Effect of diallyl disulfide on lipopolysaccharide-induced depression-like behavior in mice

Jiashu Lu (ljs534777178@163.com)
Jiangsu Taizhou People's Hospital https://orcid.org/0000-0002-4114-8090
Haiyan He
Department of Respiratory Medicine, Nantong First People's Hospital
Chao Huang
School of Pharmacy, Nantong University
Zhuo Chen
Invasive Technology Department, Nantong First People's Hospital

Research

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Abstract

Background: Depression is associated with high levels of pro-inflammatory cytokines and oxidative markers. Inhibition of neuroinflammation and oxidative stress is beneficial for depression prevention and/or therapy. Diallyl disulfide (DADS), an active compound in garlic oil, has been shown to inhibit neuroinflammation and oxidative stress. The purpose of this study is to investigate the role and mechanism of DADS in lipopolysacharide (LPS)-induced depression-like behaviors in mice.

Methods: We used behavioral tests and biochemical analysis to illustrate the role and mechanism of DADS in depression regulation.

Results: Similarly to imipramine (10 mg/kg), a clinical antidepressant, DADS (40 or 80 mg/kg), which was administered 1 h before LPS treatment (pre-LPS) or 1.5 h and 23.5 h after LPS treatment (post-LPS), prevented and reversed the LPS (100 μg/kg)-induced increase in immobility time in the tail suspension test (TST) and forced swim test (FST) in mice. Mechanistic studies revealed that DADS pre-treatment or post-treatment at the dose of 40 and 80 mg/kg prevented and reversed (i) the LPS-induced increases in interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and nitric oxide (NO) levels in the hippocampus and prefrontal cortex, (ii) the LPS-induced increases in contents of malondialdehyde (MDA), a parameter reflecting high levels of oxidative stress, as well as (iii) the LPS-induced decreases in contents of GSH, a marker reflecting weakened anti-oxidative ability, in both hippocampus and prefrontal cortexes in mice.

Conclusions: DADS is comparable to imipramine in effectively ameliorating LPS-induced depression-like behaviors in mice, providing a potential value for DADS in prevention and/or therapy of depression.

Introduction

Depression is a common mental disease, causing severe economic and social burdens [1]. The pathogenesis of depression is related to various factors, such as adverse social stress, heredity, and the decrease in monoamine levels in the brain [2]. On the basis of the latter factor, researchers have raised a monoamine dysfunction hypothesis of depression, and according to this hypothesis researchers have developed several antidepressants, such as the serotonin reuptake inhibitor and the monoamine inhibitor. However, these antidepressants usually need several weeks to produce therapeutic effects [3], and approximately one-third of patients do not respond to these drugs [4]. Further, these antidepressants may evoke suicide attempt and suicide in some cases [5]. It is therefore necessary to search novel drugs for depression regulation, including depression prevention and depression treatment.

Besides monoamine dysfunction, many other mechanisms, such as the impairment of hippocampal neurogenesis and glutamatergic neurotransmission, the dysfunction of the neuroendocrine system, and the impairment of the brain-derived neurotrophic factor (BDNF) signaling, have been raised to explain the pathogenesis of depression [6–8]. Increasing evidence has also revealed that there is a close relationship between immune activation and depression, thereby making the neuroinflammatory hypothesis be a new focus [9–13]. In depressed patients, pro-inflammatory cytokines, such as interleukin-1β (IL-1β), tumor

necrosis factor-α (TNF-α), and nitric oxide (NO), have been observed repeatedly to be increased in serum [14, 15]. Systemic administration of lipopolysaccharide (LPS), a classical activator of the immune cell, triggers depression-like behaviors in humans and rodents via inducing severe peripheral and central inflammatory responses [16, 17]. Using compounds which can inhibit neuroinflammatory response could be a potential strategy for depression prevention and/or therapy. Indeed, this hypothesis has been supported by a lot of evidence. For example, some pre-clinical compounds which possess anti-inflammatory properties have been confirmed to be capable of ameliorating depression-like behavior in rodents induced by acute immune stress or the other chronic stresses, such as the chronic unpredictable stress and the chronic social defeat stress [16–19], and some clinical anti-inflammatory drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and minocycline, can suppress depressive symptoms in patients [20, 21]. Further, the traditional antidepressants, including the paroxetine, fluoxetine, and imipramine, have been reported to reduce pro-inflammatory responses in the brain in rodents [22–24]. Mechanistic studies have shown that the pro-inflammatory cytokine triggers depression onset likely through promoting oxido-nitrosative stress in the hippocampus and prefrontal cortex [16, 25]. Interruption with neuroinflammatory response along with oxido-nitrosative stress may be an important strategy for depression prevention and/or therapy.

Garlic is a flavoring agent for diet in daily life. It has great antimicrobial effects against genera Pseudomonas, Streptococcus, and Staphylococcus [26, 27]. Garlic and/or garlic-derived compounds can also display preventive and/or therapeutic activities in central nervous system (CNS) disorders, such as headache [28], Parkinson's disease [29], and Alzheimer's disease [30]. In a rat diabetic model, garlic can ameliorate anxiety- and depressive-like behaviors via inhibiting oxidative stress [31]. Further, garlic extracts are capable of alleviating LPS-triggered oxidative stress and neuroinflammatory response in BV-2 microglia [32]. Diallyl disulfide (DADS) is an active compound derived from garlic oil. It can inhibit LPS-induced neutrophil infiltration and damage in rat intestines [33], suppress inflammation and apoptosis resistance in human Barrett's epithelial cells [34], attenuate airway inflammation via inhibiting IL-6/IL-1β production in LPS-stimulated macrophages and animals [35], and alleviate LPS-induced osteoclastogenesis via reducing pro-inflammatory cytokine production [36]. We speculate that DADS may regulate LPS-induced depression-like behavior in mice via alteration of the neuroinflammatory and oxidative status of the brain. In order to investigate this hypothesis, we evaluated the changes in depression-like behaviors in mice administered with DADS in a pre- or post-LPS model, and also evaluated whether the depression regulation effect of DADS is associated with the changes in parameters reflecting the neuroinflammatory response and oxidative stress of the mice brain (hippocampus and prefrontal cortex) following a pre- or post-LPS administration, such as IL-1β, NO, malondialdehyde (MDA), and reduced glutathione (GSH).

Materials And Methods

Materials DADS was purchased from Sigma and dissolved in dimethyl sulfoxide (DMSO). Imipramine is the product of MedChem Express (Princeton, NJ, USA). They were prepared as stock solutions and stored at -20°C. Animals Male C57BL6/J mice (6–8 weeks) were purchased from Beijing Vital River Laboratory

