trials may consider a chronically administered regimen.

Declaration of interest

All authors declare they have no conflicts of interest

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Table 1 – Animal studies investigating the basic science underlying N-acetylcysteine's (NAC) action

Author, year	**Main findings**
Ducret et. al, 2015	NAC treated rats exposed to a reinforcement schedule and later to punishment-induced abstinence displayed a lower level of cocaine use. NAC had no effects on the progression of loss of control of cocaine use nor had any effects on motivation for the drug
Reissner et. al, 2014	NAC's ability to restore the GLT-1 may be more important than the cysteine-exchanger in inhibiting reinstatement.
Corbit, 2014	NAC both normalized the excitatory postsynaptic currents (EPSCs) frequency alterations in the dorsomedial striatum of rats exposed to cocaine and reversed sensitivity to the devalued outcome, thus restoring goal-directed behavior over habitual control.
Murray et al., 2012	NAC is capable of reducing cocaine-seeking behavior both in the early and late stages of the acquisition and maintenance of the behavior in a dose dependent manner. NAC had no effect on reinforcement properties of cocaine and on general locomotor activity.
Kupchik, 2012	mGluR 2/3 antagonist LY341495 blocks inhibition of reinstatement mediated by NAC. Excitatory postsynaptic currents (ESPC) amplitude in *NAcc* responds in a biphasic manner to different NAC doses. They are reduced by low NAC doses and elevated by high doses. Reduction of ESPCs are dependent on mGluR 2/3 receptors, while elevation is dependent on mGluR 5.
Bauzo, 2012	Cocaine administration in adult squirrel monkeys elevates extracellular dopamine levels in nuclei caudate in a dose dependent manner. NAC administered 3 hours before cocaine attenuates this elevation, but this did not translate into any behavioral changes. A greater attenuation than what was achieved by the study may be necessary to affect behavior.

Reichel et al., 2011	NAC reduced cocaine-seeking behavior both after an extinction training or after abstinence without prior extinction training under multiple relapse protocols (cue or cue + cocaine-primed)
Moussawi, 2011	NAC's reduction of cocaine seeking in rats endured for at least 2 weeks after the last NAC administration. NAC's normalization of synaptic strength from prefrontal cortex to *NAcc* is dependent on an mGluR 2/3 mechanism.
LaRowe, 2010	NAC reduces lever pressing in extinction training sessions in rats trained to self- administer cocaine.
Knackstedt, 2010	NAC and Ceftriaxone restore levels of GLT-1 in rats trained to self-administer cocaine
Moussawi, 2009	NAC treatment restored Long-term potentiation (LTP) and Long-term depression (LTD) in *NAcc's* synapses. LTP restoration is possible via a mechanism dependent on elevating extrasynaptic glutamate levels, and subsequently activation of mGluR 2/3 receptors. Cotreatment with an mGluR 2/3 antagonist abolishes the ability of NAC to restore LTP. LTD is possibly mediated through the same elevated glutamate levels activating postsynaptic mGluR 5 receptors. NAC's activation of mGluR 5, actually, may promote relapse.
Kau, 2008	NAC increases activity of cystine-glutamate exchanger, reducing cocaine-primed reinstatement in rodents.
Madayag, 2007	NAC administered before cocaine use prevents the establishment of plasticity-dependent cocaine seeking, even when behavior was assayed 21 days after the last injection of N-acetylcysteine. Pretreatment with NAC prevents changes in levels of glutamate in the *NAcc* and plasticity involving cystine-glutamate exchanger, induced by cocaine.
Moran, 2005	NAC increases the level of extracellular glutamate through activation of the cystine-glutamate exchanger. Stimulation of presynaptic receptors mGluR 2/3 by nonsynaptic glutamate inhibits synaptic glutamate release and, consequently, excitatory synaptic activity.
Baker, 2003	Cocaine-withdrawn rats displayed lower levels of extrasynaptic glutamate in *NAcc*. A cystine –glutamate exchanger blocker mimics these lower levels, and NAC reverses these changes.

