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Research report

Effect of antioxidant *N*-acetyl-L-cysteine on behavioral changes and neurotoxicity in rats after administration of methamphetamine

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Abstract

Several lines of evidence suggest that oxidative stress may play a role in the behavioral changes and neurotoxicity in rats after administration of methamphetamine (MAP). *N*-acetyl-L-cysteine (NAC) is a precursor of glutathione, and it also exerts as an antioxidant. In this study, we investigated the effects of NAC on the behavioral changes (hyperlocomotion and development of sensitization) and neurotoxicity in male Wistar rats after administration of MAP. Pretreatment with NAC (30, 100 or 300 mg/kg, i.p.) attenuated significantly hyperlocomotion in rats induced by a single administration of MAP (2 mg/kg, i.p.), in a dose-dependent manner. Furthermore, pretreatment with NAC (100 mg/kg, i.p., 15 min before MAP injection, once daily for 5 consecutive days) blocked significantly the development of behavioral sensitization in rats after repeated administration of MAP (2 mg/kg, once daily for 5 consecutive days), whereas the behaviors in rats after repeated administration of NAC plus saline groups were not different from those of control (vehicle plus saline) groups. One week after administration of MAP (7.5 mg/kg × 4, 2-h intervals), levels of dopamine (DA) in rat striatum were significantly decreased as compared with control groups. Pretreatment with NAC (1, 3, 10 or 30 mg/kg, i.p., 30 min before each MAP injection) attenuated significantly the MAP-induced reduction of DA in rat striatum, in a dose-dependent manner. These results suggest that NAC could prevent the behavioral changes (acute hyperlocomotion and development of behavioral sensitization) in rats and neurotoxicity in rat striatum after administration of MAP, and that NAC would be a useful drug for treatment of several symptoms associated with MAP abuse.
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Theme: Neural basis of behavior
Topic: Drugs of abuse: amphetamine and other stimulants

Keywords: Methamphetamine; *N*-Acetyl-L-cysteine; Neurotoxicity; Oxidative stress

1. Introduction

Abuse of methamphetamine (MAP), a potent psychostimulant, is an extremely serious and growing problem in the world, and MAP is an addictive stimulant drug that strongly activates certain systems in the brain. Administration of MAP releases high levels of the neurotransmitter dopamine (DA), which stimulates brain cells, enhancing mood and body movement. A number of animal researches show that repeated administration of MAP could damage nerve terminals of DA neurons. It is well known that DA- and serotonin (5-HT)-containing neurons do not die after MAP use, but their nerve terminals are damaged and re-growth appears to be limited [7,8,10]. Recent positron emission tomography (PET) studies demonstrated that MAP use caused the marked reduction of DA transporter (DAT) in the brain of MAP abusers, suggesting a neurotoxic effect of MAP in human dopaminergic terminals [23,24,30,31]. These findings are supported by the report demonstrating that the densities of DAT are significantly decreased in the striatum of postmortem with chronic MAP users [32]. However, precise mechanisms underlying MAP-induced neurotoxicity in human dopaminergic terminals are currently unclear [7,8].

Several lines of evidence suggest that oxidative stress plays a role in the neurotoxicity of MAP in the brain

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[7,8,12]. Glutathione is an important intracellular antioxidant that protects against a variety of different antioxidant species. A disturbance of glutathione homeostasis may either lead to or result from oxidative stress in neurodegenerative disorders [21]. An important role for glutathione was proposed for the pathogenesis of neurodegenerative disorders such as Parkinson's disease, because a decrease in total glutathione concentrations in the substantia nigra has been observed in preclinical stages, at a time at which other biochemical changes are not yet detectable [4,21]. We reported that endogenous glutathione could play a role in 6-hydroxy DA (6-OHDA)-induced cell death in human neuroblastoma SK-N-SH cells, and that an antioxidant *N*-acetyl-L-cysteine (NAC), that is converted in the body into metabolites capable of stimulating glutathione synthesis, might work as a beneficial dopaminergic neuron-survival factor more efficiently than exogenous glutathione [25]. Because glutathione does not cross the blood–brain barrier, other treatment options to increase brain concentrations of glutathione including glutathione analogs (e.g., NAC as glutathione precursors) would be suitable for therapeutic drug [6,14,16,21,22].

