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REGULAR RESEARCH ARTICLE

Monophosphoryl Lipid A Tolerance Against Chronic Stress-Induced Depression-Like Behaviors in Mice

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Abstract

Backgrounds: Our recent studies reported that a single injection with lipopolysaccharide (LPS) before stress exposure prevents depression-like behaviors in stressed mice. Monophosphoryl lipid A (MPL) is a derivative of LPS that lacks the undesirable properties of LPS. We hypothesize that MPL can exert a prophylactic effect on depression.
Methods: The experimental mice were pre-injected with MPL before stress exposure. Depression in mice was induced through chronic social defeat stress (CSDS). Behavioral tests were conducted to identify depression-like behaviors. Real-time polymerase chain reaction and biochemical assays were performed to examine the gene and protein expression levels of pro-inflammatory cytokines.
Results: A single MPL injection 1 day before stress exposure at the dosages of 400, 800, and 1600 μg/kg but not 200 μg/kg prevented CSDS-induced depression-like behaviors in mice. This effect of MPL, however, vanished with the extension of the interval time between drug injection and stress exposure from 1 day or 5 days to 10 days, which was rescued by a second MPL injection 10 days after the first MPL injection or by a 4× MPL injection 10 days before stress exposure. A single MPL injection (800 μg/kg) before stress exposure prevented CSDS-induced increases in the gene expression levels of pro-inflammatory cytokines in the hippocampus and prefrontal cortex. Pre-inhibiting the innate immune stimulation by minocycline pretreatment (40 mg/kg) abrogated the preventive effect of MPL on CSDS-induced depression-like behaviors and neuroinflammatory responses in animal brains.
Conclusions: MPL, through innate immune stimulation, prevents stress-induced depression-like behaviors in mice by preventing neuroinflammatory responses.

Keywords: Innate immunization, monophosphoryl lipid A, neuroinflammatory response, prevention

Introduction

Depression is a common neuropsychological disorder with a high rate of morbidity across the world, which is associated with severe social and economic burdens (Smith and Mazure, 2021). Nowadays, most studies are focusing on the development of drugs that can ameliorate the already-developed disease symptoms, and most clinically available antidepressants in their practical use display several undesirable effects, including insomnia, sexual dysfunction, and even increased suicide ratio (Möller et al., 2008; Brietzke et al., 2019; Luft et al., 2021). An alternative strategy proposed by previous studies (Gu et al., 2021; Ji et al., 2021) suggests the prevention of the occurrence of depression from the source. This strategy may be of great

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Significance Statement

We previously reported that pre-injection with lipopolysaccharides (LPS) can prevent depression-like behaviors in chronically stressed mice, suggesting that innate immune pre-stimulation is a potential strategy for the prevention of depression. Monophosphoryl lipid A (MPL) is a chemically detoxified lipid A moiety that possesses unique immunomodulatory properties at nonpyrogenic doses. Moreover, it lacks the potential undesirable effects of LPS and provides a significant therapeutic window. We found that MPL pre-injection could prevent depression in chronically stressed mice by preventing neuroinflammation. We expect that this finding would promote the development of innate immune stimulation-based strategies for the prevention of depression and other such neuropsychiatric disorders.

significance for reducing the morbidity of depression and for ameliorating the social and economic burdens induced by this type of disease.

The innate immune system, mainly composed of peripheral macrophages and central microglia, belongs to an endogenous defense system that protects the body against environmental challenges (Griffiths et al., 2007). Its over-activation can act as the key process for the induction of cellular or tissue damage via induction of the overproduction of pro-inflammatory cytokines (Place and Kanneganti, 2020; Karki and Kanneganti, 2021). However, its moderate activation by stimulation using a low dose of innate immune stimulant has also been demonstrated to induce neuroprotective effects in rodent disease models such as cerebral ischemia (Stevens et al., 2011) and brain trauma (Longhi et al., 2011) through mechanisms such as the induction of an epigenetically immuno-suppressive phenotype in microglia, the activation of the TRIF-interferon regulatory factor 3 signaling cascade, and the displacement of the inhibitory presynaptic terminals from cortical neurons. We recently reported that a single injection of a low dose of lipopolysaccharide (LPS) or macrophage-colony stimulating factor (M-CSF) before stress exposure, possibly through the stimulation of the innate immune system, prevents the development of depression-like behaviors in chronically stressed animals by transforming the neuroinflammatory response toward an anti-inflammatory phenotype (Gu et al., 2021; Ji et al., 2021). This finding suggests that the pre-stimulation of the innate immune system may be a potential strategy for the prevention of the progression of depressive symptoms, at least in rodent models. However, LPS, especially when administered at a toxic dosage, may produce undesirable effects, such as through the over-activation of neutrophils and the induction of fever and sickness behavior (Matsuwaki et al., 2017; Liu and Sun, 2019; O'Neill et al., 2021). Thus, it may be necessary to search for a new and effective alternative that induces similar prophylactic effects on depression.

Monophosphoryl lipid A (MPL) is a chemically detoxified lipid A moiety derived from Salmonella Minnesota R595 LPS with unique immunomodulatory properties at nonpyrogenic doses (Hesam et al., 2018). MPL lacks the possible undesirable effects of LPS and offers a significant therapeutic relative to that of LPS (Elliott et al., 1998; Chilton et al., 2013). Past experimental studies reported that MPL pretreatment can induce prophylactic effects on neuronal damages in animal models of central nervous system disorders. For example, the pre-administration of animals with a low dose of MPL has been demonstrated to prevent increased infarct size and impaired cognitive function in a rat model of hippocampal ischemia, which occurred through the downregulation of inflammatory mediator nuclear factor-κ B and tumor necrosis factor-α (TNF-α) and the upregulation of anti-inflammatory mediators interferon-β, interferon regulatory factor 3, and transforming growth factor-β (Hosseini et al., 2018). The suppression of the production of pro-inflammatory cytokines in the brain, such as TNF-α, interleukin-1β (IL-1β), and IL-6, was also found to contribute to the preventive effect of MPL on seizure susceptibility and seizure-related behaviors induced by traumatic brain injury or pilocarpine (Hesam et al., 2018; Hosseinzadeh et al., 2019). Furthermore, minor stimulation of microglia by MPL pretreatment has been reported to prevent abnormally increased expression of pro-inflammatory cytokines, such as TNF-α, in the brain tissues of amyloid beta-treated rats, thereby improving the impaired long-term potentiation and spatial and working memory functions (Pourbadie et al., 2018; Yousefi et al., 2019). These studies have demonstrated that the suppression of the over-production of pro-inflammatory cytokines in the brain may be the key mechanism for the prevention of the pathological progression of central nervous system disorders by MPL pretreatment.

Based on these backgrounds, we designed an array of experiments to evaluate whether MPL, as an immune stimulant like LPS but with fewer toxicities, can prevent the development of depression-like behaviors in chronically stressed mice through a process associated with the prevention of neuroinflammation in the brain. Our study's findings may thus promote the development of innate immune stimulation-based strategies for the prevention of depression and other neuropsychiatric disorders.

METHODS

Animals

Six-week-old male C57BL6/J mice and 8-week-old male and female CD1 mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Mice were housed 5 per cage at conditions of 12-hour-light/-dark cycle, lights on from 7:00 AM to 7:00 PM, 23°C±1°C ambient temperature, 55%±10% relative humidity, and free access to food and water. The female CD1 mice were used to induce aggressive behaviors in the male CD1 mice. The male CD1 mice would attack the intruder mice when a male C57BL6/J intruder came in and a female CD1 sexual partner was removed (Goyens and Noirot, 1975). Animal experiments were approved by the University Animal Ethics Committee of Nantong University (permit no. 2110836) and conducted in accordance with internationally accepted guidelines for the use of animals in toxicology as adopted by the Society of Toxicology in 1999.

Materials

Both MPL and Hoechst 33258 are the products of Sigma (St Louis, MO, USA). Minocycline was purchased from Selleck (Shanghai, China). The anti-Iba-1 antibody was from Abcam (Cambridge, MA, USA). The MPL was dissolved in dimethyl sulfoxide (DMSO) as a stock solution and was diluted to a final concentration

100 μg/mL using Ringer's solution. The minocycline was dissolved in di-H_2O as a stock solution.

