

RESEARCH ARTICLE

Antidepressant-Like Effects of GM1 Ganglioside Involving the BDNF Signaling Cascade in Mice

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Abstract

Background: Depression is a serious psychiatric disorder that easily causes physical impairments and a high suicide rate. Monosialotetrahexosylganglioside is a crucial ganglioside for the central nervous system and has been reported to affect the function of the brain derived neurotrophic factor system. This study is aimed to evaluate whether monosialotetrahexosylganglioside has antidepressant-like effects.

Methods: Antidepressant-like effects of monosialotetrahexosylganglioside were assessed in the chronic social defeat stress model of depression, and various behavioral tests were performed. Changes in the brain derived neurotrophic factor signaling pathway after chronic social defeat stress and monosialotetrahexosylganglioside treatment were also investigated. A tryptophan hydroxylase inhibitor and brain derived neurotrophic factor signaling inhibitors were used to determine the antidepressant mechanisms of monosialotetrahexosylganglioside.

Results: Monosialotetrahexosylganglioside administration significantly reversed the chronic social defeat stress-induced reduction of sucrose preference and social interaction in mice and also prevented the increased immobility time in the forced swim test and tail suspension test. In addition, monosialotetrahexosylganglioside completely ameliorated the stress-induced dysfunction of brain derived neurotrophic factor signaling cascade in the hippocampus and medial prefrontal cortex, 2 regions closely involved in the pathophysiology of depression. Furthermore, the usage of brain derived neurotrophic factor signaling cascade inhibitors, K252a and anti-brain derived neurotrophic factor antibody, each abolished the antidepressant-like effects of monosialotetrahexosylganglioside, while the usage of a serotonin system inhibitor did not.

Conclusions: Taken together, our findings suggest that monosialotetrahexosylganglioside indeed has antidepressant-like effects, and these effects were mediated through the activation of brain derived neurotrophic factor signaling cascade.

Keywords: depression, chronic social defeat stress, hippocampus, medial prefrontal cortex

Introduction

Depression is a common illness with potentially life-threatening emotional and behavioral symptoms, and this kind of neuropsychiatric disorder will become the second-most burdensome disease in the world according to the World Health Organization (Zonana and Gorman, 2005; Vicente et al., 2006; Olesen et al., 2012). The monoamine hypothesis of depression postulates that insufficient serotonergic and noradrenergic transmission in the brain accounts for many or most symptoms of depression (Chopra et al., 2011; Prins et al., 2011). However, many patients are drug refractory or experience intolerable side effects from antidepressants regulating monoaminergic neurotransmission (McGrath et al., 2006). Moreover, these drugs all show delayed onset of action and unpredictable efficacy in individual patients (McGrath et al., 2006), prolonging the search for more effective agents with fewer side effects.

Brain derived neurotrophic factor (BDNF), a member of the neurotrophin family of growth factors, is distributed throughout the central nervous system (Hofer et al., 1990; Conner et al., 1997). BDNF induces the phosphorylation and activation of cAMP response element-binding protein (CREB) by combining the tyrosine kinase B (TrkB) receptor and then activating the MAPK-ERK and PI3K-AKT signaling pathways, 2 key downstream signaling pathways of BDNF (Shaywitz and Greenberg, 1999; Lim et al., 2008). Previous studies have already demonstrated that depression is associated with decreased BDNF signaling in the hippocampus and medial prefrontal cortex (mPFC), 2 regions closely implicated in the pathophysiology of depression, and this decrease can be normalized by antidepressant treatment (Nibuya et al., 1996; Thome et al., 2000; Blendy, 2006; Gronli et al., 2006; Castren and Rantamaki, 2010; Razzoli et al., 2011; Zhou et al., 2014). Animal studies found that administration of BDNF into the hippocampus produced antidepressant-like effects in models of depression (Shirayama et al., 2002; Hoshaw et al., 2005). Interestingly, depression is also accompanied with enhanced BDNF expression and CREB activation in the nucleus accumbens (NAc), another region involved in the pathophysiology of depression, while blockade of BDNF function in the NAc exhibits an antidepressant-like phenotype (Shirayama and Chaki, 2006).