In this study, we investigated the effects of NAC on the behavioral changes (acute hyperlocomotion and development of behavioral sensitization) in rats induced by administration of MAP and on the MAP-induced neurotoxicity in dopaminergic neurons of rat brain.

2. Materials and methods

2.1. Animals

Male Wistar rats (Nihon SLC, Hamamatsu, Shizuoka, Japan) weighing 200–220 g were used. The animals were housed in groups of three or four per cage. They were maintained under standard conditions (12/12 h light–dark cycle: lights on from 06:00 to 18:00 h; room temperature, 22 ± 2 °C; humidity, 55 ± 5%) with free access to food and water. All experiments were performed in accordance with the Guide for Animal Experimentation, Chiba University Graduate School of Medicine.

2.2. Drugs

MAP hydrochloride and NAC were purchased from Dainippon Pharmaceuticals (Osaka, Japan) and Wako (Tokyo, Japan), respectively. MAP and NAC were dissolved in saline and distilled water, respectively. Other chemicals were purchased from commercial sources.

2.3. Effects of NAC on hyperlocomotion after a single administration of MAP

Fifteen minutes after vehicle (1 ml/kg, i.p.) or NAC (30, 100 or 300 mg/kg, i.p.), MAP (2 mg/kg, i.p.) was administered intraperitoneally (i.p.) into rats. Locomotor activity was measured using an animal movement analysis system (SCANET SV-10, Melquest, Toyama, Japan) as reported previously [29].

2.4. Effects of NAC on development of behavioral sensitization after administration of MAP

Thirty-two rats were divided into the following four groups: (1) vehicle (1 ml/kg) + saline (1 ml/kg); (2) vehicle (1 ml/kg) + MAP (2 mg/kg); (3) NAC (100 mg/kg) + MAP (2 mg/kg); (4) NAC (100 mg/kg) + saline (1 ml/kg). The interval between the first and second injections was 15 min. For each animal, the same treatment was continued for 5 consecutive days. One week after the last administration, each rat was given MAP (1 mg/kg, i.p.) and the behavioral changes were observed as reported previously [29]. The intensity of stereotyped behavior was assessed using the classification method of Akiyama et al. [1] as follows: 0, asleep or still; 1, locomotion with normal exploration and normal pattern of sniffing; 2, hyperlocomotion with repetitive exploratory behavior, rearing, or increased rate of sniffing; 3, discontinuous sniffing with periodic locomotion activity, and 4, continuous compulsive sniffing without locomotion. Following drug treatment, the intensity of stereotyped behavior was assessed every 10 min for periods of 1 min by an observer blind to the treatment. The sum of nine consecutive intensity scores was used for data analysis.

2.5. Effects of NAC on neurotoxicity after repeated administration of MAP

Thirty minutes after i.p. injection of NAC (1, 3, 10 and 30 mg/kg) or vehicle (1 ml/kg), rats received four injections of MAP (7.5 mg/kg, i.p.) or vehicle (saline, 1 ml/kg, i.p.). Rats received four injections of same treatment at 2-h intervals. Rectal temperatures were recorded 30 min before the first injection and 1 h after injection of MAP. Rectal temperature was measured using a TD-320 thermometer coupled to a rectal probe (Shibaura Electronics, Saitama, Japan). Animals were sacrificed 7 days after drug treatment. The brains were quickly removed and dissected on an ice-cold glass plate. The striatum were isolated, weighed, frozen on dry ice, and stored at − 80 °C until assay.

2.6. Measurement of DA by HPLC

Tissue samples were homogenized by sonication in 0.2 M perchloric acid ($HClO_4$) containing 100 μM disodium EDTA and 100 ng/ml isoproterenol (internal standard), and were then centrifuged at 20,000 × *g* for 15 min at 4 °C. The supernatants were filtered through a 0.45 μm pore membrane (Millex-LH, 4 mm; Millipore, Tokyo, Japan) and analyzed for DA by a high-performance liquid chromatography coupled with electrochemical detection (HPLC-ECD). The HPLC system consisted of a liquid chromatograph

pump (EP-300, Eicom, Kyoto, Japan), a degasser (DG-300, Eicom), a reversed phase column: Eicompak SC-5ODS 150 × 3.0 mm (Eicom), an ECD-300 electrochemical detector (Eicom) and a data processor (EPC-300, Eicom). The mobile phase was 0.1 M acetate-citric acid buffer (pH 3.5) containing 17% methanol, 5 mg/l disodium EDTA and 190 mg/l sodium octyl sulfate.

