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# Interactive effects of N-acetylcysteine and antidepressants

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ABSTRACT

N-acetylcysteine (NAC), a glutathione precursor and glutamate modulator, has been shown to possess various clinically relevant psychopharmacological properties. Considering the role of glutamate and oxidative stress in depressive states, the poor effectiveness of antidepressant drugs (ADs) and the benefits of drug combination for treating depression, the aim of this study was to explore the possible benefit of NAC as an add on drug to treat major depression. For that matter we investigated the combination of subeffective and effective doses of NAC with subeffective and effective doses of several ADs in the mice tail suspension test. The key finding of this study is that a subeffective dose of NAC reduced the minimum effective doses of imipramine and escitalopram, but not those of desipramine and bupropion. Moreover, the same subeffective dose of NAC increased the minimum effective dose of fluoxetine in the same model. In view of the advantages associated with using the lowest effective dose of antidepressant, the results of this study suggest the potential of a clinically useful interaction of NAC with imipramine and escitalopram. Further studies are necessary to better characterize the molecular basis of such interactions, as well as to typify the particular drug combinations that would optimize NAC as an alternative for treating depression.



## 1. Introduction

Major depression (MD) is the commonest psychiatric disorder, estimated to affect as much as 120 million people worldwide (Hashimoto, 2011). Chief reasons for failure with antidepressant treatments are thought to include the lack of proper response, the considerable disabling adverse effects of ADs drugs, the length of psychiatric history, and the quality of patient–physician relationship (Keitner et al., 2006; Pampallona et al., 2004). While the need for developing more effective drugs is a consensus, alternative strategies to improve the clinical response have been explored, especially in refractory cases. Antidepressant augmentation (e.g., ADs plus pindolol, lithium, and buspirone) and combination (two ADs with different mechanisms of action) are commonly used as such strategies (Connolly and Thase, 2011; Linde et al., 2011; Rocha et al., 2012) and are advocated to improve the prognosis of patients refractory to standard treatment protocols (Bobo et al., 2011; Martín-López et al., 2011; Rojo et al., 2005), as well as those with marked suicidal ideation (Zisook et al., 2011).

In line with the monoaminergic hypothesis of depression, the mechanism of action of the majority of current ADs aims to increase/restore neurotransmitter synaptic levels. The so called glutamate hypothesis put forward at the early 90s (Trullas and Skolnick, 1990) aired the neurobiological basis of depression, raising expectations to decipher the gaps present at the more established monoaminergic hypothesis. The report that ketamine, a glutamate NMDA receptor antagonist, improves depressive symptoms in treatment resistant patients can be viewed as a landmark in this focus shift (Berman et al., 2000), with evidence pointing to the role of glutamate in mood disorders rapidly accumulating (Hashimoto, 2011; Tokita et al., 2012). The timing for clinical response with ketamine is in itself impactful: antidepressant effects are observed 120 min after a single intravenous administration, and effects were documented to last for one week (Zarate et al., 2006). Even though ketamine falls short from the ideal drug, its clinically relevant, though transient (Jordan et al., 2006), antidepressant effects were crucial to reinforce the concept that drug-induced glutamate modulation

*Abbreviations:* ADs, antidepressants; AMPA, 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid; CNS, central nervous system; ERK1/2, extracellular signal-regulated kinase 1 and 2; FST, forced swimming test; MD, major depression; NAC, N-acetylcysteine; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f] quinoxaline-2,3-dione; NMDA, N-methyl-D-aspartate; TST, tail suspension test; VGLUT1, vesicular glutamate transporter 1.

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may be a promising possibility to improve the treatment of depression. Sanacora et al. (2012, p. 64) in fact called for the recognition of glutamate as "the final common pathway of therapeutic treatment for depression and other mood/anxiety disorders".