Animal Technology Co., Ltd. (Beijing, China), and were housed 5 per cage for 1 week with free access to food and water under standard conditions (five mice per cage), which includes 12-h of light/dark cycle, lights on from 07:00 to 19:00, 55 ± 10% of relative humidity, and 23 ± 1°C of temperature. Behavioral experiments, which were approved by the University Animal Ethics Committee of Nantong University (Permit Number: 2110836), were carried out during the light phase, according to internationally accepted guidelines for the use of animals in toxicology as adopted by the Society of Toxicology in 1999. Pharmacological treatments DADS and LPS were given intraperitoneally. Imipramine (10 mg/kg) was used as a positive control. In the pre-treatment model, the mice in separated groups were administered with vehicle, DADS (40 and 80 mg/kg), or imipramine (10 mg/kg) at 1 h before a single LPS injection (100 μg/kg). In the post-treatment model, the mice in separated groups were first stimulated by a single dose of LPS (100 μg/kg), and then administered with vehicle, DADS (40 and 80 mg/kg), or imipramine (10 mg/kg) at two different time-points: 1.5 and 23.5 h after LPS injection. Behavioral assays, including the tail suspension test (TST), forced swimming test (FST), and open field test (OFT), in both pre- and post-treatment models were performed at 24 h after the single LPS injection. For biochemical assays, the mice in separated groups were sacrificed immediately after the drug treatment. Each group included 10 mice. In all of the behavioral and biochemical experiments control animals received a vehicle solution containing DMSO and water. The doses of LPS [37], DADS [38], and imipramine [39] were selected according to some previous studies [37–38]. TST This experiment was performed according to some previous studies [40,41]. Briefly, the mice in groups with or without DADS, imipramine, and/or LPS treatment were individually suspended 50 cm above the floor for 6 min by adhesive tape placed approximately 1 cm from the tip of the tail. The mice were considered immobile when they hung passively and were completely motionless (any mouse that climbed its tails was excluded from further analysis). Immobility time in different mice during the last 4 min was recorded by an investigator blinded to the experiment. FST This experiment was performed according to some previous studies [41,42]. Briefly, the mice in groups with or without DADS, imipramine, and/or LPS treatment were individually placed in a clear glass cylinder (height in 25 cm, diameter in 10 cm) filled to 10 cm with water at 25 ± 1°C for 6 min. The mice were considered immobile when they floated in the water without struggling and making only those movements necessary to keep its head above the water. Immobility time in different mice during the last 4 min was recorded by an investigator blinded to the experiment. OFT The open field arena was made of acrylic (30 × 30 × 15 cm) with transparent walls and a black floor, which is divided into nine squares of equal areas. The open field was used to evaluate the exploratory activity of the animal during 5 min. The number of squares crossed by the animal during the 5 min was recorded by an investigator blinded to the experiment. Immunoassays for IL-1β levels The concentration of IL-1β in the hippocampus and prefrontal cortex was determined by the ELISA kit (Proteintech, Wuhan, China) according to the manufacturer's protocol and expressed in pg/g tissue. Measurement of nitrite levels Total nitrite levels in the hippocampus and prefrontal cortex were measured with a Griess reagent kit according to supplier's recommendation (Bi Yuntian Biological Technology Institution, Shanghai, China). The reaction consisted of 20 μL of Griess Reagent, 150 μL of supernatant, and 130 μL of de-ionized water. After incubation of the mixture for 30 min at room temperature, nitrite levels were measured at 548 nm using anM2 spectrophotometric microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Measurement of MDA and GSH levels To assess the oxidative stress status in hippocampus and prefrontal cortex, the MDA and GSH levels were determined using method of Jangra et al. [43] and Beutler et al. [44], respectively. The estimation of MDA and GSH were expressed as μmol/g of wet tissue. The measurement of brain protein was according to the Lowry method and was expressed as μmol/mg of protein concentration [45]. Statistical analysis All analyses were performed using SPSS 13.0 software (SPSS Inc., USA), and data are presented as mean ± SEM. Differences between mean values were evaluated using one-way or two-way analysis of variance (ANOVA). Bonferroni's post hoc test was applied to assess isolated comparisons. $P < 0.05$ was considered statistically significant.

Results

Effect of DADS pre-treatment on LPS-induced depression-like behavior in mice

In order to determine whether DADS can prevent LPS-induced depression-like behavior, the mice were pretreated with DADS (40 or 80 mg/kg, i.p.) at 1 h before LPS injection (100 μg/kg, i.p., Fig. 1A). For TST, a two-way ANOVA revealed significant effects for LPS stimulation ($F_{1,72} = 5.38$, $P < 0.05$), drug pre-treatment ($F_{3,72} = 4.69$, $P < 0.01$), and LPS × drug interaction ($F_{3,72} = 11.68$, $P < 0.001$). For FST, the two-way ANOVA revealed significant effects for LPS stimulation ($F_{1,72} = 6.94$, $P < 0.05$), drug pre-treatment ($F_{3,72} = 2.79$, $P < 0.05$), and LPS × drug interaction ($F_{3,72} = 3.08$, $P < 0.05$). Post hoc analysis showed that DADS pre-treatment at the dose of 40 and 80 mg/kg markedly prevented the LPS-induced increase in immobility time in the TST and FST in mice. Similarly, imipramine pre-treatment (10 mg/kg) also prevented the LPS-induced in immobility time in the TST and FST in mice (Fig. 1B, C). The OFT results showed that both DADS and imipramine pre-treatment did not affect the locomotor activity in mice with or without LPS treatment (no significant effects for LPS stimulation ($F_{1,72} = 0.34$, $P = 0.56$), drug pre-treatment ($F_{3,72} = 0.34$, $P = 0.79$), and LPS × drug interaction ($F_{3,72} = 0.19$, $P = 0.91$), Fig. 1D). These results indicate that DADS has a prophylactic effect on LPS-induced depression-like behavior in mice.

Effect of DADS post-treatment on LPS-induced depression-like behavior in mice

We next evaluated the inhibitory effect of DADS on LPS-induced depression-like behavior in mice in a post-treatment model, in which the mice were administered with DADS (40 or 80 mg/kg, i.p.) at either 1.5 or 23.5 h after LPS treatment (100 μg/kg, i.p., Fig. 2A). For TST, a two-way ANOVA revealed significant effects for drug post-treatment ($F_{3,72} = 4.76$, $P < 0.05$) and LPS × drug interaction ($F_{3,72} = 5.09$, $P < 0.001$), but not for LPS stimulation ($F_{1,72} = 0.34$, $P = 0.56$). For FST, the two-way ANOVA revealed significant effects for LPS stimulation ($F_{1,72} = 11.84$, $P < 0.001$), drug post-treatment ($F_{3,72} = 8.68$, $P < 0.001$), and LPS × drug interaction ($F_{3,72} = 11.45$, $P < 0.001$). Post hoc analysis showed that DADS, administered at both doses (40 and 80 mg/kg) after LPS injection, markedly reversed the LPS-induced increase in immobility time in the TST and FST in mice (Fig. 2B, C). Similarly, imipramine administration at the dose of 10 mg/kg after LPS injection also suppressed the LPS-induced in immobility time in the TST and FST in mice (Fig. 2B, C). In the OFT, the locomotor activity of mice administered with LPS, DADS, or

imipramine was not affected (no significant effects for LPS stimulation ($F_{1,72}$ = 0.90, P = 0.34), drug post-treatment ($F_{3,72}$ = 0.30, P = 0.83), and LPS × drug interaction ($F_{3,72}$ = 0.18, P = 0.91), Fig. 2D). These results indicate that DADS post-treatment can reverse LPS-induced depression-like behavior in mice.

Effect of DADS pre- or post-treatment on LPS-induced increase in IL-1β and TNF-α levels in the hippocampus and prefrontal cortex

The systemic inflammation induced by LPS could lead to pro-inflammatory cytokine production in the CNS, among which IL-1β and TNF-α was closely related to the induction of depression-like behavior in mice. In this study, we evaluated the change of IL-1β and TNF-α levels in the hippocampus and prefrontal cortex in both pre- and post-treatment models. In the pre-treatment model, a one-way ANOVA for IL-1β (hippocampus: $F_{3,36}$ = 8.72, P < 0.001; prefrontal cortex: $F_{3,36}$ = 15.74, P < 0.001) and TNF-α (hippocampus: $F_{3,36}$ = 7.95, P < 0.001; prefrontal cortex: $F_{3,36}$ = 5.09, P < 0.001), and post hoc analysis showed that both imipramine pre-treatment (10 mg/kg) and DADS pre-treatment at the dose of 40 and 80 mg/kg prevented the LPS (100 μg/kg)-induced increase in IL-1β (Fig. 3A, B) and TNF-α (Fig. 4A, B) levels in the hippocampus and prefrontal cortex. In the post-treatment model, the one-way ANOVA for IL-1β (hippocampus: $F_{3,36}$ = 6.73, P < 0.01; prefrontal cortex: $F_{3,36}$ = 4.52, P < 0.01) and TNF-α (hippocampus: $F_{3,36}$ = 5.77, P < 0.01; prefrontal cortex: $F_{3,36}$ = 5.80, P < 0.01), and post hoc analysis showed that both imipramine post-treatment (10 mg/kg) and DADS post-treatment (40 and 80 mg/kg) reversed the LPS (100 μg/kg)-induced increase in IL-1β (Fig. 3C, D) and TNF-α (Fig. 4C, D) levels in the hippocampus and prefrontal cortex. These results indicate that DADS pre- and post-treatment can reverse LPS-induced neuroinflammatory responses in the brain.