2.7. Statistical analysis

The data were presented as the mean ± standard error of mean (S.E.M.). Results of acute behavioral study and rectal temperature were analyzed by two-way analysis of variance (ANOVA), for repeated measures with treatment as the between subjects factor and time as within-subjects factor. When appropriate, group means at individual time points were compared by one-way ANOVA, and post-hoc comparisons were performed using Bonferroni/Dunn test. Striatal levels of DA were analyzed by one-way ANOVA followed by Bonferroni/Dunn test for multiple comparisons. The p values <0.05 were considered statistically significant.

3. Results

3.1. Effects of NAC on hyperlocomotion after a single administration of MAP

A single administration of MAP (2 mg/kg, i.p.) caused marked hyperlocomotion in rats as compared with vehicle (1 ml/kg, i.p.)-treated rats (Fig. 1). Two-way ANOVA revealed significant differences among five groups [$F(4, 35) = 13.7$, $p < 0.0001$]. Pretreatment with NAC (30, 100 or 300 mg/kg, i.p.) attenuated hyperlocomotion in rats induced by administration of MAP (2 mg/kg, i.p.), in a dose-dependent manner (Fig. 1).

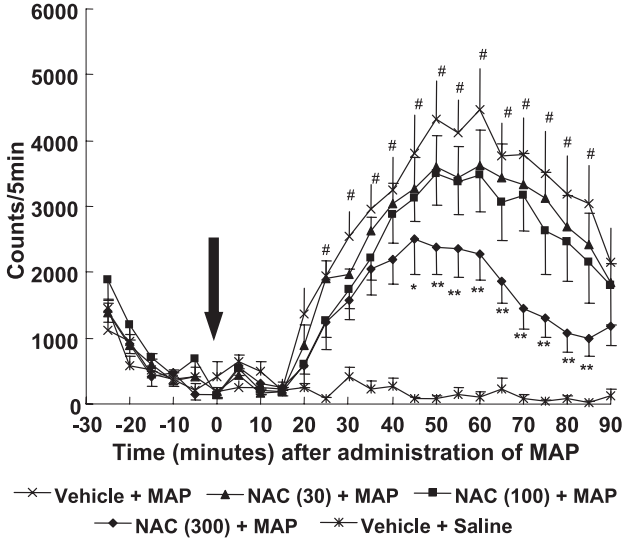

Fig. 1. Effects of NAC on time course of hyperactivity in rats after a single administration of MAP. Fifteen minutes after injection of vehicle (1 ml/kg, i.p.) or NAC (30, 100 or 300 mg/kg, i.p.), saline (1 ml/kg, i.p.) or MAP (2 mg/kg, i.p.) were administered into rats. Each value is mean ± S.E.M. ($n = 8$ per group). $^{\#}p < 0.01$ as compared with vehicle plus saline group (Bonferroni/Dunn method). $^{*}p < 0.05$, $^{**}p < 0.01$ as compared with vehicle plus MAP group (Bonferroni/Dunn method).