Table 2 – Clinical studies using N-acetylcysteine (NAC) as an intervention for cocaine dependence

Author, year	**Type of Study**	**N**	**What the study evaluates**	**Details about what was evaluated**	**Results**	**Limitations of the study**

Larowe, 2013	Randomized, Double blind, placebo controlled	111	Craving, abstinence, days to relapse	Urine samples of participants were collected and the cocaine metabolite Benzoylecognine measured over an 8-week period.	No difference between groups for cocaine metabolite levels in urine sample (abstinence). In a subgroup of individuals already abstinent, NAC prolonged time to relapse	Design of the study, which assessed NAC's role on inducing abstinence. NAC has been studied as an anti-relapse agent in animal studies instead of an abstinence inducer.
Schmaal, 2012	Crossover, open-label, randomized	8 patients and 14 controls	Effect of NAC on glutamate levels	Each subject underwent 2 Proton magnetic resonance spectroscopies with a 2-week interval. They randomly received either 2400 mg of NAC or nothing.	Maintenance of glutamate levels. Reduction of the glutamate/creatinine ratio in cocaine-dependent subjects to levels similar to non-dependent subjects after NAC treatment. Moreover, there was a positive correlation between glutamate/creatinine in the dorsal anterior cingulate cortex and impulsivity.	Open-label study (subjective impression of taking NAC changing glutamate levels); small sample; only one dose of NAC; cocaine-dependent subjects were also consuming alcohol and cigarettes;

						data on metabolites collected only from the dorsal anterior cingulate cortex
Amen, 2011	Pre-clinical single-blind trial	2 groups of 4 cocaine-addicted participants and 2 groups of 12 rats.	Effectiveness of NAC in reducing craving in humans and reinstatement in rats	Whether cocaine reinstatement in rats and craving in humans were reduced after daily NAC treatment.	Seven days of NAC produced a significant reduction in cocaine-induced reinstatement in rats. Four days of NAC (1200–2400 mg/day) reduced craving in cocaine-dependent human subjects following an experimenter-delivered intravenous injection of cocaine (20 mg/70 kg/60 s).	Lack of a placebo group compared with human dependent subjects (NAC was compared with baclofen). The subjects in the two groups were almost the same, differentiated by a short washout period between each treatment (7-14 days).

Larowe, 2007	Crossover, double-blind, placebo-controlled clinical trial	15	Whether NAC can suppress the reactivity of cocaine-dependent subjects to cues	Participants were hospitalized. One group received placebo and the other 600mg of NAC every 12h for a total of four doses (2400 mg). Participants completed a cue-reactivity procedure in response to images that portray cocaine and its use.	There was no difference in the physiological measurements (craving, desire to use, interest and time spent on each image). There was a significant difference favoring the NAC group compared to the placebo when the participants were exposed to images related to cocaine, concerning desire to use, interest and time spent on each image.	Small sample and very short period of medication use.
Markidian, 2007	Open-label clinical trial	23	Safety and tolerability of 3 different NAC doses for the treatment of cocaine dependency (1200 mg/day, 2400 mg/day and 3600 mg/day)	Treatment phase lasted 4 weeks. Adverse events and self-reported cocaine use were assessed. Cocaine use was verified by Urine Drug Screening. Vital signs, cocaine craving and abstinence symptoms (CSSA, Cocaine Selective	No serious side effects occurred. Difference of completers across the groups approached statistical significance, favoring 2400mg and 3600mg. The baseline mean of total days of use, of total dollars spent on cocaine and of the overall mean baseline CSSA for subjects who	Evaluates only short-term side effects. Seven out of the 23 participants failed to complete the trial.

				Severity Assessment) were also assessed. Participants were hospitalized twice for 3 days (for the crossover) with a 4-day interval between hospitalizations. One group received placebo and the other 600mg of NAC every 12h, for a total dose of 2400 mg. Participants were evaluated by laboratory tests, craving and abstinence scales, vital signs, EKG and EEG.	completed the study showed significant improvement. There were no serious, unexpected or significantly different side effects. Craving and withdrawal: No differences between the NAC and placebo. Only the one-tailed t-tests for the NAC condition reached significance. When the 90-days prior to hospitalization were compared to the two weeks post-hospitalization, there was a reduction in the proportion of use days and dollars spent on the drug.	
LaRowe, 2006	Double-blind, placebo-controlled crossover clinical trial	13	Safety and tolerability of NAC in healthy cocaine-dependent individuals; reduction of craving rate and abstinence symptoms			Small sample size, and too short a period of medication use.

Highlights

- We conducted a review on animal and clinical Studies investigatig N-acetylcysteine as a treatment for cocaine addiction
- NAC has been shown to reverse the disruption of glutamate homeostasis caused by long-term cocaine use
- NAC may be better suited for avoiding relapse in already abstinent subjects
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