Effect of DADS pre- or post-treatment on LPS-induced increase in nitrite levels in the hippocampus and prefrontal cortex

Since NO is a crucial molecule which can bridge the neuroinflammatory response and oxidative stress together, we evaluated the change of nitrite levels in the hippocampus and prefrontal cortex in mice administered with LPS and/or DADS. In the pre-treatment model, a one-way ANOVA (hippocampus: $F_{3,36}$ = 12.44, P < 0.001; prefrontal cortex: $F_{3,36}$ = 10.72, P < 0.001) and post hoc analysis for nitrite levels showed that both imipramine pre-treatment (10 mg/kg) and DADS pre-treatment (40 and 80 mg/kg) prevented the LPS (100 μg/kg)-induced increase in nitrite levels in the hippocampus (Fig. 5A) and prefrontal cortex (Fig. 5B). In the post-treatment model, the one-way ANOVA (hippocampus: $F_{3,36}$ = 15.91, P < 0.001; prefrontal cortex: $F_{3,36}$ = 10.30, P < 0.001) and post hoc analysis for nitrite levels showed that both imipramine post-treatment (10 mg/kg) and DADS post-treatment at the dose of 40 and 80 mg/kg reversed the LPS (100 μg/kg)-induced increase in nitrite levels in the hippocampus (Fig. 5C) and prefrontal cortex (Fig. 5D).

Effect of DADS pre- or post-treatment on LPS-induced alterations in markers reflecting the oxidative and anti-oxidative status of the hippocampus and prefrontal cortex

Finally, we measured the change of MDA and GSH, two markers reflecting the levels of oxidative and anti-oxidative status, in the hippocampus and prefrontal cortex in mice administered with LPS and/or DADS. In the pre-treatment model, a one-way ANOVA and post hoc analysis for MDA (hippocampus: $F_{3,36} = 6.97$, $P < 0.001$; prefrontal cortex: $F_{3,36} = 7.77$, $P < 0.001$) and GSH (hippocampus: $F_{3,36} = 4.32$, $P < 0.05$; prefrontal cortex: $F_{3,36} = 4.66$, $P < 0.01$) levels showed that both imipramine pre-treatment (10 mg/kg) and DADS pre-treatment at the dose of 40 and 80 mg/kg prevented the LPS (100 μg/kg)-induced increase in MDA levels (Fig. 6A, B) as well as the LPS-induced decrease in GSH levels (Fig. 7A, B) in the hippocampus and prefrontal cortex. In the post-treatment model, the one-way ANOVA and post hoc analysis for MDA (hippocampus: $F_{3,36} = 7.79$, $P < 0.001$; prefrontal cortex: $F_{3,36} = 6.02$, $P < 0.01$) and GSH (hippocampus: $F_{3,36} = 6.52$, $P < 0.01$; prefrontal cortex: $F_{3,36} = 4.43$, $P < 0.01$) levels showed that both imipramine post-treatment (10 mg/kg) and DADS post-treatment (40 and 80 mg/kg) reversed the LPS (100 μg/kg)-induced increase in MDA levels (Fig. 6C, D) as well as the LPS-induced decrease in GSH levels (Fig. 7C, D) in the hippocampus and prefrontal cortex. These results indicate that both DADS pre- and post-treatment are capable of inhibiting high levels of oxidative stress in the brain.

Discussion

The present study for the first time reports that DADS, a major component in garlic oil, displays both prophylactic and therapeutic effects on depression-like behaviors in mice induced by acute LPS administration, while did not affect the locomotor activity of the same mice in the OFT. The prophylactic and therapeutic effect of DADS in this depression model is similar with that of imipramine, a clinical available antidepressant. Furthermore, both DADS and imipramine were found to attenuate the pathological changes in neuroinflammatory responses and oxidative stress in the hippocampus and prefrontal cortex in mice. These results indicate that DADS and imipramine, at least in the present experimental model, may have similar properties in depression regulation.

Our results showed that DADS pre-treatment (1 h before a single LPS injection) prevented while DADS post-treatment (1.5 h and 23.5 h after a single LPS injection) reversed the LPS-induced depression-like behavior in mice, suggesting that in the present experimental model a relatively short-term drug exposure is enough for DADS to induce depression regulation effect. LPS is an endotoxin which is produced endogenously via gut microbiota dysfunction [46]. The increased LPS would enter into the blood via the impaired gut barrier and activate the peripheral and central immune system, thereby inducing sickness and depression-like behavior in both rodents and humans [16, 47, 48]. The acute behavioral response to endotoxin exposure, including the sickness and depression-like behavior, is well-known to cope with environmental challenges. However, repeated or chronic endotoxin exposure may induce an accumulated behavioral response, which has been shown to be tightly associated with the progression of chronic brain damage [49, 50]. Suppression of behavioral abnormalities upon endotoxin challenge may help prevent the occurrence or progression of chronic brain damage. Our results demonstrate that DADS supplementation may be a potential strategy in accord with that purpose because both DADS pre-treatment and post-treatment were found to affect LPS-induced increase in immobility time in the TST

and FST. Furthermore, since DADS is mainly extracted from garlic oil, our results may show a great significance for the application of DADS and DADS-containing garlic or the other edible plants in depression prevention and therapy, especially at the condition of endotoxin presence. The gastrointestinal system, at all times, faces various detrimental challenges, such as Escherichia coli infection, food poisoning, and intestinal cold. These detrimental factors would induce endotoxin accumulation, which may promote the occurrence of repeated behavioral damages. Daily intake of DADS or DADS-containing plants may help suppress the potential damage in the brain induced by gut-derived endotoxin. On the basis of these contexts, the significance of the present study should be strengthened particularly.

Besides the classical monoamine dysfunction hypothesis, the neuroinflammatory hypothesis has been raised to explain the pathogenesis of depression in the past decades. This hypothesis appears to be a new focus for depression study in recent years because the actively involvement of neuroinflammation in depression onset and/or progression has been supported by more and more studies [14, 15, 19, 48, 51, 52]. For example, the over-accumulation of pro-inflammatory cytokines in the blood and/or brain have been observed repeatedly in depressed patients and animals [14, 15, 19], and stimulation of animals with pro-inflammatory cytokines, such as IL-1β and TNF-α, can induce typical depression-like behaviors [51, 52]. Researchers have observed that peoples who accept a low dose of endotoxin immune (24 h) develop sickness and depressive behaviors [48]. Moreover, some clinical available anti-inflammatory drugs, such as NSAIDs and minocycline, can produce obvious antidepressant effects in human individuals and rodent models [20, 21, 53]. Therefore, inhibition of neuroinflammation may be a potential mechanism for the antidepressant effect of the clinical and pre-clinical drugs. Our results showed that DADS pre-treatment markedly prevented while DADS post-treatment reversed the LPS-induced increases in IL-1β and TNF-α levels in the hippocampus and prefrontal cortex in mice, suggesting inhibition of IL-1β and TNF-α production in the brain may mediate the pharmacological effect of DADS in depression prevention and therapy. Further clarifying the mechanism for the regulatory effect of DADS pretreatment and post-treatment on IL-1β and TNF-α expression and/or release in conditions of endotoxin exposure may help develop DADS as a preventive and/or therapeutic drug for depression induced by endotoxin as well as endotoxin-producing factors.