Gangliosides are sialic acid-containing glycosphingolipids synthesized by neuronal and glial cells, and are abundant in the brain (Svennerholm, 1956; Suzuki, 1965; Derry and Wolfe, 1967). Gangliosides are crucial for brain development and plasticity (Ferrari and Greene, 1998; Hadjiconstantinou and Neff, 1998; Lim et al., 2011). Previous studies have shown that accumulation of gangliosides in the brain leads to neurite outgrowth, while lack of gangliosides causes neurodegeneration (Purpura and Suzuki, 1976; Sparrow et al., 1984; Yamashita et al., 2005). Moreover, exogenously administered gangliosides have been shown to affect the survival of different neuronal types, including glutamatergic, dopaminergic, and cholinergic neurons (Sofroniew et al., 1986; Favaron et al., 1988; Hadjiconstantinou and Neff, 1988; Schneider et al., 1992). Experimental data have shown that one ganglioside, monosialotetrahexosylganglioside (GM1), exhibit properties similar to neurotrophins (Mocchetti, 2005), and several reports found that the neurotrophic activity of GM1 derives from its ability to activate Trks, the family of high-affinity neurotrophin receptors (Mutoh et al., 1995; Ferrari and Greene, 1996; Duchemin et al., 2002; Rabin et al., 2002; Duchemin et al., 2008). GM1 also induces the synthesis and release of BDNF and in turn activates an autocrine loop (Lim et al., 2011; Valdomero et al., 2015).

Since GM1 has important physiological properties such as activating neurotrophin receptors and promoting BDNF release, here we hypothesize that GM1 may have antidepressant-like

effects. In this study, we firstly assessed the antidepressant-like effects of GM1 using the chronic social defeat stress (CSDS) model of depression and explored the molecular mechanisms responsible for these effects.

Materials and Methods

Animals

Adult male C57BL/6J mice (6–8 weeks old) were obtained from the Experimental Animal Center of Medical College, Nantong University. Before use, mice were housed 5 per cage under standard conditions (12-h-light/-dark cycle; lights on from 7:00 AM to 7:00 PM; $24 \pm 1^\circ\text{C}$ ambient temperature; $55 \pm 10\%$ relative humidity) for 1 week with free access to food and water. Behavioral experiments were carried out during the light phase. The experimental procedures involving animals and their care were conducted in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Materials

Monosialotetrahexosylganglioside (GM1, purity >95%), fluoxetine and p-chlorophenylalanine methyl ester (PCPA) were purchased from Sigma (St. Louis, MO). K252a was purchased from Alomone Laboratories (Jerusalem, Israel). Chicken anti-BDNF neutralizing anti-body (G1641) and chicken IgY control Ig (G1161) were purchased from Promega (Madison, WI). The repeated drug treatment of mice was performed once daily between 9:30 and 10:30 AM. The dosages of GM1, fluoxetine, K252a, anti-BDNF neutralizing antibody, and PCPA were chosen based on previous reports (Jiang et al., 2015a; Valdomero et al., 2015). GM1, fluoxetine, K252a, and PCPA were administered i.p. in a volume of 10 mL/kg. Chicken anti-BDNF neutralizing antibody and chicken IgY control Ig were intracerebroventricularly infused.

CSDS

Social defeat and avoidance testing were performed according to previously reported methods (Berton et al., 2006; Donahue et al., 2014). C57BL/6 mice were exposed to different CD1 aggressor mice each day for 10 minutes for a total of 10 days. After the 10 minutes of contact, C57BL/6 mice were separated from the aggressors by plastic dividers with holes, where they were exposed to chronic stress in the form of threat for the next 24 hours. Nondefeated control mice were handled daily. Twenty-four hours after the last session, defeated C57BL/6 mice were subjected to the social interaction test and sorted into either susceptible or unsusceptible phenotype based on interaction scores. All the CSDS-unsusceptible mice were removed. Then the CSDS-susceptible mice were housed individually and received daily injections of vehicle or tested compounds for another 14 days. After that, various behavioral tests were performed. All the mice were first anaesthetized using 0.5% pentobarbital sodium (10 mL/kg) and then sacrificed by cervical dislocation.

Additional experimental procedures are available online in the supplemental Information.

Data Analysis

All analyses were performed using SPSS 13.0 software (SPSS Inc) and data are presented as the mean \pm SEM. Differences between

mean values were evaluated using 1-way ANOVA (posthoc LSD test) or 2-way ANOVA (posthoc Bonferroni's test), as appropriate. $P < .05$ was considered statistically significant.