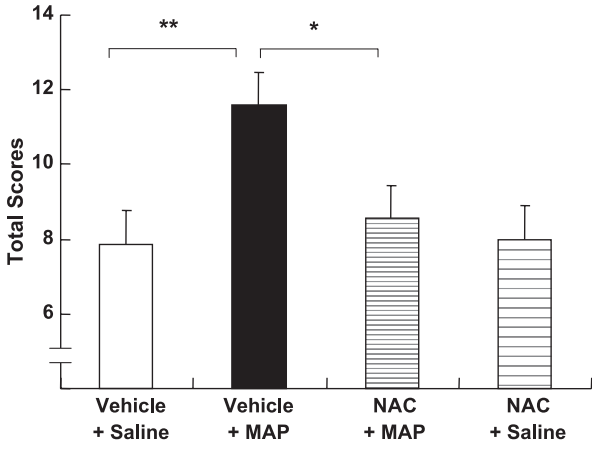

Fig. 2. Effects of NAC on development of sensitization in rats after repeated administration of MAP. Vehicle (1 ml/kg) plus saline (1 ml/kg), vehicle (1 ml/kg) plus MAP (2 mg/kg), NAC (100 mg/kg) plus MAP (2 mg/kg), NAC (100 mg/kg) plus saline (1 ml/kg, i.p.) were administered daily for consecutive 5 days. A week after a final administration, MAP (1 mg/kg, i.p.) was administered into all rats. Behavioral evaluations in rats after administration of MAP were measured. Each value is mean ± S.E.M. ($n = 8$–10 per group). $^{*}p < 0.05$, $^{**}p < 0.01$ as compared with vehicle plus MAP groups (Bonferroni/Dunn method).

3.2. Effects of NAC on development of behavioral sensitization after administration of MAP

Repeated administration of MAP (2 mg/kg, once daily for 5 consecutive days) increased significantly ($p < 0.01$) total scores of stereotyped behaviors in rats as compared with those of vehicle plus saline treated groups, indicating the development of behavioral sensitization by repeated treatment with MAP (Fig. 2). One-way ANOVA revealed significant differences among four groups [$F(3, 31) = 3.59$, $p = 0.024$], and post-hoc analysis indicated that pretreatment with NAC (100 mg/kg, i.p.) attenuated significantly ($p < 0.05$) the development of behavioral sensitization in rats after repeated administration of MAP (2 mg/kg). In contrast, total scores of stereotyped behaviors of NAC (100 mg/kg) plus saline treated groups were not different from those of vehicle plus saline treated groups (Fig. 2).

3.3. Effects of NAC on neurotoxicity after repeated administration of MAP

Administration of MAP increased significantly the body temperature in rats (Fig. 3). Two-way ANOVA revealed significant differences among five groups [$F(5, 37) = 35.4$, $p < 0.0001$]. Post-hoc analysis indicated that pretreatment with NAC (10 or 30 mg/kg, i.p., 30 min before each MAP

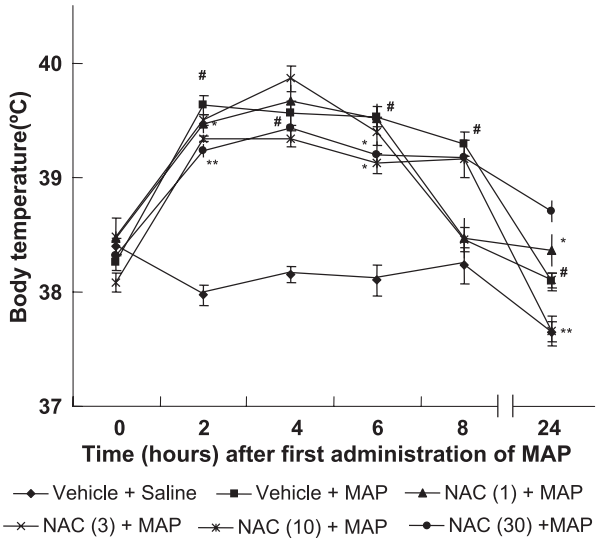

Fig. 3. Effects of NAC on MAP-induced hyperthermia in rats. Rats received four injections of MAP (7.5 mg/kg, i.p.) every 2 h alone or in combination with NAC (1, 3, 10 or 30 mg/kg). NAC were injected i.p. 30 min before the injection of MAP. Rectal temperatures were recorded 30 min before the first injection of MAP or saline and 1 h after each MAP injection. Each value is mean ± S.E.M. ($n = 6–14$ per group). #$p < 0.001$ as compared with vehicle plus saline group (Bonferroni/Dunn method). *$p < 0.05$, **$p < 0.01$ as compared with vehicle plus MAP group (Bonferroni/Dunn method).

injection) attenuated significantly MAP-induced increase of body temperature in rats at 2 and 6 h after first administration of MAP (Fig. 4).

One-way ANOVA revealed significant differences among four groups [$F(5, 37) = 5.15$, $p = 0.0011$]. Repeated administration of MAP (7.5 mg/kg × 4, 2-h intervals) decreased significantly levels of DA in rat striatum (Fig. 4). Pretreatment with NAC (1, 3, 10 or 30 mg/kg, i.p., 30 min before each MAP injection) attenuated the reduction of DA levels in rat striatum by repeated administration of MAP, in a dose-dependent manner (Fig. 4).