N-acetylcysteine (NAC), long available in the clinic as a mucolytic and antidote for acetaminophen poisoning, also induces clinically useful effects in a variety of psychiatric conditions (Dean et al., 2011). The basis for NAC psychopharmacological effects is suggested to be related to its antioxidant properties, as well as its modulation of glutamate pathways (Baker et al., 2003; Berk et al., 2008a). Relevant to this study are the reports of NAC benefits as an add-on antidepressant treatment in bipolar patients (Berk et al., 2008b, 2011; Magalhães et al., 2011). Noteworthily, good tolerability and lack of significant side effects, based upon toxicological and pharmacokinetic data, are all well established for NAC (Whyte et al., 2007), which is available in the market at low cost formulas. Though specific trials for antidepressant effects in unipolar depression are still lacking, NAC shows antidepressant-like activity in several pre-clinical models, including the rat forced swimming test (FST) (Ferreira et al., 2008), mice tail suspension test (TST) (Linck et al., 2012), FST in bulbectomized rats (Smaga et al., 2012), and was recently reported to reverse the stress-induced sweet food consumption decrease in rats (interpreted as an animal correlate of anhedonia) (Arent et al., 2012). The results of these preclinical data were associated with the reduction of oxidative stress (Arent et al., 2012; Ferreira et al., 2008; Smaga et al., 2012), and the AMPA glutamate receptors (Linck et al., 2012).

Considering the increasing attention given to the role of glutamate and oxidative stress in depressive states, the poor outcome of ADs and the advocated superiority of drug combination for treating depression, the aim of this study was to explore the validity of NAC as an add-on medication for MD. We hypothesized that positive interactions could result by combining NAC with ADs, depending on specificities of ADs mechanisms of action. We here tested different doses of NAC in combination with desipramine, bupropion, imipramine, escitalopram, and fluoxetine, in the mice tail suspension test.

## 2. Materials and methods

### 2.1. Animals

Experiments were performed with male (CF1) 2-month-old albino mice (40–45 g) obtained from Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS). Mice were maintained at the Pharmacology Department animal facility under controlled environmental conditions ($22 \pm 1$ °C, 12 h-light/dark cycle, free access to food [Nuvilab CR1] and water) for at least two weeks before the experiments. All procedures were carried out according to institutional policies on experimental animals handling; the project was approved by the University ethics committee (approval #19981).

### 2.2. Drugs

N-acetylcysteine (NAC), imipramine, bupropion and desipramine were acquired from Sigma-Aldrich (St Louis, Missouri, USA). Escitalopram and fluoxetine were used from commercial sources (Lexapro®, H. Lundbeck A/S Laboratories, RJ, Brazil and Daforin®, Laboratório EMS, SP, Brazil). All drugs were solubilized in saline (NaCl 0.9%), used as the negative control; injection volume was 0.1 ml/10 g of body weight. All drugs were administered intraperitoneally.

### 2.3. Tail suspension test (TST)

We used the tail suspension test method as described by Steru et al. (1985). In a soundproof room, mice were suspended by the tail with a piece of adhesive tape applied to a wooden frame 50 cm above the worktable. Mice were observed for 6 min, during which the immobility time was recorded with a stopwatch; mice were considered immobile when hanging passively and motionless. Animals were immediately returned to their housing after the experiments.

Effective doses of NAC and standard ADs in the mice TST have been previously identified in our laboratory (Linck et al., 2012); subeffective doses were defined by pilot experiments, in which doses increasingly lower than those found to significantly reduce immobility were used. The experiment was designed to verify if subeffective doses of NAC combined with subeffective and/or effective doses of ADs resulted in effects different from ADs alone. NAC 5.0 mg/kg was administered 1 h prior to test; desipramine (2.5, 5.0 and 10.0 mg/kg), bupropion (1.0, 2.0, and 10.0 mg/kg), imipramine (5.0, 10.0 and 20.0 mg/kg), escitalopram (1.0, 2.0 and 3.0 mg/kg), and fluoxetine (24.0, 28.0 and 32.0 mg/kg) were administered 30 min prior to test ($n = 8–10$). The time courses for drug administrations were chosen due to pharmacokinetic reasons (Ferreira et al., 2008). Pilot experiments showed that NAC was effective 1 h after administration, and antidepressants were effective within 30 min.

A second experiment was designed to evaluate the effects of combining the effective dose of 25 mg/kg NAC in the TST (Linck et al., 2012) with effective doses of imipramine and escitalopram in the same test.

### 2.4. Locomotion

Locomotion was assessed in activity cages ($45 \times 25 \times 20$ cm, Albarsch Electronic Equipments, Porto Alegre, Brazil) equipped with four parallel photocells. The number of crossings was automatically recorded for 15 min, considering the first 5 min as exploration and the final 10 min as locomotion (Linck et al., 2009). The doses of standard ADs (administered 30 min prior to test) and NAC (administered 1 h prior to test) were the same as those used for the TST.