In the neuroinflammation hypothesis, the oxidative/nitrosative stress constitutes an important aspect of depression pathogenesis [54, 55]. The activated immune system in the brain first induces a pro-inflammatory cascade, which includes a tremendous production of various pro-inflammatory cytokines, such as IL-1β and TNF-α, and then the increased pro-inflammatory cytokines promote the production of reactive oxygen species via enhancing nitrite synthesis and release [56]. The increased nitrite together with reactive oxygen species subsequently causes oxido-nitrosative stress which leads to behavioral abnormalities [57]. This hypothesis was evidenced further by our present findings that markers reflecting oxidative/nitrosative stress, such as MDA and nitrite, in the hippocampus and prefrontal cortex were increased markedly by a single LPS injection, and meanwhile the levels of GSH in the hippocampus and prefrontal cortex were reduced by LPS injection. Reversing the harmful changes upon endotoxin exposure would be beneficial for depression prevention and/or therapy. DADS administration may be a potential strategy for that purpose because both DADS pre-treatment and post-treatment were found to attenuate

the LPS-induced increase in MDA and nitrite levels as well as the LPS-induced decrease in GSH levels in the hippocampus and prefrontal cortex.

In depression regulation, most anti-inflammatory compounds require a relatively long time to produce significant regulatory effects [16, 37]. However, in the present study we found that an acute treatment is enough for DADS to produce prophylactic and therapeutic effects on LPS-induced depression-like behaviors in mice. What's the reason for this phenomenon? We noticed that in a previous study by Mello et al. (2013), researchers have reported a similar effect for doxycycline, an antibiotic used in clinic [39]. In that study, the preventive and reversing effects of imipramine and doxycycline on LPS-induced depression-like behavior in mice are considered to be associated with their anti-microbial activities [39]. It is well-known that the change in microbiota in the brain-gut axis is tightly linked with depression pathogenesis [58], and some antidepressants, such as ketamine and IL-6 receptor antagonist, have been shown to rapidly ameliorate depression-like behavior and alter the composition of the gut microbiota in depressed animals [59, 60]. Given that DADS can inhibit bacteria growth [26, 27], it is reasonable to speculate that the herein observed prophylactic and reversing effect of DADS on LPS-induced depression-like behavior in mice may be mediated by its inhibitory effect on gut bacteria. This hypothesis should be clarified clearly in order to explain why DADS benefits the human health.

Conclusion

This study was designed to search drugs that prevent and/or rescue behavioral abnormalities induced by endotoxin exposure, and shows for the first time that similarly to imipramine both DADS pre- and post-treatment ameliorate LPS-induced depression-like behavior in mice, suggesting that daily supplementation of DADS or DADS-containing garlic might be an adjuvant method for the prevention of brain damages induced by repeated endotoxin exposure, a condition which could be observed in various pathological conditions, such as gut bacterial toxicosis.

Declarations

Availability of supporting data

All data generated or analyzed during this study are included in this work.

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Ethics approval and consent to participate

The study was approved by the University Animal Ethics Committee of Nantong University (Permit Number: 2110836), were carried out in each group during the light phase, according to internationally accepted guidelines for the use of animals in toxicology as adopted by the Society of Toxicology in 1999.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author contributions

JL, HH, CH, and ZC performed the experiments and analyzed the data; JL and ZC designed the experiments and wrote the paper. All authors reviewed and approved the manuscript.

Author details

[1]Department of Pharmacy, The People's Hospital of Taizhou, The Fifth Affiliated Hospital of Nantong University, # 210, Yingchun Road, Taizhou, 225300, Jiangsu, China; [2]Department of Respiratory Medicine, Nantong First People's Hospital, the Second Affiliated Hospital of Nantong University, #6 North Road Hai'er Xiang, Nantong, 226001, Jiangsu, China; [3]Department of Pharmacology, School of Pharmacy, Nantong University, 19# Qixiu Road, Nantong, 226001, Jiangsu, China; [4]Invasive Technology Department, Nantong First People's Hospital, the Second Affiliated Hospital of Nantong University, #6 North Road Hai'er Xiang, Nantong, 226001, Jiangsu, China

Abbreviations

ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; DADS, diallyl disulfide; DMSO, dimethyl sulfoxide; FST, forced swimming test; GSH, glutathione; IL-1β, interleukin-1β; LPS, lipopolysaccharide; MDA, malondialdehyde; NO, nitric oxide; NSAIDs, nonsteroidal anti-inflammatory drugs; OFT, open field test; TNF-α, tumor necrosis factor-α; TST, tail suspension test