Pharmacological Treatment and Behavioral Procedures

In dose-dependent analyses, a single MPL was administered i.p. at doses of 200, 400, 800, and 1600 μg/kg 1 day before stress exposure (Fig. 1A). In the time-interval experiment, a second MPL injection (800 μg/kg) was administered 10 days after the first MPL injection or with a 4× MPL injection 10 days before stress exposure at the dose of 800 μg/kg, as shown in Fig. 2–4A. The mice were allocated into the vehicle, MPL, vehicle + chronic social defeat stress (CSDS), and MPL + CSDS groups to evaluate the effect of a single MPL injection (800 μg/kg) on CSDS-induced neuroinflammatory response (n=8, in each group; Fig. 5A). The dose range of MPL was selected in accordance with previous suggestions (Watts et al., 2019, 2020). To evaluate the role of the innate immune stimulation on the preventive effect of MPL on mouse behavior and neuroinflammatory response, the mice were allocated into the vehicle, vehicle + CSDS, MPL+CSDS, minocycline+CSDS, and minocycline+MPL+CSDS groups, and, in the present experiment, the mice received 2 days of minocycline treatment (40 mg/kg) before MPL injection, which was followed by another 2 days of post-treatment (Fig. 6D). The social interaction test (SIT), tail suspension test (TST), and forced swim test (FST), were conducted 1, 2, and 3 days after the discontinuation of defeat stress, respectively. The brain tissues, which were prepared for the detection of mRNA and Iba-1-positive microglia, were collected immediately after the discontinuation of the behavioral assays. To investigate whether minocycline pretreatment indeed inhibited the immune activity, the experimental mice were pre-treated with 2 days of minocycline (40 mg/kg) (Fig. 6A), and the blood was collected 5 hours after MPL injection by the enzyme linked immunosorbent assay. The herein-used dose of minocycline could efficiently inhibit the innate immune cells (Ye et al., 2020; Gu et al., 2021). All behavioral tests were performed during the light phase. As DMSO may have immune-modifying effects (Huang et al., 2020) and as

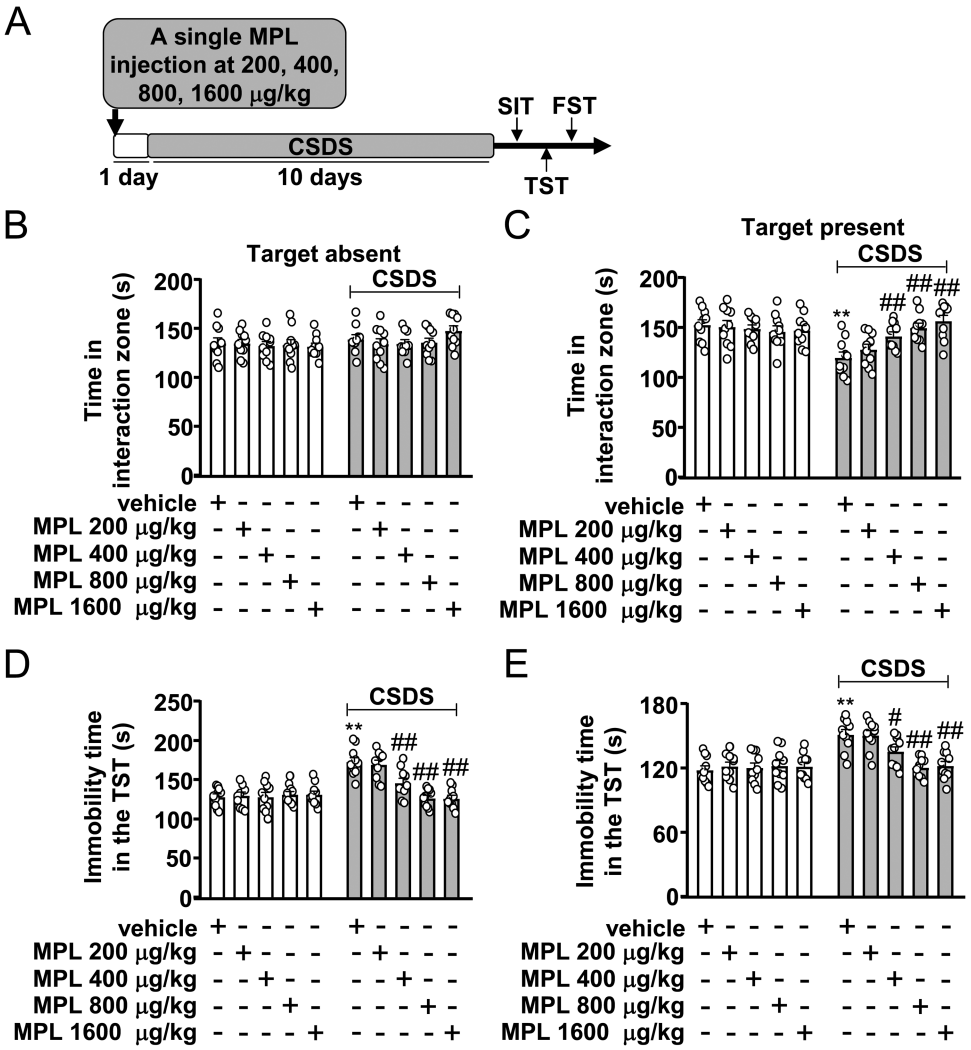

Figure 1. Dose-dependent effect of MPL pre-injection on CSDS-induced depression-like behaviors. (A) A schematic diagram showing the experimental arrangement for the evaluation of the effect of MPL pre-injection at different dosages on CSDS-induced depression-like behaviors in mice. (B, C) Quantitative analysis showing the effect of MPL pre-injection (200, 400, 800, or 1600 μg/kg) 1 day before stress exposure on CSDS-induced changes in the time spent in the interaction zone in the SIT with target absence (B) or presence (C) (n=10, **P<.01 vs vehicle; ##P<.01 vs vehicle + CSDS). (D, E) Quantitative analysis showing the effect of MPL pre-injection (200, 400, 800, or 1600 μg/kg) 1 day before stress exposure on CSDS-induced increases in immobility time in the TST (D) and FST (E) (n=10, **P<.01 vs vehicle, #P<.05 or ##P<.01 vs vehicle + CSDS). Data are shown as mean±SEM. CSDS, chronic social defeat stress; FST, forced swimming test; MPL, monophosphoryl lipid A; SEM, standard error of mean; SIT, social interaction test; TST, tail suspension test.