### 2.5. Statistical analysis

Data were analyzed by one- or two-way analysis of variance (ANOVA), followed by Newman–Keuls post hoc test. GraphPad Prism 5.0 for Windows was used for the statistical analysis. Statistical significance was set at $p<0.05$. Values were expressed as mean and standard error of mean.

## 3. Results

Fig. 1(A–E) shows that 5 mg/kg NAC alone did not decrease immobility in the TST. No significant interaction was observed for the combination of this subeffective dose of NAC with progressive doses of desipramine ($F_{3,67} = 0.84$, $p > 0.05$) (Fig. 1A) or bupropion ($F_{3,73} = 0.86$, $p > 0.05$) (Fig. 1B). However, statistically significant interactions were verified for the combination of this subeffective dose of NAC with progressive doses of imipramine ($F_{3,68} = 5.6$, $p<0.001$) (Fig. 1C), escitalopram ($F_{3,65} = 3.92$, $p<0.01$) (Fig. 1D), or fluoxetine ($F_{3,66} = 3.75$, $p<0.01$) (Fig. 1E). The interaction is such that the combination with NAC decreased the minimum effective doses of imipramine and escitalopram, while increased the minimum effective dose of fluoxetine.

Fig. 2 shows that the combination of 25 mg/kg NAC, a dose that diminishes immobility at the TST, with likewise active doses of imipramine (Fig. 2A) or escitalopram (Fig. 2B) did not alter the effect of any of the drugs alone ($p > 0.05$).

None of the drugs at doses used in TST experiments interfered with mice locomotion ($F_{12,90} = 1.77$, $p > 0.05$ for antidepressants; $F_{2,21} = 0.302$, $p > 0.05$ for NAC) (data not shown).