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Figures

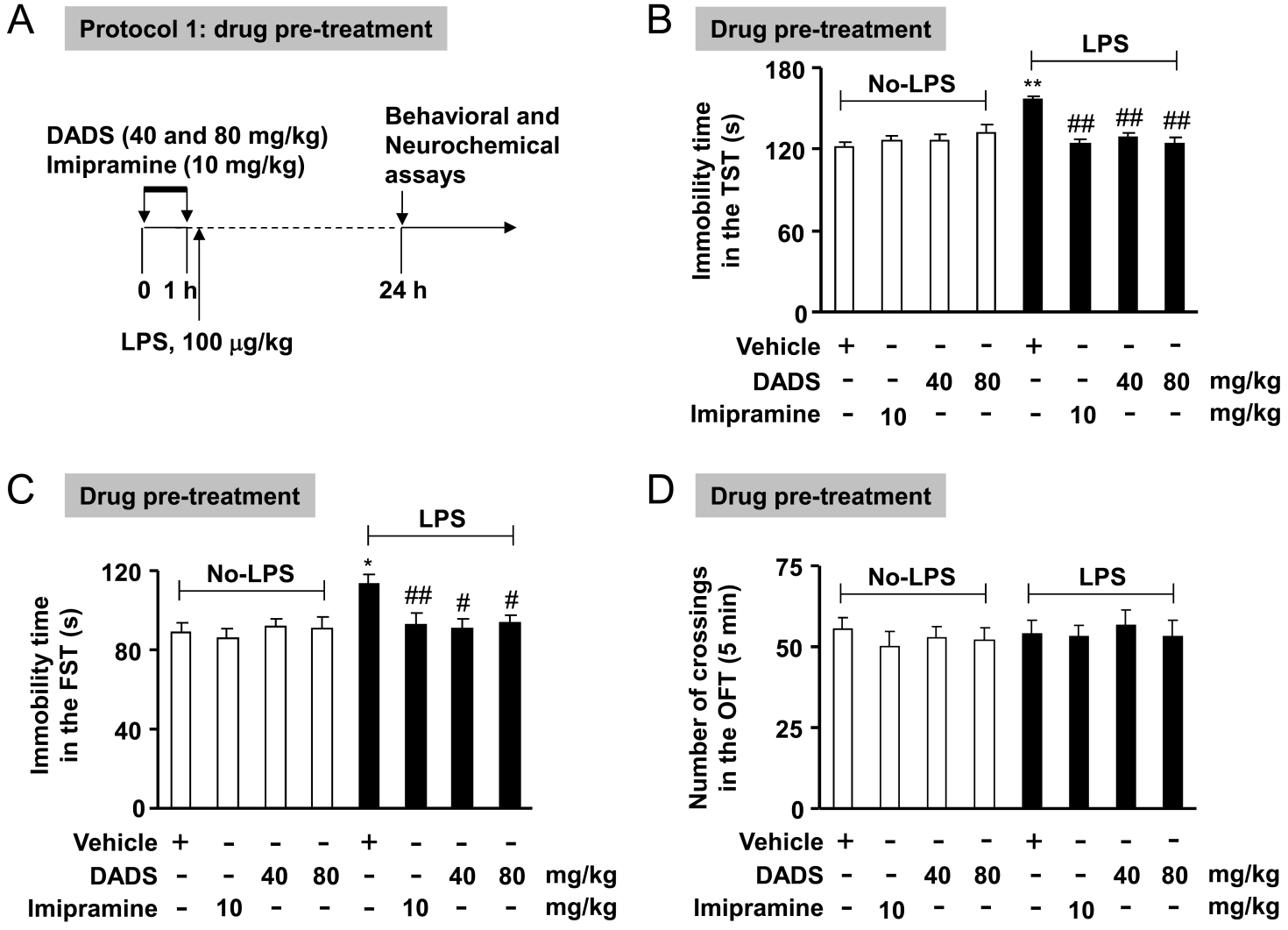

Figure 1

Effect of DADS and imipramine pre-treatment on LPS-induced behavioral alterations in mice. a A schematic diagram showing the study plan for DADS pre-treatment and behavioral assays. b, c Quantitative analysis showing the effect of DADS pre-treatment at the dose of 40 and 80 mg/kg on LPS (100 μg/kg)-induced increase in immobility time in the TST (b, n = 10, **P < 0.01 vs. vehicle, ##P < 0.01 vs. LPS + vehicle) and FST (c, n = 10, *P < 0.05 vs. vehicle, #P < 0.05 or ##P < 0.01 vs. LPS + vehicle). d Quantitative analysis showing the effect of DADS pre-treatment (40 and 80 mg/kg) and/or LPS treatment (100 μg/kg) on the number of crossings of mice in the OFT (n = 10). Data are expressed as mean ± SEM.

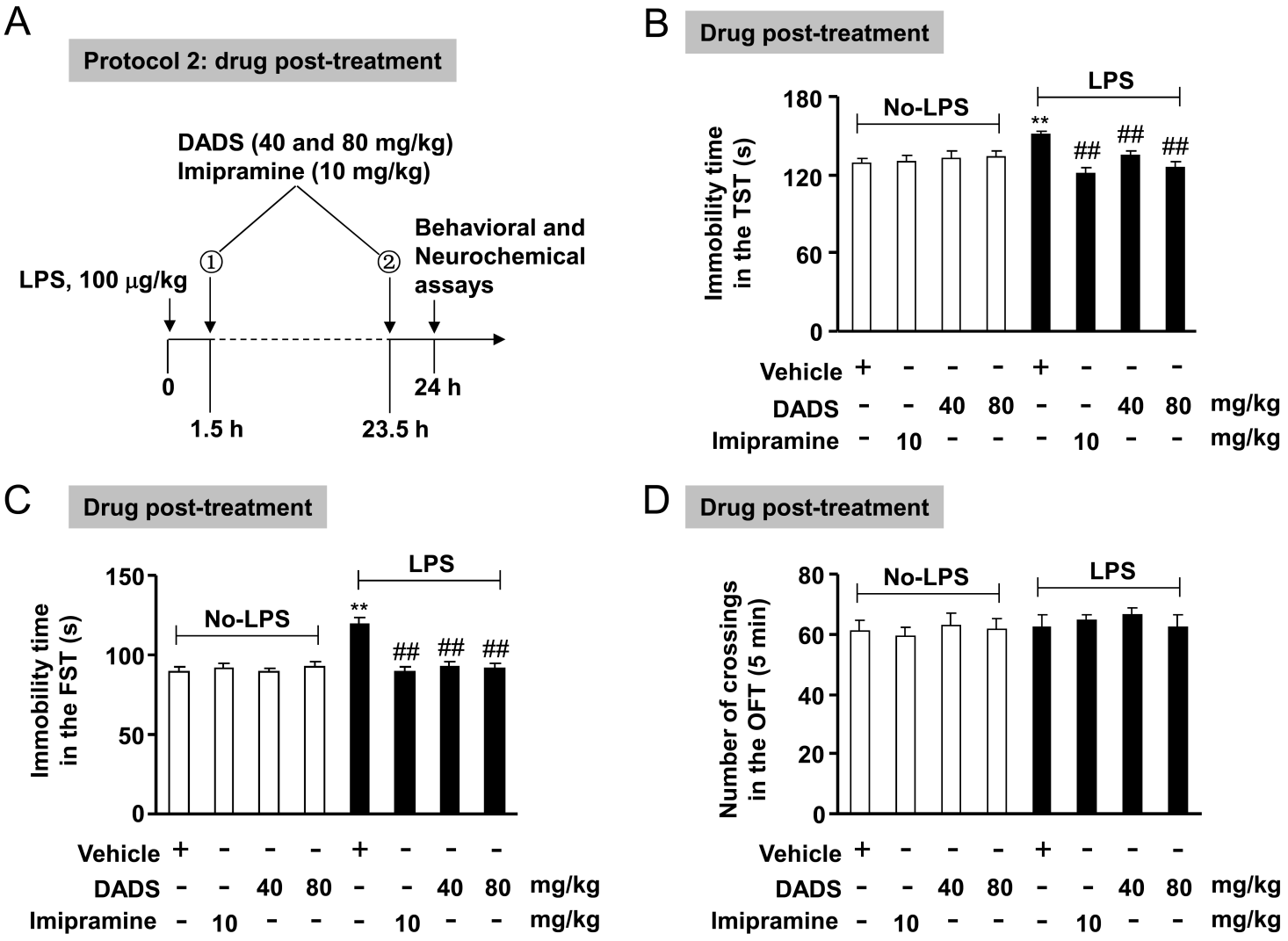

Figure 2

Effect of DADS and imipramine post-treatment on LPS-induced behavioral alterations in mice. a A schematic diagram showing the study plan for DADS post-treatment and behavioral assays. b, c Quantitative analysis showing the effect of DADS post-treatment at the dose of 40 and 80 mg/kg on LPS (100 μg/kg)-induced increase in immobility time in the TST (b, n = 10, **P < 0.01 vs. vehicle, ##P < 0.01 vs. LPS + vehicle) and FST (c, n = 10, **P < 0.01 vs. vehicle, ##P < 0.01 vs. LPS + vehicle). d Quantitative analysis showing the effect of DADS post-treatment (40 and 80 mg/kg) and/or LPS treatment (100 μg/kg) on the number of crossings of mice in the OFT (n = 10). Data are expressed as mean ± SEM.