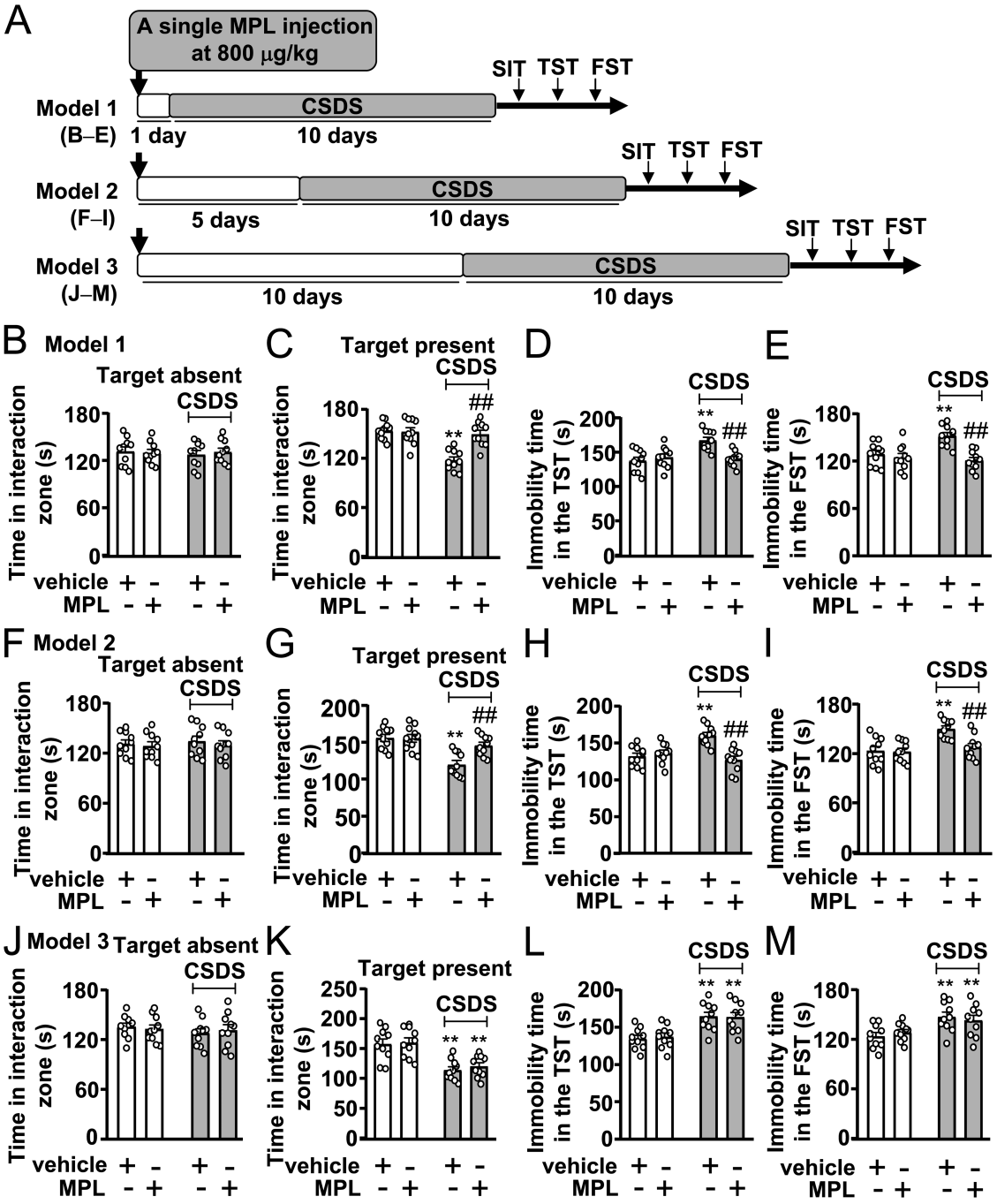

Figure 2. Influence of time interval between MPL pre-injection and stress exposure on CSDS-induced depression-like behaviors. (A) A schematic diagram showing the experimental arrangement for the evaluation of the influence of different time intervals between MPL pre-injection and stress exposure on CSDS-induced depression-like behaviors. (B–E) Quantitative analysis showing the influence of a 1-day interval between MPL pre-injection (800 μg/kg) and stress exposure on CSDS-induced decrease in the time spent in the interaction zone in the SIT with target absence (B) or presence (C) and CSDS-induced increases in the immobility time in the TST (D) and FST (E) (n = 10, $^{**}P < .01$ vs vehicle; $^{\#\#}P < .01$ vs vehicle + CSDS). (F–I) Quantitative analysis showing the influence of a 5-day interval between MPL pre-injection (800 μg/kg) and stress exposure on CSDS-induced decrease in the time spent in the interaction zone in the SIT with target absent (F) or present (G) and CSDS-induced increases in the immobility time in the TST (H) and FST (I) (n = 10, $^{**}P < .01$ vs vehicle; $^{\#\#}P < .01$ vs vehicle + CSDS). (J–M) Quantitative analysis showing the influence of a 10-day interval between MPL pre-injection (800 μg/kg) and stress exposure on CSDS-induced decrease in the time spent in the interaction zone in the SIT with target absence (J) or presence (K) and CSDS-induced increases in the immobility time in the TST (L) and FST (M) (n = 10, $^{**}P < .01$ vs vehicle). Data are shown as mean ± SEM. CSDS, chronic social defeat stress; FST, forced swimming test; MPL, monophosphoryl lipid A; SEM, standard error of mean; SIT, social interaction test; TST, tail suspension test.

its concentrations in different MPL doses may vary widely, we selected the diluted Ringer's solution, which contained the DMSO that was used to dissolve the highest dose of MPL (1600 μg/kg) as a vehicle control. Additional experimental procedures are available online in the supplementary Materials.

Statistical Analysis

Statistical analyses were performed using Graphpad Prism 8 (Graphpad Software, Inc., La Jolla, CA, USA). Differences between the mean values of data were evaluated by a 2-way ANOVA). The

post-hoc Bonferroni test was used to assess isolated comparisons. $P < .05$ was considered statistically significant. Data are presented as mean ± standard error of mean (SEM).

RESULTS

Dose-Dependent Effect of MPL Pretreatment on Depression-Like Behaviors in CSDS Mice

We first evaluated the dose-dependent effect of MPL pretreatment (200, 400, 800, and 1600 μg/kg) on CSDS-induced depression-like behaviors. A 2-way ANOVA was applied for the time spent in the interaction zone when the target was absent in the SIT, which showed no significant effects for CSDS exposure ($F_{1,90} = 2.65$, $P = .11$), MPL treatment ($F_{4,90} = .58$, $P = .68$), and the CSDS × MPL interaction ($F_{4,90} = .99$, $P = .42$) (Fig. 1B). On the other hand, when the target was present, the 2-way ANOVA for the same index demonstrated significant effects for CSDS exposure ($F_{1,90} = 9.17$, $P < .01$), MPL pretreatment ($F_{4,90} = 3.00$, $P < .05$), and the CSDS × MPL interaction ($F_{4,90} = 5.82$, $P < .001$) (Fig. 1C). In the TST, the 2-way ANOVA showed significant effects for CSDS exposure ($F_{1,90} = 37.01$, $P < .001$), MPL pretreatment ($F_{4,90} = 10.04$, $P < .001$), and the CSDS × MPL interaction ($F_{4,90} = 12.09$, $P < .001$) (Fig. 1D), while, in the FST, the application of 2-way ANOVA revealed significant effects for CSDS exposure ($F_{1,90} = 33.22$, $P < .001$), MPL treatment ($F_{4,90} = 5.49$, $P < .001$), and the CSDS × MPL interaction ($F_{4,90} = 7.13$, $P < .001$) (Fig. 1E). Post-hoc analysis showed that a single MPL injection at the dose of 200 μg/kg did not affect CSDS-induced decreases in the time spent in the interaction zone in the SIT (Fig. 1B–C) as well as the increases in the immobility time in the TST (Fig. 1D) and FST (Fig. 1E). At the doses of 400, 800, or 1600 μg/kg, MPL pretreatment prevented the decreased time spent in the interaction zone in the SIT (Fig. 1B–C) and the increased immobility time in the TST (Fig. 1D) and FST (Fig. 1E) in CSDS mice. The effect at 800 μg/kg was found to be like that observed at 1600 μg/kg; thus, the 800-μg/kg dosage was used in further studies. Under stress-naïve conditions, a single MPL injection at any dose did not affect the mouse behaviors in the SIT (Fig. 1B–C), TST (Fig. 1D), and FST (Fig. 1E) groups.

Effect of Time Interval on MPL-Induced Prevention of Depression-Like Behaviors in CSDS Mice

According to the above-mentioned dose-dependent observations, we assumed that the time interval between MPL injection and stress exposure may affect the preventive effect of MPL pretreatment on CSDS-induced depression-like behaviors (Fig. 2A). As shown in Fig. 2B–C, a 2-way ANOVA involving a 1-day interval experiment in the SIT for the time spent in the interaction zone when the target was absent indicated no significant effects for CSDS exposure ($F_{1,36} = .07$, $P = .96$), MPL treatment ($F_{1,36} = .02$, $P = .89$), and the CSDS × MPL interaction ($F_{1,36} = .34$, $P = .56$). On the other hand, in the presence of the target, the 2-way ANOVA for the same index indicated significant effects for CSDS exposure ($F_{1,36} = 20.59$, $P < .001$), MPL treatment ($F_{1,36} = 12.54$, $P < .01$), and the CSDS × MPL interaction ($F_{1,36} = 15.04$, $P < .001$). The 2-way ANOVA involving the 1-day interval experiment in the TST revealed significant effects for CSDS exposure ($F_{1,36} = 8.81$, $P < .01$), MPL treatment ($F_{1,36} = 5.66$, $P < .05$), and the CSDS × MPL interaction ($F_{1,36} = 11.66$, $P < .01$) (Fig. 2D). In addition, in the FST, the 2-way ANOVA for the 1-day interval experiment showed significant effects for CSDS exposure ($F_{1,36} = 5.00$, $P < .05$), MPL treatment ($F_{1,36} = 16.00$, $P < .001$), and the CSDS × MPL interaction ($F_{1,36} = 8.98$, $P < .01$) (Fig. 2E). Post-hoc analysis revealed that a single MPL injection at the dose of 800 μg/kg 1 day before stress exposure prevented the decreased time spent in the interaction zone in the SIT (Fig. 2B–C) and the increased immobility time in the TST (Fig. 2D) and FST (Fig. 2E) in the CSDS mice.

In the 5-day interval experiment for SIT, a 2-way ANOVA performed for the time spent in the interaction zone in the absence of the target revealed no significant effects for CSDS exposure ($F_{1,36} = .15$, $P = .70$), MPL treatment ($F_{1,36} = .53$, $P = .47$), and the CSDS × MPL interaction ($F_{1,36} = .06$, $P = .80$). On the other hand, in the presence of the target, the 2-way ANOVA for the same index revealed significant effects for CSDS exposure ($F_{1,36} = 21.35$, $P < .001$), MPL treatment ($F_{1,36} = 6.53$, $P < .05$), and the CSDS × MPL interaction ($F_{1,36} = 7.03$, $P < .05$) (Fig. 2F–G). In the TST for the 5-day-interval experiment, the 2-way ANOVA revealed significant effects for CSDS exposure ($F_{1,36} = 4.66$, $P < .05$), MPL treatment ($F_{1,36} = 10.54$, $P < .01$), and the CSDS × MPL interaction ($F_{1,36} = 17.70$, $P < .001$) (Fig. 2H). In the FST for the 5-day-interval experiment, the 2-way ANOVA revealed significant effects for CSDS exposure ($F_{1,36} = 13.75$, $P < .001$), MPL treatment ($F_{1,36} = 7.09$, $P < .05$), and the CSDS × MPL interaction ($F_{1,36} = 5.91$, $P < .05$) (Fig. 2I). Post-hoc analysis revealed that a single MPL injection (800 μg/kg) 5 days before the stress exposure could prevent the decreased time spent in the interaction zone in the SIT (Fig. 2F–G) and the increased immobility time in the TST (Fig. 2H) and FST (Fig. 2I) in the CSDS mice.