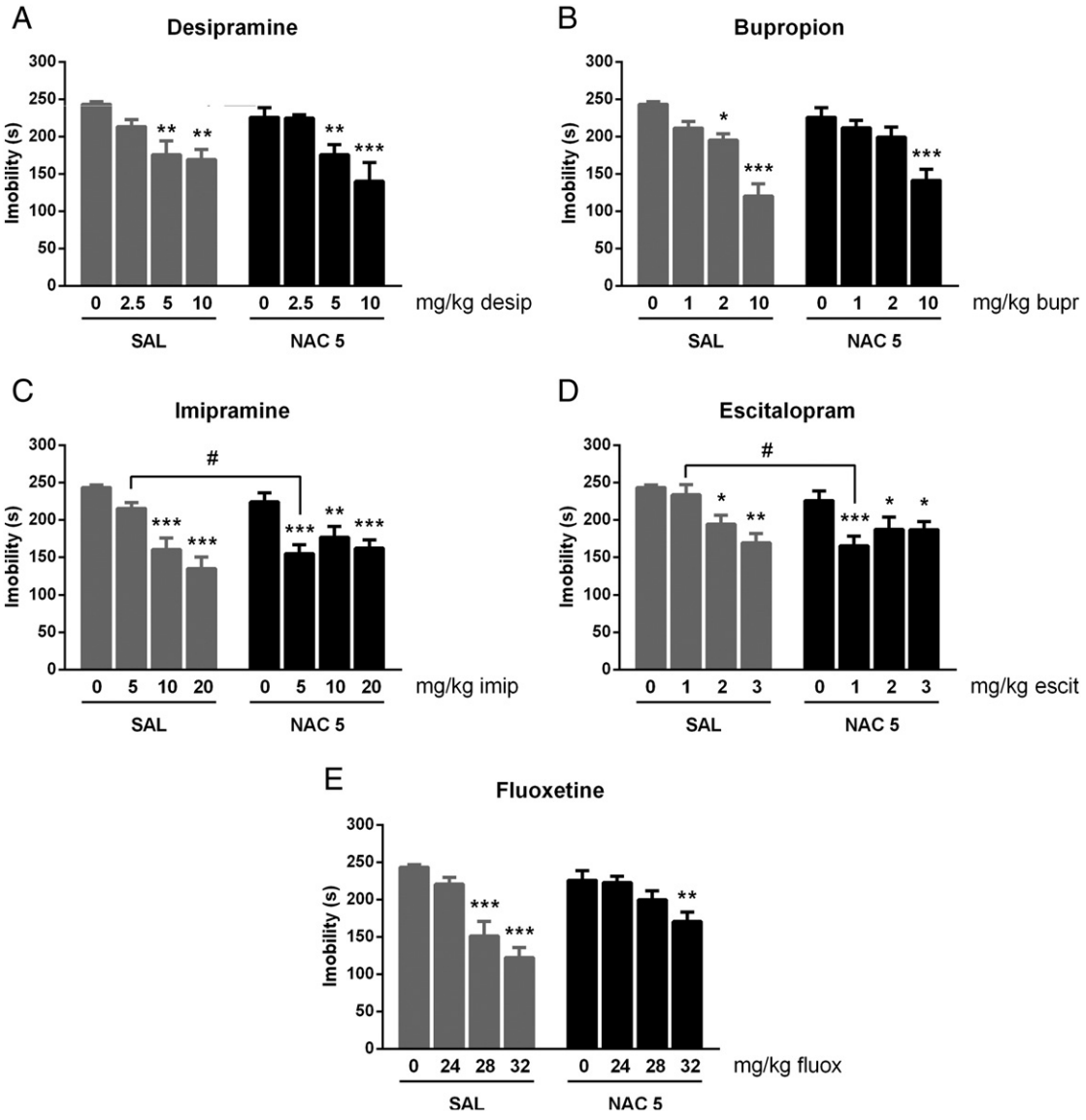


**Fig. 1.** Effects of a subeffective dose of N-acetylcysteine (NAC, 5 mg/kg) combined with progressive doses of (A) desipramine (n = 8–10), (B) bupropion (n = 7–13), (C) imipramine (n = 8–11), (D) escitalopram (n = 7–11), and (E) fluoxetine (n = 8–10). Data represent mean + SEM. *p<0.05, **p<0.01, ***p<0.001 compared to saline–saline; #p<0.01 for the comparisons shown. Two-way ANOVA/Newman–Keuls.

## 4. Discussion

The key finding of this study is that a dose of NAC devoid of antidepressant-like effects in the mice TST, a model with established predictive value (Willner et al., 2003), reduces the minimum effective doses of imipramine and escitalopram, but not those of desipramine and bupropion in the same model. Moreover, the same subeffective dose of NAC increases the minimum effective dose of fluoxetine. Considering the advantages consequent to lowering antidepressant effective doses the results of this study suggest a potentially clinical useful interaction of NAC with imipramine and escitalopram.

Highlighting the role of glutamate in depressive states and the mode of action of various classes of ADs, it has been shown that NMDA antagonists not only possess antidepressant activity but also potentiate the effects of standard ADs (Maj et al., 1992; Petrie et al., 2000; Trullas and Skolnick, 1990). In comparison with known NMDA antagonists that show antidepressant effects, but also a range of unwanted effects that hinder its clinical use, given its safety and tolerability profile (Whyte et al., 2007) NAC may be an ideal candidate to translate to clinical setting the concept of potentiating ADs effects with glutamate antagonists. The availability of glutamate to its various receptors is primarily determined by the astrocytic sodium-dependent glutamate transport (Diamond, 2001; Dunlop, 2006; Huang and Bergles, 2004; Huang et al., 2004). Glutamate availability can be additionally tuned by the astrocytic cystine–glutamate exchanger, a mechanism of non-vesicular glutamate release into the extrasynaptic compartment (Baker et al., 2002). By controlling extrasynaptic glutamate levels, the cystine–glutamate exchanger modulates group II metabotropic glutamate autoreceptors, ultimately leading to reduced glutamate synaptic release (Moran et al., 2005). It is through the astrocytic cystine–glutamate exchanger that NAC is thought to modulate glutamate pathways in a clinically relevant manner (Baker et al., 2002; Dean et al., 2011). This subtle but effective regulation of glutamate release would be beneficial to CNS diseases accompanied by hyperglutamatergic states, such as addiction (Schmaal et al., 2012), schizophrenia (Jordan et al., 2006), and depression (Sanacora et al., 2012).

It has been suggested that, despite differences in primary mechanisms of action, ADs might work by stabilizing glutamate neurotransmission in key brain areas, such as the hippocampus (Bonanno et al., 2005; Hashimoto, 2011; Sanacora et al., 2012). Our data show that NAC interacts selectively with different ADs, indicating that specificities in how different agents affect glutamate function are of relevance.