4. Discussion

The major findings of the present study are that the antioxidant NAC blocks behavioral changes (acute hyperlocomotion and development of sensitization) in rats by administration of MAP, and that NAC shows protective effects on MAP-induced neurotoxicity in rat striatum, suggesting the role of oxidative stress in the behavioral changes and neurotoxicity after administration of MAP. Psychostimulants such as MAP cause behavioral changes and the development of behavioral sensitization via pre- and/or postsynaptic dopaminergic systems [13,19,20]. It is suggested that the large release of DA produced by MAP may be implicated in the behavioral changes after administration of MAP [7,15,28], and that formation of DA-quinones and free radicals by autooxidation of DA may contribute to MAP-induced behavioral and/or neurotoxic actions, supporting the evidence of oxidative stress in this model [4,15,28]. Thus, it seems that attenuation of oxidative stress (e.g., glutathione reduction or reactive oxygen species) by NAC might be implicated in the protective effects of NAC on the behavioral changes and neurotoxicity after administration of MAP although the precise mechanisms underlying protective effects of NAC are currently unclear. Therefore, our findings suggest that oxidative stress might be implicated in the development of behavioral sensitization in rats after repeated administration of MAP although further studies should be necessary.

It is known that neurotoxic doses of MAP cause hyperthermia and that hypothermia could suppress MAP-induced neurotoxicity, suggesting the role of body temperature in the MAP-induced dopaminergic neurotoxicty [2,3]. In this study, high doses (10 and 30 mg/kg) of NAC attenuated significantly hyperthermia in rats induced by administration of MAP although these doses did not alter the body temperature at other times (4 and 8 h). Thus, it seems that decrease of body temperature by NAC may play, in part, a

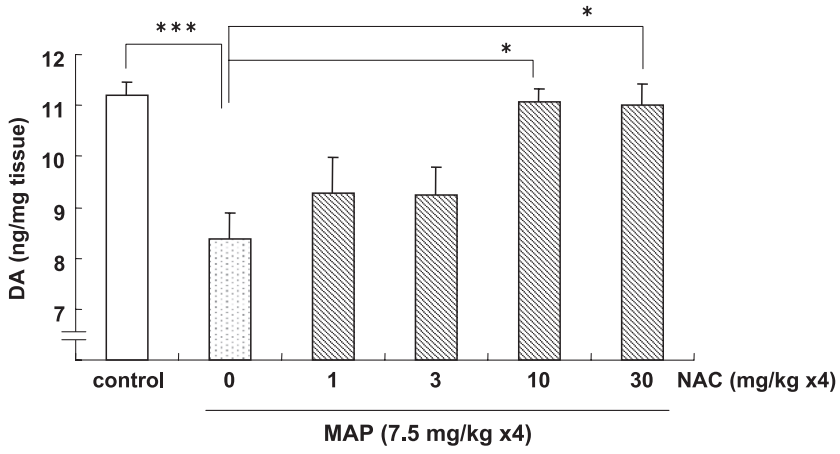

Fig. 4. Effects of NAC on striatal DA levels after administration of MAP. Rats received NAC 30 min before the four injections of MAP. Measurement of DA levels in rat striatum was performed 1 week after administration of MAP. Each value is mean ± S.E.M. ($n = 6–14$ per group). *$p < 0.05$, ***$p < 0.001$ (Bonferroni/Dunn method).

role in the protection of MAP-induced neurotoxicity by NAC.

Administration of L-cysteine (500 mg/kg, i.p.) markedly attenuated the persistent decreases in striatal DA levels in rats 1 week after the administration of a single dose of amphetamine (9.2 mg/kg, i.p.) to iprindole-treated animals [27]. The protective dose (500 mg/kg) of L-cysteine in amphetamine-induced neurotoxicity is much higher than that (1–30 mg/kg) of NAC in our study. In the cell culture model of 6-OHDA-induced cell death, we reported that NAC was more potent inhibitor than L-cysteine and glutathione [25]. Thus, it is possible that NAC might work as a beneficial dopaminergic neuron-survival factor more efficiently than glutathione or L-cysteine since NAC has higher cell permeability than glutathione and other precursors (e.g., L-cysteine)[25]. Interestingly, our recent PET study demonstrates that treatment with NAC can attenuate significantly the reduction of DAT in monkey striatum after repeated administration of MAP [11]. Furthermore, it is also reported that levels of endogenous antioxidant glutathione in rat striatum are significantly decreased following a high neurotoxic dose of MAP [17], supporting the notion that oxidative stress is involved in the mechanism of MAP-induced neurotoxicity in rat striatum. Taken together, our findings suggest that NAC would be a suitable drug for treatment of MAP-induced neurotoxicity in human brain.