In the 10-day interval experiment for SIT, a 2-way ANOVA performed for the time spent in the interaction zone in the absence of the target showed no significant effects for CSDS exposure ($F_{1,36} = .70$, $P = .41$), MPL treatment ($F_{1,36} = .004$, $P = .95$), and the CSDS × MPL interaction ($F_{1,36} = .35$, $P = .56$). In the presence of the target, 2-way ANOVA for the time spent in the interaction zone revealed a significant effect for CSDS exposure ($F_{1,36} = 35.22$, $P < .001$), but not for MPL treatment ($F_{1,36} = .39$, $P = .53$) and the CSDS × MPL interaction ($F_{1,36} = .07$, $P = .79$) (Fig. 2J–K). For the TST, the 2-way ANOVA revealed a significant effect for CSDS exposure ($F_{1,36} = 26.21$, $P < .001$), but not for MPL treatment ($F_{1,36} = .04$, $P = .84$) and the CSDS × MPL interaction ($F_{1,36} = .12$, $P = .74$) (Fig. 2L). For the FST, the 2-way ANOVA revealed a significant effect for CSDS exposure ($F_{1,36} = 14.04$, $P < .001$), but not for MPL treatment ($F_{1,36} = .001$, $P = .97$) and the CSDS × MPL interaction ($F_{1,36} = .87$, $P = .36$) (Fig. 2M). Post-hoc analysis revealed that a single MPL injection (800 μg/kg) administered 10 days before stress exposure failed to prevent a CSDS-induced decrease in time spent in the interaction zone in the SIT (Fig. 2J–K) and increase in the immobility time in the TST (Fig. 2L) and FST (Fig. 2M).

Preventive Effect of a Second MPL Injection on Depression-Like Behaviors in CSDS Mice

As in the 10-day-interval experiment the preventive effect of MPL vanished at a long-term time point after a single MPL injection, we speculated whether a repeat injection of MPL 10 days after the first MPL injection still could induce a preventive effect (Fig. 3A). In the SIT, a 2-way ANOVA for the time spent in the interaction zone in the absence of the target indicated no significant effects for CSDS exposure ($F_{1,36} = .94$, $P = .34$), MPL treatment ($F_{1,36} = .004$, $P = .95$), and the CSDS × MPL interaction ($F_{1,36} = .19$, $P = .66$). In the presence of the target, the 2-way ANOVA for the same index revealed significant effects for CSDS exposure ($F_{1,36} = 16.79$, $P < .001$), MPL treatment ($F_{1,36} = 6.19$, $P < .05$), and the CSDS × MPL interaction ($F_{1,36} = 9.30$, $P < .01$) (Fig. 3B–C). For the TST, the 2-way ANOVA revealed significant effects for CSDS exposure ($F_{1,36} = 13.55$, $P < .01$), MPL treatment ($F_{1,36} = 5.23$, $P < .05$), and the CSDS × MPL interaction ($F_{1,36} = 8.09$, $P < .01$) (Fig. 3D). For the FST, the 2-way ANOVA demonstrated significant effects for CSDS exposure ($F_{1,36} = 10.75$, $P < 0.01$), MPL treatment ($F_{1,36} = 5.73$,

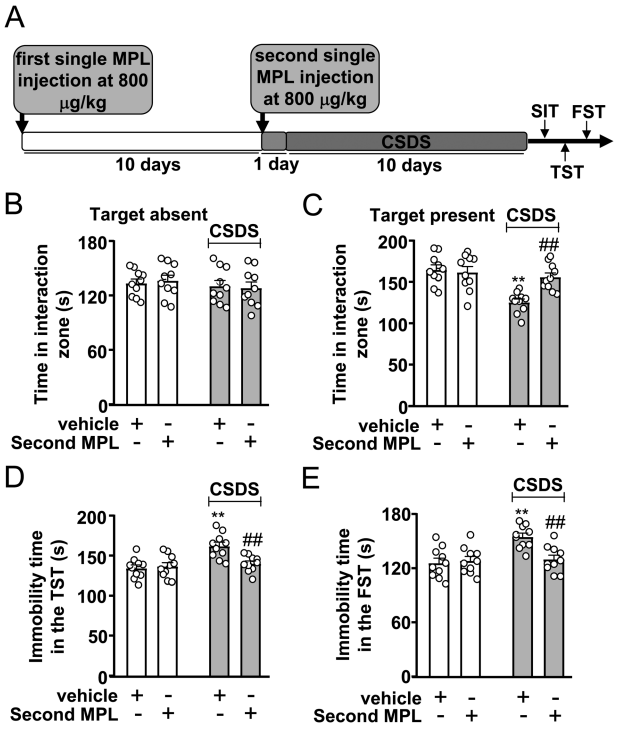

Figure 3. Effect of a second MPL injection 10 days after the first MPL injection on CSDS-induced depression-like behaviors. (A) A schematic diagram showing the experimental arrangement for the evaluation of the effect of the first and second MPL injection with a 10-day interval on CSDS-induced depression-like behaviors. (B–E) Quantitative analysis showing the effect of a second MPL injection (800 μg/kg, 1 day before stress exposure) 10 days after the first MPL injection on CSDS-induced decrease in the time spent in the interaction zone in the SIT with target absence (B) or presence (C) and CSDS-induced increases in the immobility time in the TST (D) and FST (E) in mice (n = 10, **P < .01 vs vehicle, ##P < .01 vs vehicle + CSDS). Data are shown as mean ± SEM. CSDS, chronic social defeat stress; FST, forced swimming test; MPL, monophosphoryl lipid A; SEM, standard error of mean; SIT, social interaction test; TST, tail suspension test.

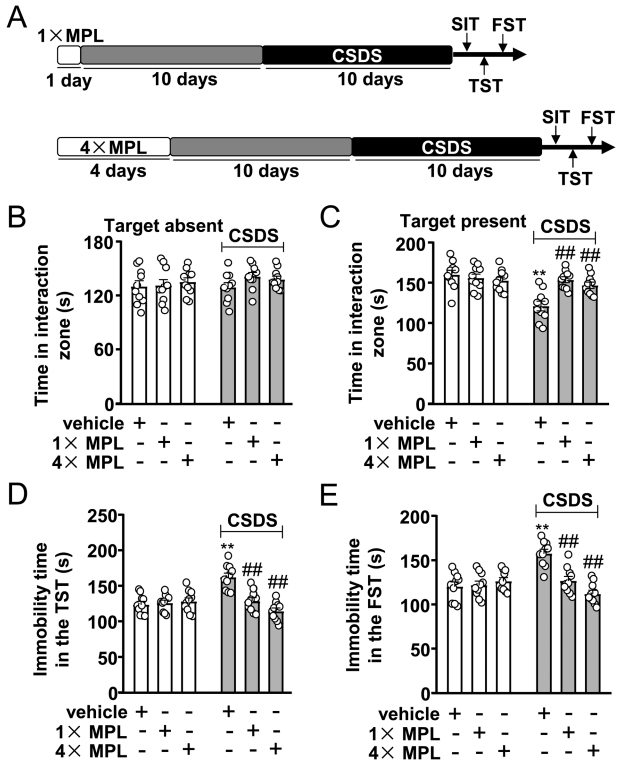

Figure 4. Effect of repeated MPL injections 10 days before stress exposure on CSDS-induced depression-like behaviors. (A) A schematic diagram showing the experimental arrangement for the evaluation of the effect of the 1× and 4× MPL injection ten days before stress exposure on CSDS-induced depression-like behaviors. (B–E) Quantitative analysis showing the differential effect of the 1× and 4 × MPL injection (800 μg/kg) 10 days before stress exposure on CSDS-induced decrease in the time spent in the interaction zone in the SIT with target absence (B) or presence (C) and CSDS-induced increases in the immobility time in the TST (D) and FST (E) in mice (n = 10, **P < .01 vs vehicle, ##P < .01 vs vehicle + CSDS). Data are shown as mean ± SEM. CSDS, chronic social defeat stress; FST, forced swimming test; MPL, monophosphoryl lipid A; SEM, standard error of mean; SIT, social interaction test; TST, tail suspension test.

P < .05), and the CSDS × MPL interaction ($F_{1,36}$ = 8.77, P < .01) (Fig. 3E). Post-hoc analysis showed that a second MPL injection (800 μg/kg) 10 days after the first MPL injection still prevented CSDS-induced decrease in time spent in the interaction zone in the SIT (Fig. 3B–C) and CSDS-induced increases in the immobility time in the TST (Fig. 3D) and FST (Fig. 3E).