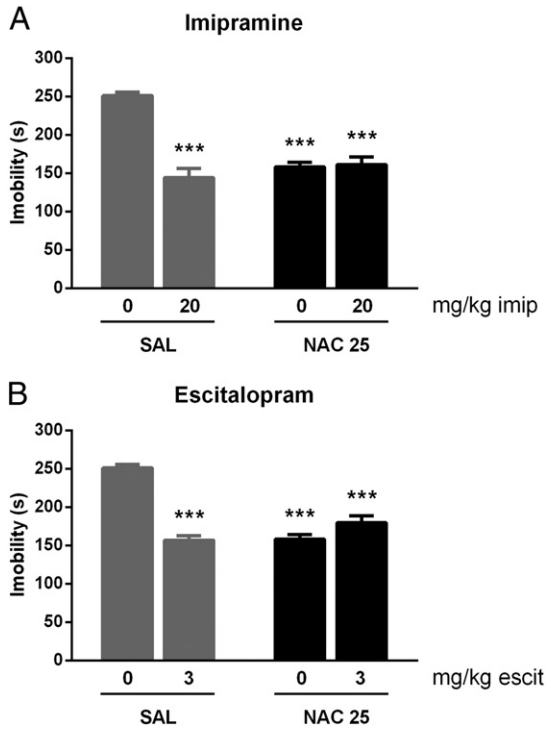


**Fig. 2.** Effects of an active dose of N-acetylcysteine (NAC, 25 mg/kg) combined with active doses of (A) imipramine (20 mg/kg, n = 8), and (B) escitalopram (3 mg/kg, n = 8). Data represent mean + SEM. ***p<0.001 compared to saline-saline. One-way ANOVA/Newman–Keuls.

Barbon et al. (2006) concluded that desipramine exerts moderate but selective effects on glutamate receptor expression and editing. FST-induced increase in glutamate levels (mouse dorsolateral prefrontal cortex) was reversed by acute desipramine administration (Kim et al., 2010). Relevant to this discussion, Bouron and Chatton (1999) showed that in cultured hippocampal neurons desipramine rapidly enhanced the spontaneous vesicular release of glutamate. The effects of bupropion in glutamate transmission are less clear. While it has been shown that bupropion inhibits glutamate release in rat cerebral cortex nerve terminals (Lin et al., 2011), acute and chronic exposure of rats to bupropion results in enhanced striatal overflow of glutamate (Santamaría and Arias, 2010). The increase in glutamate release induced by these two ADs may be the basis for their lack of interaction with NAC, which decreases glutamate release.

Though the mechanism of action of imipramine is related to the blockade of serotonin and norepinephrine reuptake, it has been shown that it also induces marked changes in glutamate: imipramine decreases the spontaneous release of glutamate in rats pre-frontal cortex (Tokarski et al., 2008), decreases potassium-stimulated glutamate outflow (Michael-Titus et al., 2000), and, chronically administered, reduces radioligand binding to NMDA receptors in the same area (Nowak et al., 1993, 1996; Skolnick et al., 1996). An increased expression of AMPA receptors combined with a reduced function of NMDA have been postulated as part of imipramine mechanism of action (Martinez-Turrillas, 2002; Skolnick, 1999). Escitalopram, the S isomer of citalopram, is a selective inhibitor of serotonin reuptake extensively used in the clinic (Höschl and Svestka, 2008). Its effect in the mice FST was shown to be dependent on the inhibition of NMDA receptors (Zomkowski et al., 2010). Microdialysis experiments indicates that citalopram (acute or for two weeks) significantly inhibited the release of glutamate and aspartate (Gołembiowska and Dziubina, 2000), which is likely to occur with escitalopram at lower doses. The overall effect of these ADs in reducing glutamate transmission is coherent with the leftward shift observed with the combination with NAC.

The effects of fluoxetine in glutamate transmission seem, at best, unclear. It was found to increase astrocytic glutamate efflux (mouse prefrontal cortex acute slices, Schipke et al., 2011), as well as the expression of vesicular glutamate Transporter-1 (VGLUT1) when chronically administered (C57BL/6 but not in the BALB/c mice, Farley et al., 2012). Selective changes in glutamate receptor subunits of NMDA and AMPA receptors are also documented in rodents (Ampuero et al., 2010; Barbon et al., 2006). Glutamate signaling through extracellular signal-regulated kinase 1 and 2 (ERK1/2) in astrocytes was shown to be abolished by fluoxetine (Li et al., 2011b). Our data indicates that, in the presence of NAC, higher doses of fluoxetine are required for a statistically significant reduction of immobility in the TST, indicating that diminished glutamate release and/or postsynaptic glutamate receptors activation impairs fluoxetine activity. A possible reading is that an adequate level of synaptic glutamate is required for optimizing fluoxetine effects.