Results

Chronic GM1 Treatment Restores the CSDS-Induced Depressive-Like Symptoms

We firstly considered the possibility that GM1 could reverse depression in an animal model of depression using a chronic social defeat stress protocol that mimics many symptoms of depression in humans (Berton et al., 2006). After a brief (10 minutes) daily exposure to a highly aggressive resident mouse for 10 days, CSDS-susceptible mice were selected and received 14-day treatment of GM1/fluoxetine/vehicle, with behavioral tests then performed. For the social interaction test, ANOVA analysis revealed a significant interaction [$F(3, 88) = 61.437, P < .01$] with significant effects for CSDS [$F(1, 88) = 56.319, P < .01$] and drug treatment [$F(3, 88) = 14.895, P < .01$]. In the absence of an aggressor, all mice spent similar amounts of time in the interaction zone. Compared with nonstressed control mice, CSD-susceptible mice spent about 70% less time in the interaction zone when an aggressor was introduced into the cage ($n = 12, P < .01$ vs control) (Figure 1C). The 14-day treatment of fluoxetine reversed this avoidance behavior, increasing the interaction time such that it was close to that of control mice ($n = 12, P < .01$ vs CSDS susceptible + vehicle; Figure 1C), in agreement with previous reports (Tsankova et al., 2006). Interestingly, while GM1 treatment had no significant effects in control animals, 14-day treatment of GM1 significantly increased the interaction time of CSDS susceptible mice relative to control mice ($n = 12, P < .01$ vs CSDS susceptible + vehicle; Figure 1C), similar to fluoxetine. Further analysis revealed that the interaction time was increased by $126.9 \pm 14.4\%$ and $169.2 \pm 11.3\%$ for 15 mg/kg and 30 mg/kg GM1, respectively.

We then performed the sucrose preference test. Data are summarized in Figure 1D, and 2-way ANOVA also indicated a significant interaction [$F(3, 88) = 22.034, P < .01$] with significant effects for CSDS [$F(1, 88) = 34.509, P < .01$] and drug treatment [$F(3, 88) = 11.163, P < .01$]. CSDS induced a $58.7 \pm 6.2\%$ decrease in sucrose preference, compared with control group ($n = 12, P < .01$ vs control). While GM1 produced no significant effects in naive mice, 14-day treatment with GM1 in CSDS-susceptible mice caused a robust increase of sucrose intake in animals also resulting in an antidepressant-like effect ($n = 12, P < .01$ vs CSDS susceptible + vehicle) (Figure 1D). Further analysis showed that sucrose intake was increased by $64.5 \pm 7.1\%$ and $109.7 \pm 9.6\%$ with administration of 15 mg/kg and 30 mg/kg GM1, respectively.

Additionally, the forced swim test (FST) and tail suspension test (TST), 2 widely used behavioral tests for assessing potential antidepressant-like effects (Cryan and Holmes, 2005; Cryan and Slattery, 2007), were performed. The FST and TST data are summarized in Figures 1E and F, respectively. For the FST data, ANOVA analysis indicated a significant interaction [$F(3, 88) = 70.664, P < .01$] with significant effects for CSDS [$F(1, 88) = 56.841, P < .01$] and drug treatment [$F(3, 88) = 21.828, P < .01$]. For the TST data, ANOVA also revealed a significant interaction [$F(3, 88) = 64.266, P < .01$] with significant effects for CSDS [$F(1, 88) = 54.772, P < .01$] and drug treatment [$F(3, 88) = 18.856, P < .01$]. CSDS stress robustly increased the immobility time of susceptible mice in the FST and TST compared with control group ($n = 12, P < .01$ vs control), while these changes were fully reversed by 30 mg/kg GM1 administration ($n = 12, P < .01$ vs CSDS susceptible + vehicle), similar to fluoxetine. Moreover, it was

found that GM1 treatment also reduced the immobility duration of naive mice in the FST and TST tests ($n = 12, P < .01$ vs control).