Effect of Repeated MPL Injections 10 Days Before Stress Exposure on Depression-Like Behaviors in CSDS Mice

The preventive effect of MPL increased in parallel with an increase in the MPL dosage, which suggested that a repeated injection in a short-term period could prolong the persistence time for the preventive effect of MPL. We compared the effect between the 1× and 4× MPL injections in the herein-used depression model (Fig. 4A). A 2-way ANOVA for the time spent in the interaction zone in the absence of a target in the SIT revealed no significant effects for CSDS exposure ($F_{1,54}$ = .90, P = .35), 4 × MPL injections ($F_{2,54}$ = 1.15, P = .33), and CSDS × MPL interaction ($F_{2,54}$ = .56, P = .58). On the other hand, in the presence of the target, the 2-way ANOVA for the time spent in the interaction zone indicated significant effects for CSDS exposure ($F_{1,54}$ = 16.40, P < .001), 4× MPL injections ($F_{2,54}$ = 4.75, P < .05), and the CSDS × MPL interaction ($F_{2,54}$ = 9.04, P < .001) (Fig. 4B–C). For the TST, the 2-way ANOVA showed significant effects for CSDS exposure ($F_{1,54}$ = 6.00, P < .05), 4× MPL injections ($F_{2,54}$ = 11.75, P < .001), and the CSDS × MPL interaction ($F_{2,54}$ = 16.96, P < .001) (Fig. 4D). For the FST, the 2-way ANOVA showed significant effects for CSDS exposure ($F_{1,54}$ = 7.00, P < .05), 4× MPL injections ($F_{2,54}$ = 11.22, P < .001), and the CSDS × MPL interaction ($F_{2,54}$ = 18.06, P < .001) (Fig. 4E). Unlike the effect of a single MPL injection (post-hoc analysis), the 4× MPL injections (on each day for 4 consecutive days at the dose of 800 μg/kg) prevented the decreased time spent in the interaction zone in the SIT (Fig. 4B–C) and the increased immobility time in the TST (Fig. 4D) and FST (Fig. 4E) in CSDS mice.

Effect of MPL on Enhanced Neuroinflammatory Response in CSDS Mice

Based on our previous reports, innate immune stimulants can prevent chronic stress-induced depression-like behaviors by preventing neuroinflammatory response (Gu et al., 2021), and the MPL may produce similar effects (Fig. 5A). We collected brain tissues from mice treated without or with MPL and/or CSDS immediately after the discontinuation of stress exposure. Regarding the levels of IL-1β mRNA in the hippocampus and prefrontal cortex, 2-way ANOVA revealed significant effects for CSDS exposure (hippocampus: $F_{1,28}$ = 83.90, P < .001; cortex: $F_{1,28}$ = 54.53, P < .001), MPL injection (hippocampus: $F_{1,28}$ = 59.01, P < .001; cortex: $F_{1,28}$ = 44.58, P < .001), and the CSDS × MPL interaction

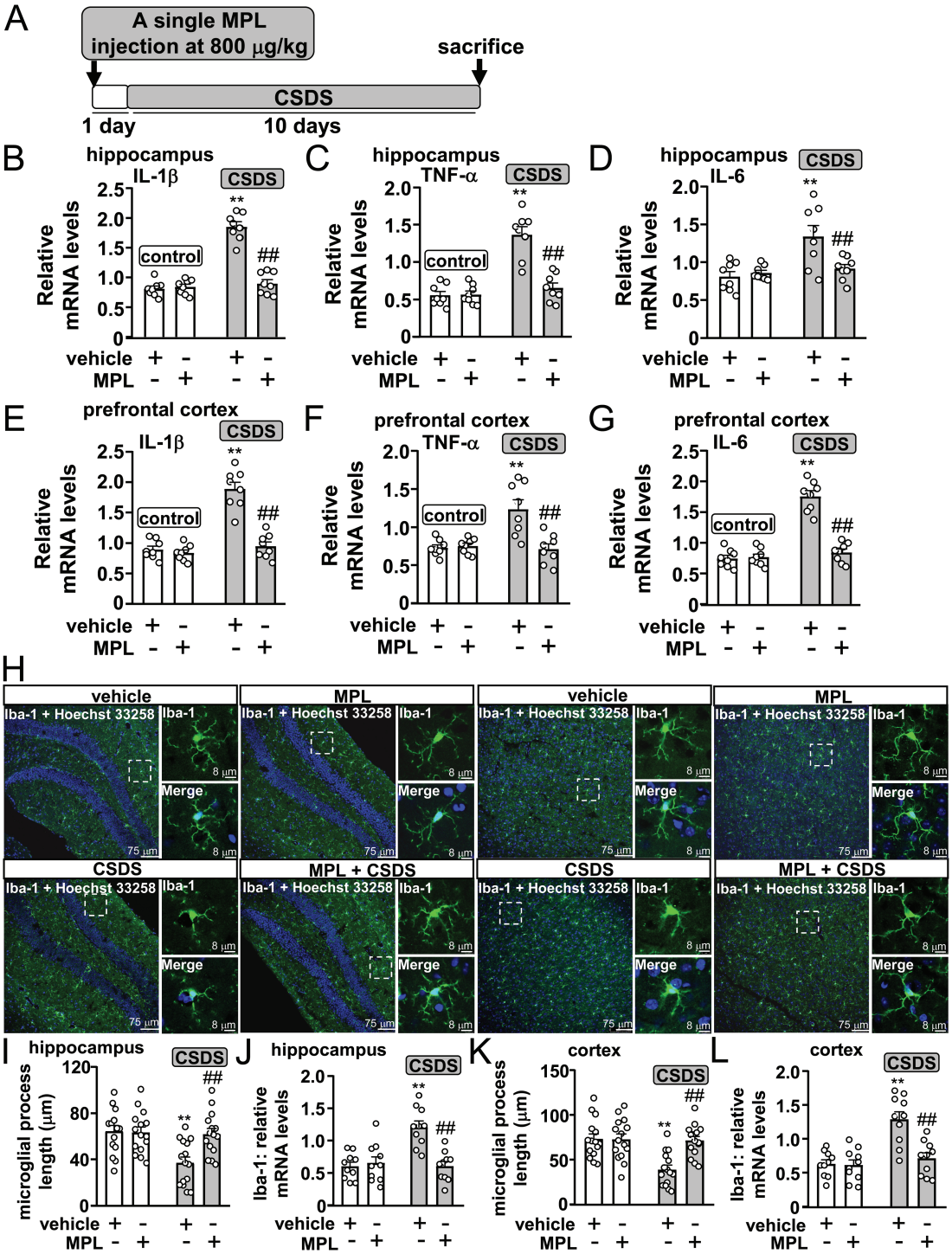

Figure 5. Effect of MPL preconditioning on CSDS-induced neuroinflammatory response. (A) A schematic diagram showing the experimental arrangement for the evaluation of the effect of MPL injection (800 μg/kg) 1 day before stress exposure on CSDS-induced neuroinflammatory responses in the brain. (B–G) Quantitative analysis showing the preventive effect of MPL pre-injection (800 μg/kg) 1 day before stress exposure on CSDS-induced increases in the levels of IL-1β, TNF-α, and IL-6 mRNA in the hippocampus (B–G) and prefrontal cortex (E–G) (n = 8, **P < .01 vs vehicle; ##P < .01 vs vehicle + CSDS). (H) Representative images showing the preventive effect of MPL pre-injection (800 μg/kg) 1 day before stress exposure on CSDS-induced changes in microglial morphology in the hippocampus and prefrontal cortex. Iba-1: microglia; Hoechst 33258: nucleus; scale bars: 8 or 75 μm. (I–L) Quantitative analysis showing the preventive effect of MPL pre-injection (800 μg/kg) 1 day before stress exposure on CSDS-induced shortening of microglial process (hippocampus: I; cortex: K; n = 15) and CSDS-induced increases in the expression levels of Iba-1 mRNA (hippocampus: J; cortex: L; n = 10) (**P < .01 vs vehicle; ##P < .01 vs vehicle + CSDS) in the hippocampus and prefrontal cortex. Data are shown as mean ± SEM. CSDS, chronic social defeat stress; Iba-1, ionized calcium bindingadaptor molecule-1; IL-1β, interleukin-1β; MPL, monophosphoryl lipid A; SEM, standard error of mean; TNF-α, tumor necrosis factor-α.

(hippocampus: $F_{1,28} = 67.76$, $P < .001$; cortex: $F_{1,28} = 34.99$, $P < .001$) (Fig. 5B, E). Regarding the levels of TNF-α mRNA in the hippocampus and prefrontal cortex, the 2-way ANOVA showed significant effects for CSDS exposure (hippocampus: $F_{1,28} = 40.09$, $P < .001$; cortex: $F_{1,28} = 9.90$, $P < .01$), MPL injection (hippocampus: $F_{1,28} = 24.29$, $P < .001$; cortex: $F_{1,28} = 11.53$, $P < .01$), and the CSDS × MPL

interaction (hippocampus: $F_{1,28}=25.69$, $P<.001$; cortex: $F_{1,28}=13.66$, $P<.001$) (Fig. 5C, F). As for the levels of IL-6 mRNA in the hippocampus and prefrontal cortex, the results of 2-way ANOVA demonstrated significant effects for CSDS exposure (hippocampus: $F_{1,28}=11.90$, $P<.01$; cortex: $F_{1,28}=74.53$, $P<.001$), MPL injection (hippocampus: $F_{1,28}=4.84$, $P<.05$; cortex: $F_{1,28}=50.19$, $P<.001$), and the CSDS × MPL interaction (hippocampus: $F_{1,28}=7.75$, $P<.01$; cortex: $F_{1,28}=54.93$, $P<.001$) (Fig. 5D, G). Post-hoc analysis suggested that a single MPL pretreatment 1 day before stress exposure at the dose of 800 μg/kg could prevent CSDS-induced increases in the levels of IL-1β, TNF-α, and IL-6 mRNA in the hippocampus (Fig. 5B–D) and prefrontal cortex (Fig. 5E–G).