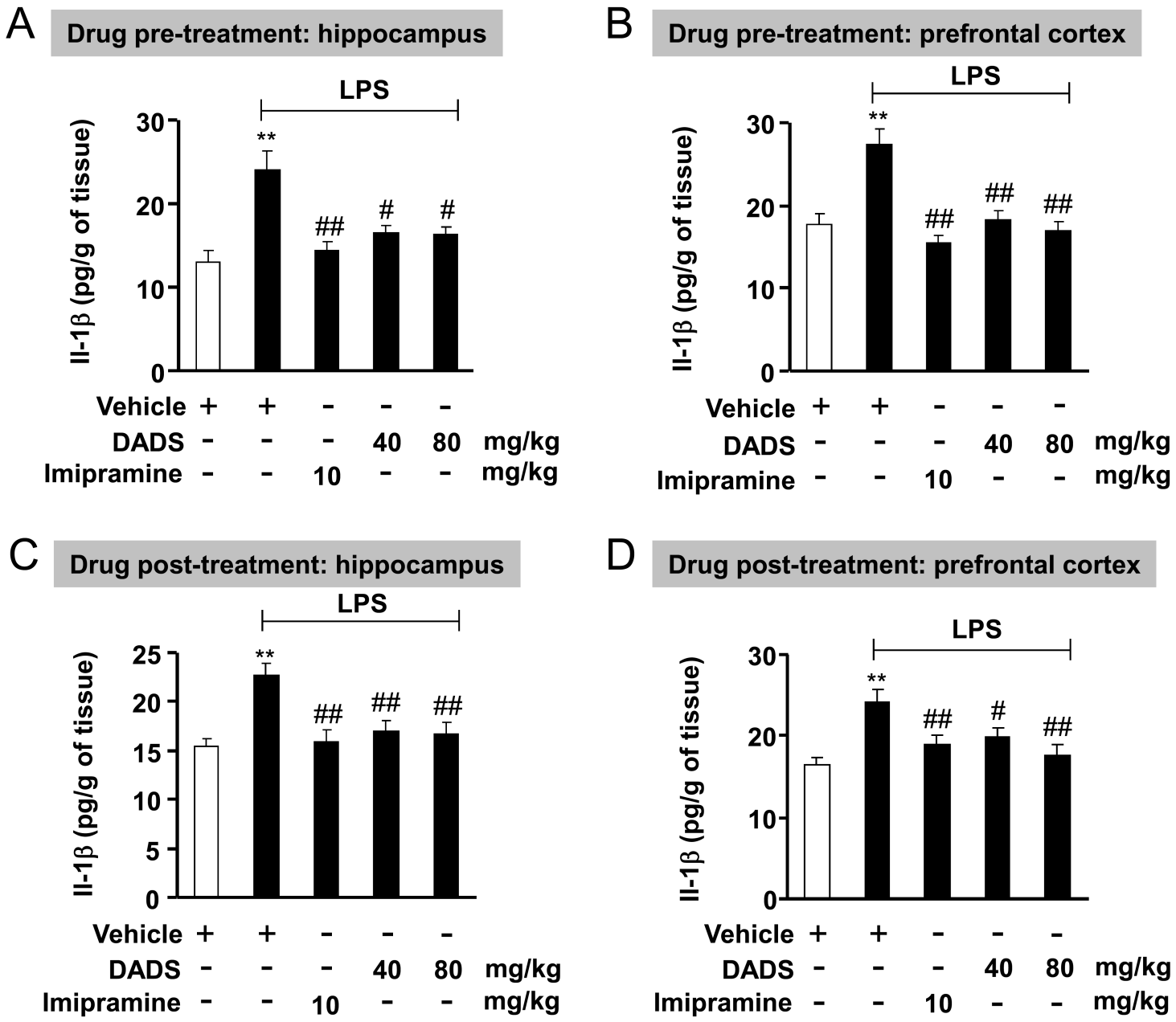

Figure 3

Effect of DADS/imipramine pre- and post-treatment on LPS-induced increase in IL-1β levels in the hippocampus and prefrontal cortex in mice. a, b Quantitative analysis showing the preventive effect of DADS pre-treatment (40 and 80 mg/kg) on LPS (100 μg/kg)-induced increase in IL-1β levels in the hippocampus (a, n = 10, **P < 0.01 vs. vehicle, #P < 0.05 or ##P < 0.01 vs. LPS + vehicle) and prefrontal cortex (b, n = 10, **P < 0.01 vs. vehicle, ##P < 0.01 vs. LPS + vehicle) in mice. c, d Quantitative analysis showing the reversing effect of DADS post-treatment (40 and 80 mg/kg) on LPS (100 μg/kg)-induced increase in IL-1β levels in the hippocampus (c, n = 10, **P < 0.01 vs. vehicle, ##P < 0.01 vs. LPS + vehicle) and prefrontal cortex (d, n = 10, **P < 0.01 vs. vehicle, #P < 0.05 or ##P < 0.01 vs. LPS + vehicle) in mice. Data are expressed as mean ± SEM.

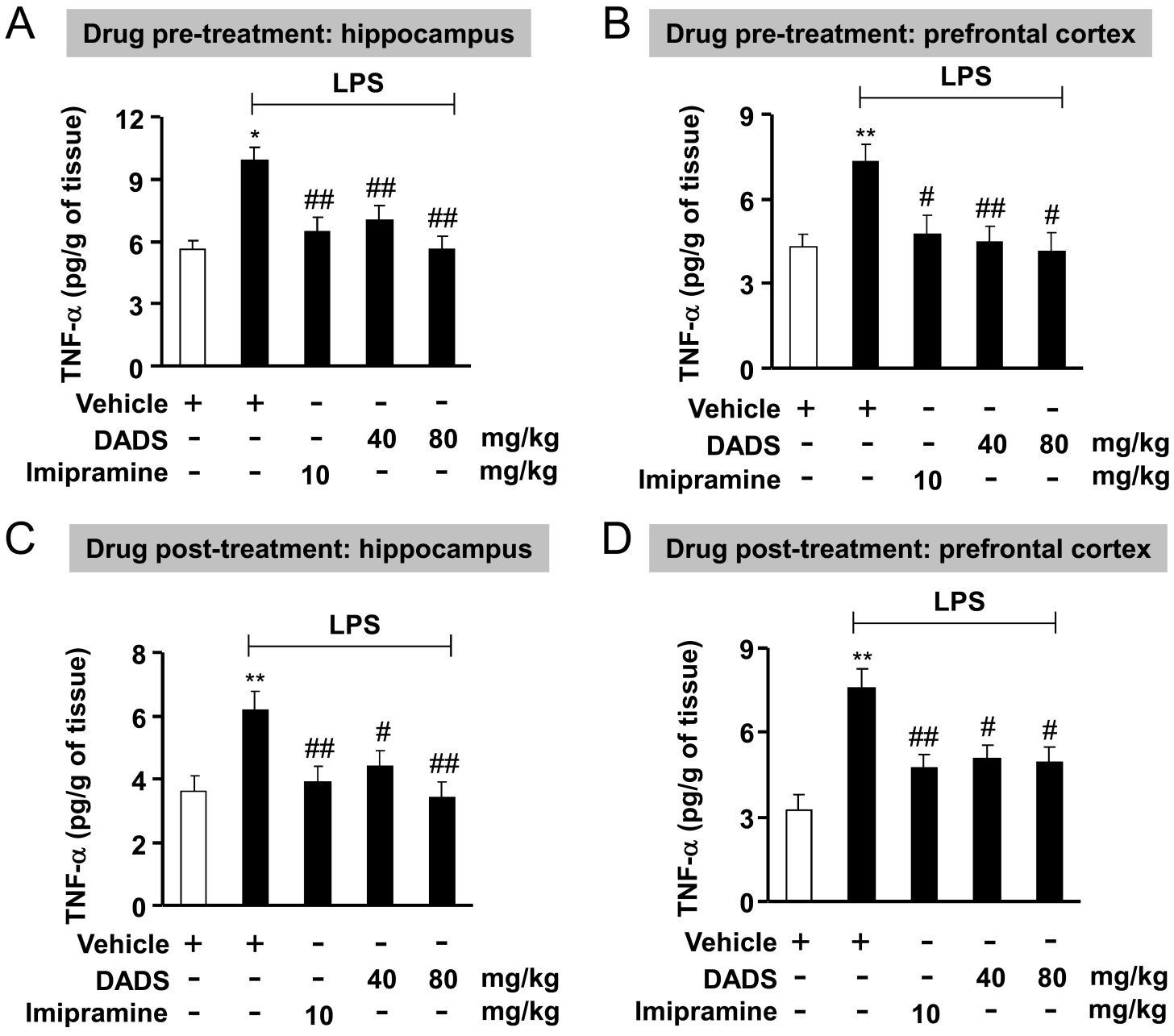

Figure 4

Effect of DADS/imipramine pre- and post-treatment on LPS-induced increase in TNF-α levels in the hippocampus and prefrontal cortex in mice. a, b Quantitative analysis showing the preventive effect of DADS pre-treatment (40 and 80 mg/kg) on the LPS (100 μg/kg)-induced increase in TNF-α levels in the hippocampus (a, n = 10, *P < 0.05 vs. vehicle, ##P < 0.01 vs. LPS + vehicle) and prefrontal cortex (b, n = 10, **P < 0.01 vs. vehicle, #P < 0.05 or ##P < 0.01 vs. LPS + vehicle) in mice. c, d Quantitative analysis showing the reversing effect of DADS post-treatment (40 and 80 mg/kg) on the LPS (100 μg/kg)-induced increase in TNF-α levels in the hippocampus (c, n = 10, **P < 0.01 vs. vehicle, #P < 0.05 or ##P < 0.01 vs. LPS + vehicle) and prefrontal cortex (d, n = 10, **P < 0.01 vs. vehicle, #P < 0.05 or ##P < 0.01 vs. LPS + vehicle) in mice. Data are expressed as mean ± SEM.