Counteracting oxidative stress (Berk et al., 2008a; Ferreira et al., 2008) and modulating glutamate (Dean et al., 2011; Linck et al., 2012; Schmaal et al., 2012) are the two key NAC properties under consideration regarding its postulated antidepressant effects. The antidepressant-like effects of NAC in bulbectomized rats (Smaga et al., 2012) and unpredictable chronic stress (Arent et al., 2012) were accompanied by reduced markers of oxidative stress, and it has been documented that significant oxidative stress exist after FST and accompanies human depressive states (Behr et al., 2012; Ferreira et al., 2012). Nevertheless, the notion that antidepressant effects are the result of antioxidative properties is contradictory to the data showing that a single ketamine administration increased lipid peroxidation, nitrite content and catalase activity, while decreased glutathione levels in mice prefrontal cortex (da Silva et al., 2010).

It has been shown that acute stress (foot shock, tail pinch, forced swimming, and restraint) induced marked increase in glutamate release (Musazzi et al., 2010; Popoli et al., 2012). Specifically for foot shock-induced stress, patch-clamp recordings of pyramidal neurons in the prefrontal cortex revealed that stress increased glutamatergic transmission through both pre- and postsynaptic mechanisms; moreover, various antidepressants counteracted this glutamate increase (Musazzi et al., 2010). Though subeffective doses of imipramine and escitalopram became active when combined with NAC, no significant interaction in terms of reduced immobility was observed when associating effective doses of any of these antidepressants with NAC. It has been shown that a single dose of NAC reduces the glutamate/creatine ratio in the anterior cingulate cortex in cocaine addicts, but not in healthy subjects (Schmaal et al., 2012). Likewise, intra-accumbens infusion of cystine was shown to restore extracellular glutamate levels in cocaine withdrawn rats, but not in control rats (Baker et al., 2002). Coherently with NAC subtle modulation of glutamate levels, our data also suggest that NAC modifies glutamate levels in a relevant manner only whenever glutamate homeostasis is disturbed.

We showed that the effect of NAC in the mice TST was partially reversed by pretreatment with NMDA and completely reversed by the AMPA antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl benzo[f] quinoxaline-2,3-dione (NBQX), supporting the hypothesis that the modulation of glutamate by NAC is a crucial component of NAC antidepressant properties (Linck et al., 2012). It was likewise shown that NBQX attenuated ketamine-induced antidepressant-like behaviors, and the regulation of hippocampal phosphorylated GluR1 AMPA subunit (Maeng et al., 2008). Accordingly, it has been speculated that enhanced AMPA receptor activity is key to the spine morphogenesis and rapid antidepressive response to ketamine (Li et al., 2011a). Interestingly, it has been argued that the modulation of glutamatergic synapses trough potentiation of AMPA function is important for the clinical effects of electroconvulsive shock treatment (Tokita et al., 2012). Taken together, the data point to

glutamate modulation as the key mechanism for NAC antidepressant effects as well as for the interactions of NAC with the antidepressants here reported.

The drawbacks of antidepressant treatments have been extensively discussed, among which are the long latency for clinically significant changes in mood, the less than desirable therapeutic response, and the range of side effects often determining poor adhesion to treatment (Dupuy et al., 2011). This study presents various limitations. With the experimental design used in this study no significant increase in reduced immobility was observed with the combination of ADs with NAC, though the study is obviously not exhaustive regarding dose–effect curves or NAC/antidepressants dose combinations (e.g., isobolograms). Moreover, the TST is inadequate to assess if the interaction of NAC with imipramine and escitalopram would result in shorter latencies for antidepressant effects. Nevertheless, the data suggest a potential benefit in antidepressant treatment effectiveness with NAC as add on medication, either resulting from the use of lower doses with diminished side effects and/or a potential shortening of the latency to clinical response suggested by the apparent key role of AMPA glutamate receptors for a rapid antidepressant response.

## 5. Conclusions

This study shows a differential interaction of N-acetylcysteine and antidepressants agents with distinct pharmacodynamics basis. The positive interaction with imipramine and escitalopram is of special clinical interest. Further studies are necessary to better characterize the molecular foundation underlying the positive interactions here identified, as well as to typify the particular drug combinations that would provide optimized alternatives for treating depression.

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