Considering that microglial activation can mediate the progression of neuroinflammatory response, we then examined the activation state of microglia by measuring the length of microglial process and the gene expression levels of ionized calcium bindingadaptor molecule-1 (Iba-1) in the brain in mice treated with or without CSDS and/or MPL. Regarding the length of microglial process in the hippocampus and prefrontal cortex, the 2-way ANOVA showed significant effects for CSDS exposure (hippocampus: $F_{1,56}=8.86$, $P<.01$; cortex: $F_{1,56}=11.62$, $P<.01$), MPL injection (hippocampus: $F_{1,56}=6.02$, $P<.05$; cortex: $F_{1,56}=9.39$, $P<.01$), and the CSDS × MPL interaction (hippocampus: $F_{1,56}=7.14$, $P<.01$; cortex: $F_{1,56}=9.69$, $P<.01$) (Fig. 5H, I, K). Regarding the gene expression levels of Iba-1 in the hippocampus and prefrontal cortex, the 2-way ANOVA showed significant effects for CSDS exposure (hippocampus: $F_{1,36}=11.34$, $P<.01$; cortex: $F_{1,36}=20.24$, $P<.001$), MPL injection (hippocampus: $F_{1,36}=11.21$, $P<.01$; cortex: $F_{1,36}=12.45$, $P<.01$), and the CSDS × MPL interaction (hippocampus: $F_{1,36}=15.86$, $P<.001$; cortex: $F_{1,36}=11.11$, $P<.01$) (Fig. 5J, L). Post-hoc analysis showed that a single MPL pretreatment 1 day before stress exposure at the dose of 800 μg/kg prevented CSDS-induced decrease in microglial process length (Fig. 5H, I, K) and CSDS-induced increase in the expression levels of Iba-1 mRNA (Fig. 5J, L) in the hippocampus and prefrontal cortex in mice. Taken together, these results demonstrated that MPL pretreatment prevents CSDS-induced neuroinflammatory responses in the brain.

Innate Immunization Mediates the Preventive Effect of MPL on Depression

Considering that the MPL is known to stimulate the innate immune system, we examined whether the innate immunization mediates the preventive effect of MPL on depression (Fig. 6A,D). The results listed in Fig. B–C indicated that minocycline pretreatment (40 mg/kg) prevented a single MPL injection (800 μg/kg)-induced increase in the blood IL-1β (2-way ANOVA: significant effects for MPL injection [$F_{1,36}=143.00$, $P<.001$], minocycline pretreatment [$F_{1,36}=88.82$, $P<.001$], and the MPL × minocycline interaction [$F_{1,36}=81.88$, $P<.001$]) (Fig. 6B) and IL-6 (2-way ANOVA: significant effects for MPL injection [$F_{1,36}=115.40$, $P<.001$], minocycline pretreatment [$F_{1,36}=60.86$, $P<.001$], and the MPL × minocycline interaction [$F_{1,36}=59.53$, $P<.001$]) (Fig. 6C) levels, suggesting that the herein-used minocycline was effective in the suppression of the endogenous inflammatory response. For behavioral assays in the SIT, 2-way ANOVA for the time in the interaction zone in the absence of the target revealed no significant effects for MPL injection ($F_{1,36}=1.80$, $P=.19$), minocycline pretreatment ($F_{1,36}=1.04$, $P=.31$), and the MPL × minocycline interaction ($F_{1,36}=.002$, $P=.97$) (Fig. 6E). On the other hand, in the presence of the target, the 2-way ANOVA revealed significant effects for MPL injection ($F_{1,36}=4.13$, $P<.05$), minocycline pretreatment ($F_{1,36}=11.83$, $P<.01$), and MPL × minocycline interaction ($F_{1,36}=16.43$, $P<.001$) (Fig. 6F). In the TST, the 2-way ANOVA for the immobility time revealed

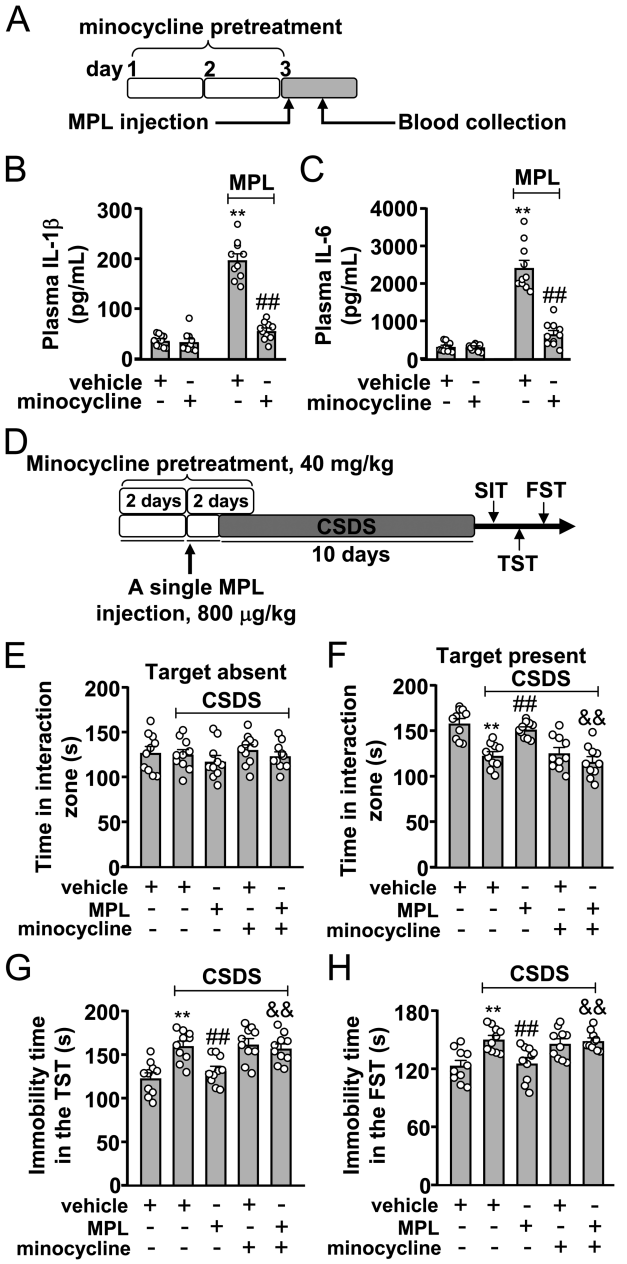

Figure 6. Effect of minocycline on the preventive effect of MPL on CSDS-induced depression-like behaviors. (A) A schematic diagram showing the set-up for minocycline pretreatment and acute MPL injection in stress-naïve mice. (B, C) Quantitative analysis showing the preventive effect of minocycline pretreatment on acute MPL injection (800 μg/kg)-induced increases in serum IL-1β and IL-6 levels (n = 10, $^{**}P<.01$ vs vehicle; $^{\#\#}P<.01$ vs vehicle + MPL). (D) A schematic diagram showing the set-up for minocycline pretreatment, MPL injection, and stress exposure in stress-naïve and CSDS mice. (E–H) Quantitative analysis showing the abrogation effect of minocycline pretreatment (40 mg/kg) on MPL pre-injection (800 μg/kg)-induced prevention of CSDS-induced decrease in the time spent in the interaction zone in the SIT with target absence (E) or presence (F) as well as CSDS-induced increases in the immobility time in TST (G) and FST (H) (n = 10, $^{**}P<.01$ vs vehicle; $^{\#\#}P<.01$ vs vehicle + CSDS; $^{\&\&}P<.01$ vs MPL + CSDS). Data are shown as mean ± SEM. CSDS, chronic social defeat stress; FST, forced swimming test; Iba-1, ionized calcium bindingadaptor molecule-1; IL-1β, interleukin-1β; MPL, monophosphoryl lipid A; SEM, standard error of mean; SIT, social interaction test; TNF-α, tumor necrosis factor-α; TST, tail suspension test.

significant effects for MPL injection ($F_{1,36}=9.29$, $P<.01$), minocycline pretreatment ($F_{1,36}=6.46$, $P<.05$), and the MPL × minocycline interaction ($F_{1,36}=4.58$, $P<.05$) (Fig. 6G. In the FST, the 2-way ANOVA for

the immobility time revealed significant effects for MPL injection ($F_{1,36}=5.91$, $P<.05$), minocycline pretreatment ($F_{1,36}=4.27$, $P<.05$), and the MPL × minocycline interaction ($F_{1,36}=9.32$, $P<.01$) (Fig. 6H). Post-hoc analysis showed that under minocycline pretreatment (40 mg/kg), the single MPL injection (800 μg/kg) failed to prevent CSDS-induced decrease in the time spent in the interaction zone in the SIT (Fig. 6F) and increases in the immobility time in the TST (Fig. 6G) and FTS (Fig. 6H) in the experimental mice. Furthermore, treatment with minocycline alone tended to induce an increase in the time spent in the interaction zone in the SIT (Fig. 6F, $P=.69$) as well as a decrease in the immobility time in TST (Fig. 6G, $P=.79$) and FST (Fig. 6H, $P=.49$) in CSDS mice, although these data did not show a statistical significance.