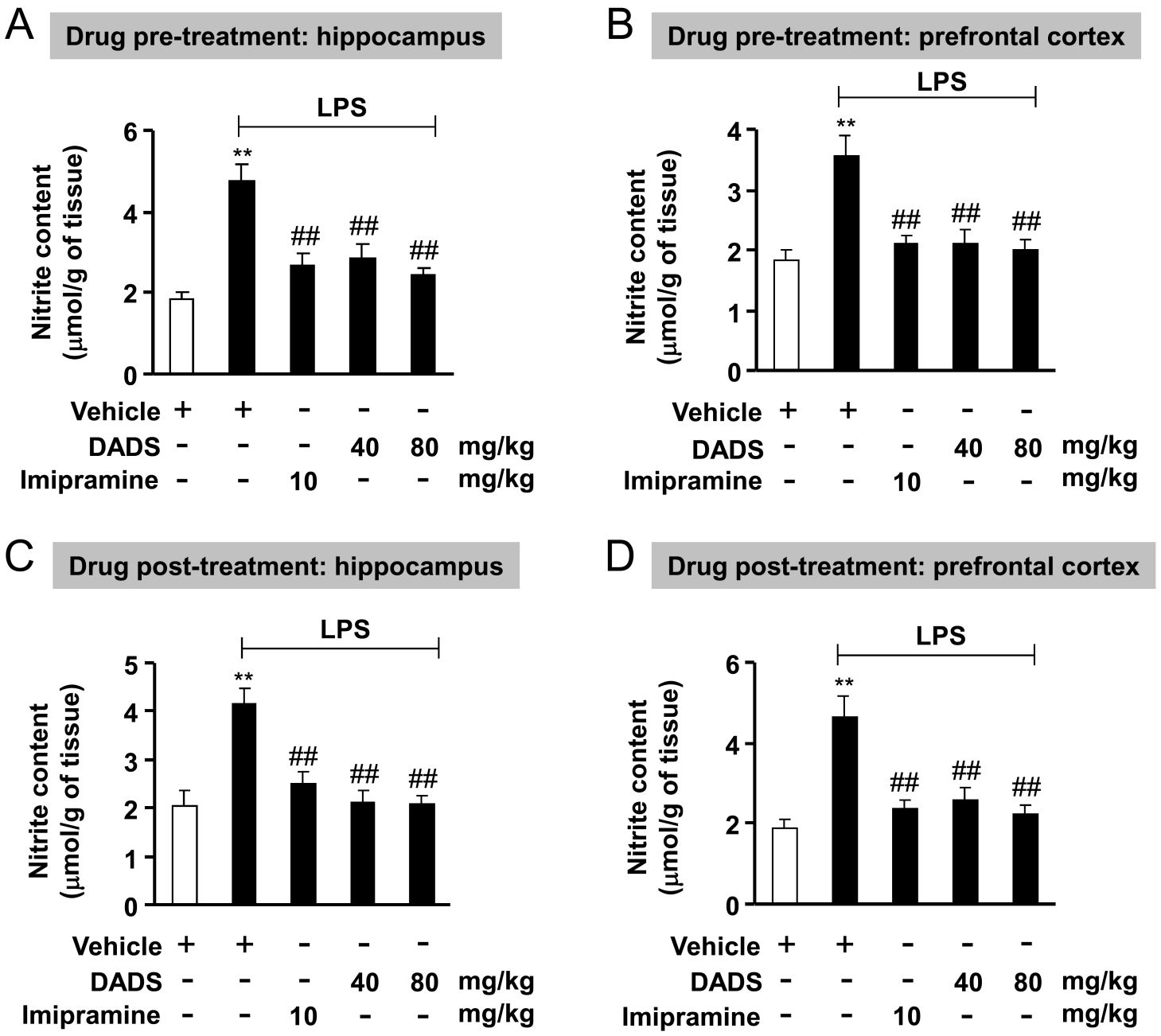

Figure 5

Effect of DADS/imipramine pre- and post-treatment on LPS-induced increase in nitrite levels in the hippocampus and prefrontal cortex in mice. a, b Quantitative analysis showing the preventive effect of DADS pre-treatment (40 and 80 mg/kg) on the LPS (100 μg/kg)-induced increase in nitrite levels in the hippocampus (a, n = 10, **P < 0.01 vs. vehicle, ##P < 0.01 vs. LPS + vehicle) and prefrontal cortex (b, n = 10, **P < 0.01 vs. vehicle, ##P < 0.01 vs. LPS + vehicle) in mice. c, d Quantitative analysis showing the reversing effect of DADS post-treatment (40 and 80 mg/kg) on the LPS (100 μg/kg)-induced increase in nitrite levels in the hippocampus (c, n = 10, **P < 0.01 vs. vehicle, ##P < 0.01 vs. LPS + vehicle) and prefrontal cortex (d, n = 10, **P < 0.01 vs. vehicle, ##P < 0.01 vs. LPS + vehicle) in mice. Data are expressed as mean ± SEM.