Withdrawal from repeated cocaine treatment reduced in vivo extracellular glutamate in the nucleus accumbens of rats by decreasing the exchange of extracellular cystine for intracellular glutamate. The reduced levels of extracellular glutamate in cocaine-pretreated rats were restored by increasing cystine/glutamate exchange, either by supplying the extracellular substrate cystine directly into the nucleus accumbens, or systemic administration of the cysteine prodrug NAC [5]. Furthermore, the elevation in glutamate required cystine/glutamate exchange, as blocking the exchanger with intra-accumbens perfusion of the cystine/glutamate exchange inhibitor (*S*)-4-carboxyphenylglycine (CPG) inhibited the NAC-induced rise in glutamate [5]. These findings support an effect of NAC on cystine/glutamate exchange that is selective for the potentially pathogenic situation where extracellular glutamate has been reduced by repeated administration of cocaine [5]. Therefore, it would be of interest to examine whether the cystine/glutamate exchange is altered in the brain of rats pretreated with MAP.

It has been reported that expression of the tumor necrosis factor-α (TNF-α) gene was induced 3 h after administration of MAP and remained elevated for up to 6 h of MAP exposure [9]. In addition, stimulation of the TNF-α gene was associated with increased TNF-α protein production in the frontal cortex, suggesting that signaling pathways leading to upregulation of TNF-α might play a role in MAP-induced disturbances in cellular redox status in vivo [9]. Furthermore, repeated treatment with MAP (2 mg/kg/day for 5 days) in rats induced a significant increase in TNF-α mRNA and protein expression in the brain. Exogenous TNF-α (1–4 μg) blocked locomotor-stimulating and rewarding effects of MAP in mice [18]. Moreover, TNF-α (−/−) mice showed enhanced responses to the locomotor-sensitizing, rewarding, and neurotoxic effects of MAP compared with wild-type (+/+) mice, suggesting that TNF-α plays a neuroprotective role in MAP-induced behavioral changes and neurotoxicity [18]. Treatment of rat primary astrocytes with TNF-α led to marked alteration in cellular redox (decrease in intracellular glutathione), and pretreatment of astrocytes with NAC prevented TNF-α-induced decrease in glutathione and degradation of sphingomyelin to ceramide, whereas treatment of astrocytes with diamide, a thiol-depleting agent, alone caused degradation of sphingomyelin to ceramide, suggesting that the intracellular level of glutathione may play a critical role in the regulation of TNF-α-induced generation of ceramide leading to apoptosis of brain cells [26]. Taken together, it seems that inhibition of inflammatory genes such as TNF-α by NAC might be implicated in the protection of MAP-induced neurotoxicity by NAC although further studies regarding the role of TNF-α in the protective action of NAC should be necessary.

NAC, the acetylated variant of the amino acid L-cysteine, is an excellent source of sulfhydryl (SH) groups and is converted in the body into metabolites capable of stimulating glutathione synthesis, promoting detoxification, and acting directly as free radical scavengers [14]. In addition to its mucolytic action, NAC has been studied and utilized in conditions characterized by decreased glutathione or oxidative stress such as HIV infection, cancer, and heart disease [14]. Therefore, NAC has been used as a tool for investigating the role of reactive oxidative stress in numerous biological and pathological processes [6,14,21,33]. Thus, it is likely that NAC would be a suitable drug for potential treatment of diseases associated with MAP use.

In conclusion, our findings suggest that the antioxidant NAC could block behavioral changes (acute hyperlocomotion and development of sensitization) in rats by administration of MAP, and that NAC shows protective effects on MAP-induced neurotoxicity in rat striatum. Therefore, it is likely that NAC would be a suitable drug for potential treatment of diseases (e.g., psychosis, neurotoxicity) associated with MAP abuse.

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