Innate Immunization Mediates the Preventive Effect of MPL on Neuroinflammatory Response in CSDS Mice

Considering that the prevention of depression through MPL pretreatment is accompanied by a decrease in the neuroinflammatory response, we next investigated whether innate immunization mediates the preventive effect of MPL on the neuroinflammatory response in CSDS mice. As shown in Fig. 7A and D, a 2-way ANOVA for the IL-1β mRNA expression levels in the hippocampus and prefrontal cortex revealed significantly affected MPL injection (hippocampus: $F_{1,28}=13.52$, $P<.001$; cortex: $F_{1,28}=15.41$, $P<.001$), minocycline pretreatment (hippocampus: $F_{1,28}=5.12$, $P<.05$; cortex: $F_{1,28}=10.44$, $P<.01$), and the MPL × minocycline interaction (hippocampus: $F_{1,28}=11.26$, $P<.01$; cortex: $F_{1,28}=23.92$, $P<.001$). For the expression levels of TNF-α mRNA in the hippocampus and prefrontal cortex (Fig. 7B,E), the 2-way ANOVA revealed significant effects for MPL injection (hippocampus: $F_{1,28}=10.71$, $P<.01$; cortex: $F_{1,28}=9.88$, $P<.01$), minocycline pretreatment (hippocampus: $F_{1,28}=7.53$, $P<.05$; cortex: $F_{1,28}=7.89$, $P<.01$), and the MPL × minocycline interaction (hippocampus: $F_{1,28}=13.54$, $P<.001$; cortex: $F_{1,28}=6.49$, $P<.05$). For the expression levels of IL-6 mRNA in the hippocampus and prefrontal cortex (Fig. 7C,F), 2-way ANOVA revealed significant effects for MPL injection (hippocampus: $F_{1,28}=17.11$, $P<.001$; cortex: $F_{1,28}=8.89$, $P<.01$), minocycline pretreatment (hippocampus: $F_{1,28}=6.03$, $P<.05$; cortex: $F_{1,28}=4.74$, $P<.05$), and the MPL × minocycline interaction (hippocampus: $F_{1,28}=6.88$, $P<.05$; cortex: $F_{1,28}=5.14$, $P<.05$). Post-hoc analysis revealed that, under minocycline pretreatment (40 mg/kg), a single MPL injection (800 μg/kg) failed to reverse CSDS-induced increases in the expression levels of IL-1β (hippocampus: Fig. 7A; cortex: Fig. 7D), TNF-α (hippocampus: Fig. 7B; cortex: Fig. 7E), and IL-6 (hippocampus: Fig. 7C; cortex: Fig. 7F) mRNA in the hippocampus and prefrontal cortex. Furthermore, treatment minocycline alone appeared to affect the CSDS-induced changes in the levels of IL-1β (hippocampus: Fig. 7A, $P=.53$; cortex: Fig. 7D, $P=.29$), TNF-α (hippocampus: Fig. 7B, $P=.57$; cortex: Fig. 7E, $P=.88$), and IL-6 (hippocampus: Fig. 7C, $P=.91$; cortex: Fig. 7F, $P=.95$) mRNA in the hippocampus and prefrontal cortex, although no statistical significance was recorded.

We also evaluated the abrogation effect of minocycline on the preventive effect of MPL pre-injection on CSDS-induced neuroinflammatory responses by evaluating the activation state of microglia. Regarding the length of microglial process in the hippocampus and prefrontal cortex, the 2-way ANOVA showed significant effects for MPL injection (hippocampus: $F_{1,56}=9.07$, $P<.01$; cortex: $F_{1,56}=26.10$, $P<.001$), minocycline pretreatment (hippocampus: $F_{1,56}=4.60$, $P<.05$; cortex: $F_{1,56}=6.63$, $P<.05$), and the MPL × minocycline interaction (hippocampus: $F_{1,56}=6.79$, $P<.05$; cortex: $F_{1,56}=8.25$, $P<.01$) (Fig. 7G, H, J). Regarding the expression levels of Iba-1 mRNA in the brain, the 2-way ANOVA showed significant effects for MPL injection (hippocampus: $F_{1,36}=7.83$, $P<.01$; cortex: $F_{1,36}=16.40$, $P<.001$), minocycline pretreatment (hippocampus: $F_{1,36}=6.70$, $P<.05$; cortex: $F_{1,36}=9.33$, $P<.01$), and the MPL × minocycline interaction (hippocampus: $F_{1,36}=5.94$, $P<.05$; cortex: $F_{1,36}=9.06$, $P<.01$) (Fig. 7I, K). Post-hoc analysis revealed that, under minocycline pretreatment (40 mg/kg), the single MPL injection (800 μg/kg) did not prevent CSDS-induced reductions in microglial process length (Fig. 7G, H, J) and CSDS-induced increases in the expression levels of Iba-1 mRNA (Fig. 7I, K) in the hippocampus and prefrontal cortex. These results demonstrated that minocycline pretreatment can abrogate the preventive effect of MPL pre-injection on CSDS-induced neuroinflammatory responses in the brain.

Discussion

In our past studies, the innate immune stimulants such as LPS and M-CSF reportedly prevented the development of depression-like behaviors in chronically stressed mice (Gu et al., 2021; Ji et al., 2021), which suggested that drugs with innate immune-stimulating activities may possess the potential to prevent the occurrence of depression. One of the major contributions of the present study was the confirmation of a preventive effect of MPL—a chemically detoxified lipid A moiety with less undesirable actions and a greater therapeutic window compared with LPS (Elliott, 1998; Chilton et al., 2013)—on chronic stress–induced depression-like behaviors in mice. As MPL is already commercialized as a vaccine adjuvant (Didierlaurent et al., 2009), our findings indicate the possibility of the use of MPL as a vaccine-like drug for the prevention of depression, which may contribute to the reduction of morbidity from depression.

Similar to past findings hinting at the involvement of depression prevention by LPS or M-CSF (Gu et al., 2021; Ji et al., 2021), the preventive effect of MPL was also found to be dependent on its using dosage. No effect of a relatively low dose of 200 μg/kg MPL on CSDS-induced decrease in social interaction and increase in immobility time in the TST and FST was noted in dose-dependent analyses, whereas a relatively high dose of MPL pre-injection (400, 800, or 1600 μg/kg) displayed a preventive effect on CSDS-induced depression-like behaviors. The preventive effect of a single MPL pretreatment on depression in CSDS mice vanished with the extension of the observation time after administering MPL. If the time interval between drug treatment and stress exposure was 10 days, a single MPL injection did not prevent CSDS-induced depression-like behaviors in the mice. These findings present with 2 opinions: first, sufficient dosage of MPL that likely induces a proper activation of the innate immune system, which is required for the preventive effect of MPL on depression; second, the innate immunization effect of a single MPL injection cannot persist to induce a long-term preventive effect; and the disappearance of the preventive effect of MPL on depression suggests limited plasticity for the innate immune system against stress stimulation. These viewpoints are supported by several multiple injection experiments, wherein consecutive 4× MPL injections 10 days before stress exposure were found to successfully prevent decreased social interaction in the SIT and the increased immobility time in the TST and FST in CSDS mice. The 4× injections enabled the MPL to reach a level that could induce a stronger activation of the innate immune system and thereby prolonged the time interval with protective effect between the drug treatment and stress exposure. In other words, after multiple MPL injections, the body may acquire a relatively long-term ability and immune plasticity to protect the brain against stress-induced depression. Future studies should