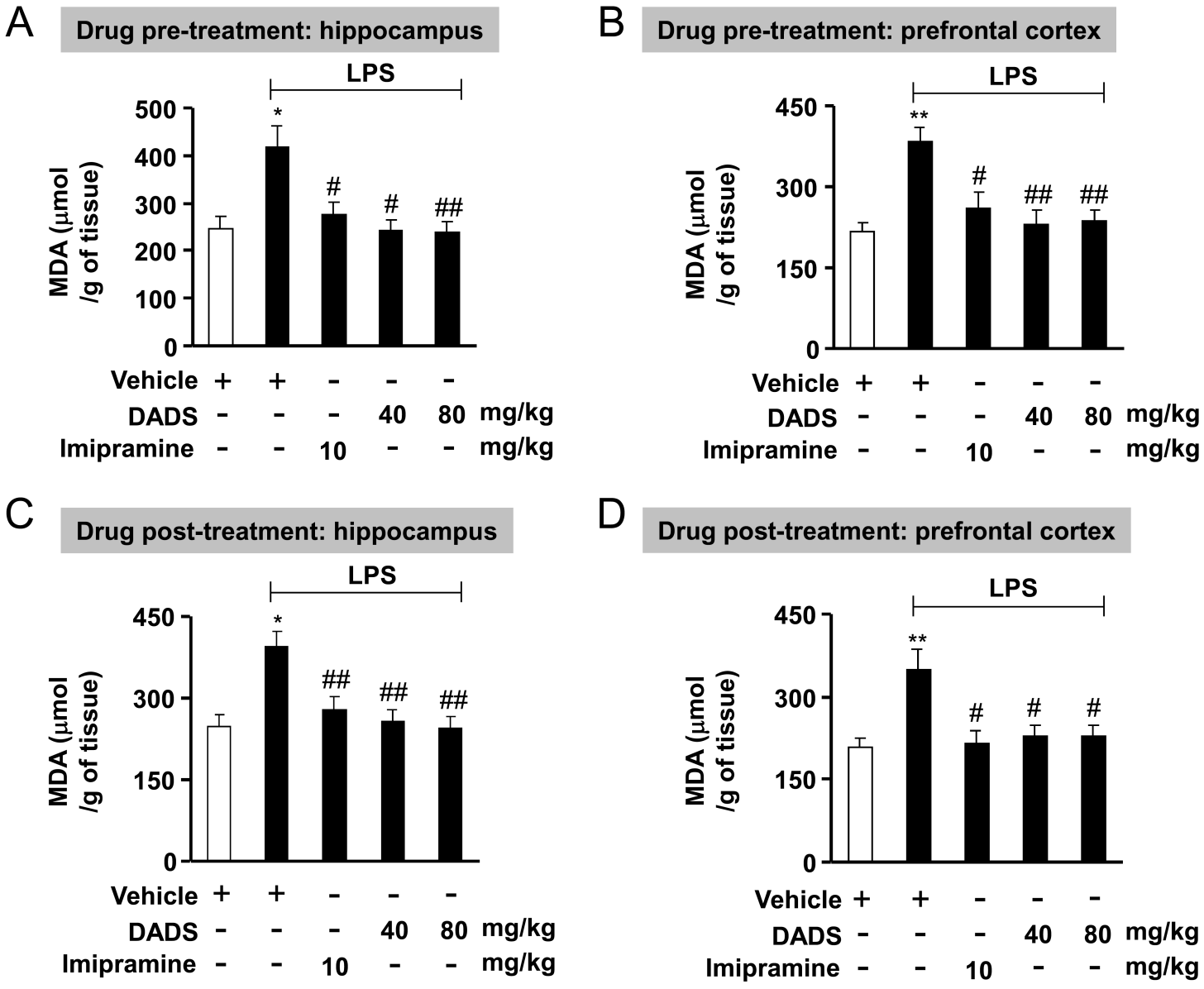

Figure 6

Effect of DADS/imipramine pre- and post-treatment on LPS-induced increase in MDA levels in the hippocampus and prefrontal cortex in mice. a, b Quantitative analysis showing the preventive effect of DADS pre-treatment (40 and 80 mg/kg) on the LPS (100 µg/kg)-induced increase in MDA levels in the hippocampus (a, n = 10, *P < 0.05 vs. vehicle, #P < 0.05 or ##P < 0.01 vs. LPS + vehicle) and prefrontal cortex (b, n = 10, **P < 0.01 vs. vehicle, #P < 0.05 or ##P < 0.01 vs. LPS + vehicle) in mice. c, d Quantitative analysis showing the reversing effect of DADS post-treatment (40 and 80 mg/kg) on the LPS (100 µg/kg)-induced increase in MDA levels in the hippocampus (c, n = 10, *P < 0.05 vs. vehicle, ##P < 0.01 vs. LPS + vehicle) and prefrontal cortex (d, n = 10, **P < 0.01 vs. vehicle, #P < 0.05 vs. LPS + vehicle) in mice. Data are expressed as mean ± SEM.

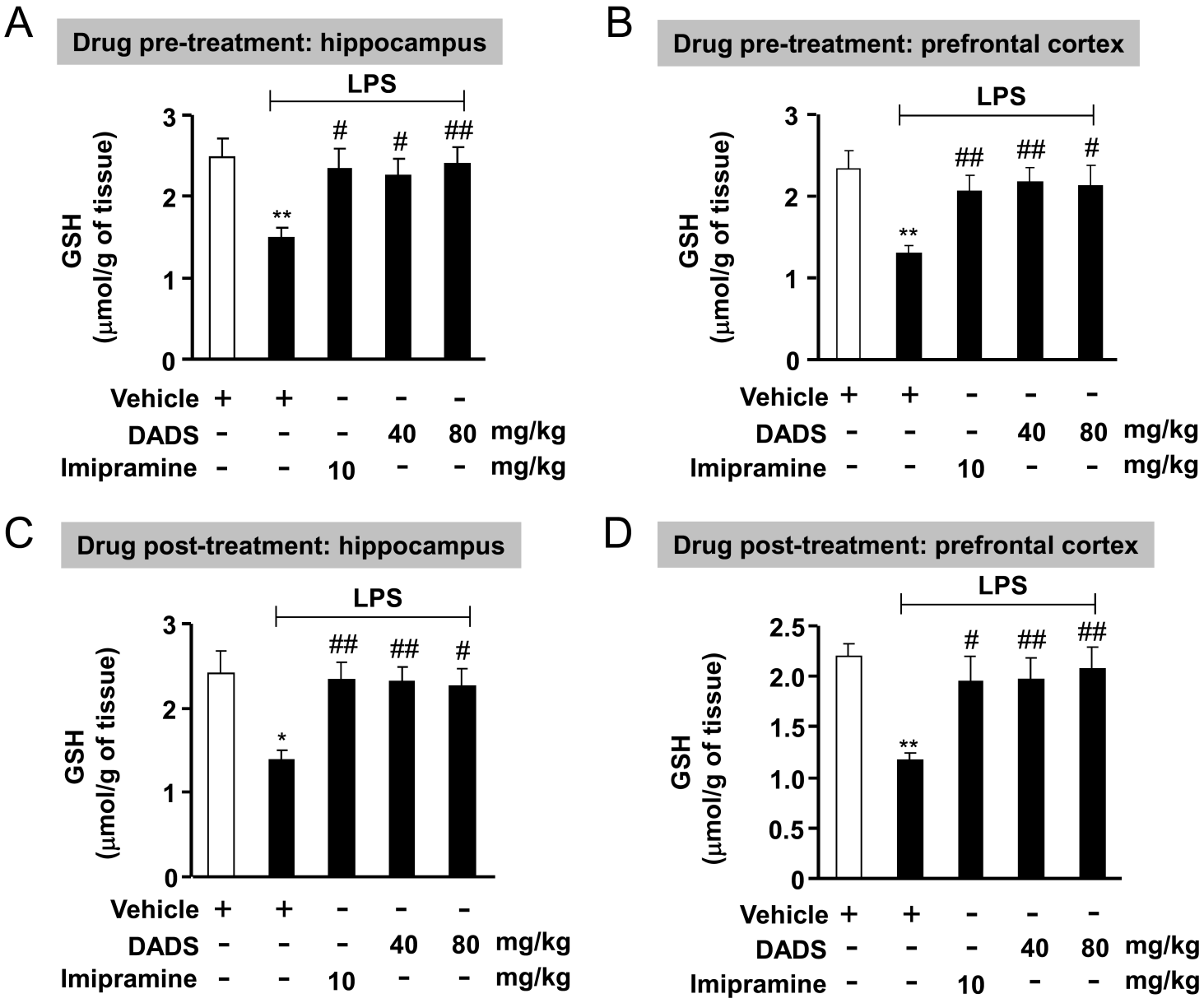

Figure 7

Effect of DADS/imipramine pre- and post-treatment on LPS-induced decrease in GSH levels in the hippocampus and prefrontal cortex in mice. a, b Quantitative analysis showing the preventive effect of DADS pre-treatment (40 and 80 mg/kg) on the LPS (100 μg/kg)-induced increase in GSH levels in the hippocampus (a, n = 10, **P < 0.01 vs. vehicle, #P < 0.05 or ##P < 0.01 vs. LPS + vehicle) and prefrontal cortex (b, n = 10, **P < 0.01 vs. vehicle, #P < 0.05 or ##P < 0.01 vs. LPS + vehicle) in mice. c, d Quantitative analysis showing the reversing effect of DADS post-treatment (40 and 80 mg/kg) on the LPS (100 μg/kg)-induced increase in GSH levels in the hippocampus (c, n = 10, *P < 0.05 vs. vehicle, #P < 0.05 or ##P < 0.01 vs. LPS + vehicle) and prefrontal cortex (d, n = 10, **P < 0.01 vs. vehicle, #P < 0.05 or ##P < 0.01 vs. LPS + vehicle) in mice. Data are expressed as mean ± SEM.