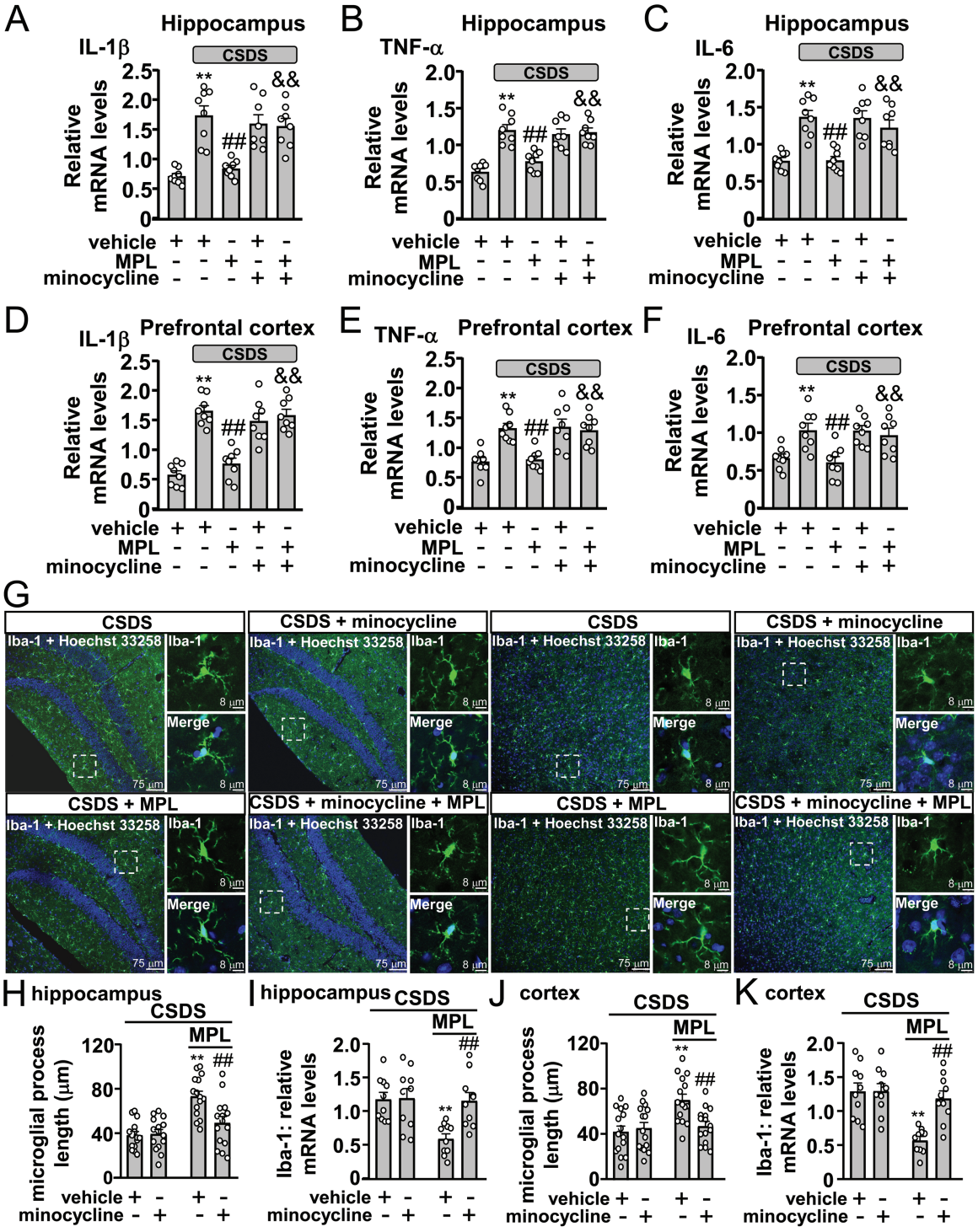

Figure 7. Effect of minocycline on the preventive effect of MPL on CSDS-induced neuroinflammatory response. (A–F) Quantitative analysis showing the abrogation effect of minocycline on the preventive effect of pre-injection (800 μg/kg) on CSDS-induced increases in the levels of IL-1β, TNF-α, and IL-6 mRNA in the hippocampus (A: IL-1β, B: TNF-α, C: IL-6) and prefrontal cortex (D: IL-1β, E: TNF-α, F: IL-6) (n = 8, **P < .01 vs vehicle; ##P < .01 vs vehicle + CSDS; &&P < .01 vs MPL + CSDS). (G) Representative images showing the abrogation effect of minocycline on the preventive effect of pre-injection (800 μg/kg) on CSDS-induced shortening of microglial process in the hippocampus and prefrontal cortex. Iba-1: microglia; Hoechst 33258: nucleus; scale bars: 8 or 75 μm. (H–K) Quantitative analysis showing the abrogation effect of minocycline on the preventive effect of pre-injection (800 μg/kg) on CSDS-induced shortening of microglial process (hippocampus: H; cortex: J; n = 15) and CSDS-induced increases in the expression levels of Iba-1 mRNA (hippocampus: I; cortex: K; n = 10) (**P < .01 vs vehicle + CSDS; ##P < .01 vs MPL + CSDS) in the hippocampus and prefrontal cortex. Data are shown as mean ± SEM. CSDS, chronic social defeat stress; Iba-1, ionized calcium bindingadaptor molecule-1; IL-1β, interleukin-1β; MPL, monophosphoryl lipid A; SEM, standard error of mean; TNF-α, tumor necrosis factor-α.

be conducted to identify the cellular and molecular mechanisms underlying the induction of persistent ability through repeated MPL injections to protect the brain against stress stimulation.

Immune preconditioning is an old but still new concept. It describes a phenomenon in which a brief and moderate innate immune stimulation confers protection against subsequent

stimuli (Larochelle et al., 2015; McDonough and Weinstein, 2020). No preventive effect of MPL on CSDS-induced depression-like behaviors was, however, observed in mice receiving minocycline (an inhibitor of the innate immune system) pretreatment, which suggests that adequate innate immunization is necessary to facilitate the preventive effect of MPL on depression. The innate immune activation is mediated by several different types of immune cells in the body, such as macrophages, microglia, and T cells (Martins-Ferreira et al., 2021; Morvan et al., 2021; Shamaei et al., 2021). The contributions of these cells to the protective effect of innate immune stimulants in different types of pathological models have been revealed in past studies. For example, macrophage preconditioning with a synthetic malaria pigment has been demonstrated to prevent pro-inflammatory cytokine production (Taramelli et al., 2000). Microglial activation has also been confirmed to be essential for the neuroprotective effect of a low dose of LPS preconditioning in traumatic brain injury models and epilepsy models (Mirrione et al., 2010; Chen et al., 2014). Reber et al. (2016) reported that the suppression of the T-cell function could abrogate the behavioral improvement effect of a heat-killed preparation of Mycobacterium vaccae in a psychiatric model induced by social defeat stress. In future studies, we should consider the cellular basis behind the preventive effect of MPL on depression.

Although the monoamine dysfunction remains a focus for explaining the pathogenesis of depression, a variety of newly proposed hypotheses in recent years have attracted increasing attention, which includes the popular neuroinflammation hypothesis (Turner et al., 2020). According to this hypothesis, the overproduction of pro-inflammatory cytokines can mediate the pathogenesis of depression and the suppression of the neuroinflammatory response progression, which is considered to be helpful in the prevention and/or treatment of depression (Yirmiya et al., 2015; Benatti et al., 2016). Our results revealed that (1) a single MPL pre-injection could prevent microglial process shortening (an activation phenotype of microglia) and an abnormal increase in the expression levels of pro-inflammatory cytokine and Iba-1 (a classical maker for microglia) mRNA in the hippocampus and the prefrontal cortex in CSDS mice; and (2) minocycline pretreatment abrogated the preventive effect of MPL on CSDS-induced depression-like behaviors, microglial process shortening, and increase in levels of pro-inflammatory cytokine and Iba-1 mRNA in the brain. These findings suggest a possibility that MPL pretreatment can modulate inflammatory damage after chronic social defeat exposure in mice, possibly by preventing microglial over-activation. This hypothesis is supported by numerous evidence. For example, past studies have shown that the pre-stimulation of the innate immune system by LPS can induce the microglia to transform into an epigenetically regulated immune-suppressive phenotype (Schaafsma et al., 2015). However, it is worth indicating, besides the microglia, peripheral monocytes and macrophages or the other nervous system cells such as astrocytes are also involved in this process as (1) stress stimulation, overactivated peripheral monocytes, and macrophages can migrate to the brain to induce behavioral abnormalities (Wohleb et al., 2013); (2) astrocyte dysfunction have been widely reported to mediate the pathogenesis of depression (Gómez-Galán et al., 2013; Zhang et al., 2020); and (3) adoptive transfer of monocytes isolated from LPS-preconditioned mice into naive mice after cerebral ischemia has been shown to reduce brain injury (Garcia-Bonilla et al., 2018).

Collectively, our results suggest that a single MPL pretreatment renders mice tolerance against social defeat stress–induced depression-like behaviors by preventing microglial activation and neuroinflammation. This ability vanished at a 10-day interval between MPL treatment and stress exposure and could be rescued with a second MPL injection 10 days after the first MPL injection or by a repeated-MPL injection 1 day before stress exposure. As MPL is a potent innate immune stimulant with lesser toxicity than its parent molecule LPS (Ribi, 1984), it has been approved as a vaccine adjuvant for clinical use (Didierlaurent et al., 2009). Our findings thus provide a promising alternative for the development of drugs towards the prevention of depression, especially for patients with high risks of detrimental stress exposure.

Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

Acknowledgments

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