To exclude the possible effects of GM1 on spontaneous locomotor activity which may contribute to immobility in the FST and TST (Bourin et al., 2001), naive mice and CSDS susceptible mice were administered GM1, and then exposed to the open-field test. ANOVA revealed no significant effects for either CSDS [$F(1, 88) = 1.074, P = .397$] or drug treatment [$F(3, 88) = 0.816, P = .397$], and there was no significant differences found in the number of squares an animal crossed in either the peripheral area or central area between all groups ($n = 12$) (supplemental Figure 1). Together, these results suggest that GM1 is able to reverse the CSDS-induced behavioral symptoms.

GM1 Treatment Reverses the CSDS-Induced Decrease of BDNF Signaling Pathway in the Hippocampus and mPFC

The BDNF-ERK/AKT-CREB signaling cascade plays an important role in the pathophysiology of depression (Gourley et al., 2008; Lee and Son, 2009; Castren and Rantamaki, 2010), and GM1 has been reported to possess neurotrophic activity (Mutoh et al., 1995; Rabin et al., 2002; Lim et al., 2011). Therefore, we performed western-blot analysis to measure the levels of BDNF signaling pathway proteins in the hippocampus and mPFC following CSDS and GM1 treatment. The mature BDNF protein level is expressed as a ratio of the expression of mature BDNF to β -actin. As shown in Figure 2A, ANOVA indicated a significant interaction [$F(3, 22) = 35.659, P < .01$] with significant effects for CSDS [$F(1, 22) = 97.548, P < .01$] and drug treatment [$F(3, 22) = 25.155, P < .01$], CSDS robustly reduced the mature BDNF expression in the hippocampus ($n = 6, P < .01$ vs control), and 14-day treatment of GM1 increased the mature BDNF protein level by $148.1 \pm 13.5\%$ and $185.2 \pm 9.8\%$ at a dosage of 15 mg/kg and 30 mg/kg ($n = 6, P < .01$ vs CSDS susceptible + vehicle), respectively. These results are similar to those of 20 mg/kg fluoxetine. Similarly, as shown in Figure 2B, ANOVA revealed a significant interaction [$F(3, 22) = 28.769, P < .01$] with significant effects for CSDS [$F(1, 22) = 92.445, P < .01$] and drug treatment [$F(3, 22) = 19.618, P < .01$], CSDS also decreased the mature BDNF expression in the mPFC ($n = 6, P < .01$ vs control; Figure 2B), and chronic GM1 treatment enhanced the mature BDNF protein level by $152.7 \pm 10.9\%$ and $200 \pm 14.6\%$ at a dosage of 15 mg/kg and 30 mg/kg ($n = 6, P < .01$ vs CSDS susceptible + vehicle; Figure 2B), respectively. In addition, GM1 administration promoted the mature BDNF expression in the hippocampus [ANOVA: $F(3, 20) = 29.569, P < .01$] and mPFC [ANOVA: $F(3, 20) = 34.206, P < .01$] of naive mice ($n = 6, P < .01$ vs control) (supplemental Figure 2), consistent with previous findings (Pitto et al., 1998; Duchemin et al., 2002; Lim et al., 2011).

Furthermore, the activated (and phosphorylated) forms of TrkB (pTrkB), ERK1/2 (pERK1/2), AKT (pAKT), and CREB (pCREB) that have been linked to the activation of BDNF signaling cascade were examined in the hippocampus and mPFC (Lim et al., 2008). As shown in Figure 2A, chronic GM1 treatment significantly reversed the CSDS-induced decrease in hippocampal pTrkB [ANOVA: CSDS, $F(1, 22) = 103.187, P < .01$; drug treatment, $F(3, 22) = 26.348, P < .01$; interaction, $F(3, 22) = 35.971, P < .01$], pERK1/2 [ANOVA: CSDS, $F(1, 22) = 110.764, P < .01$; drug treatment, $F(3, 22) = 28.894, P < .01$; interaction, $F(3, 22) = 39.361, P < .01$], pAKT [ANOVA: CSDS, $F(1, 22) = 138.711, P < .01$; drug treatment, $F(3, 22) = 33.605, P < .01$; interaction, $F(3, 22) = 44.472, P < .01$], and pCREB [ANOVA: CSDS, $F(1, 22) = 99.759, P < .01$; drug treatment, $F(3, 22) = 23.373, P < .01$; interaction, $F(3, 22) = 32.841, P < .01$] expression ($n = 6, P < .01$ vs CSDS susceptible + vehicle).

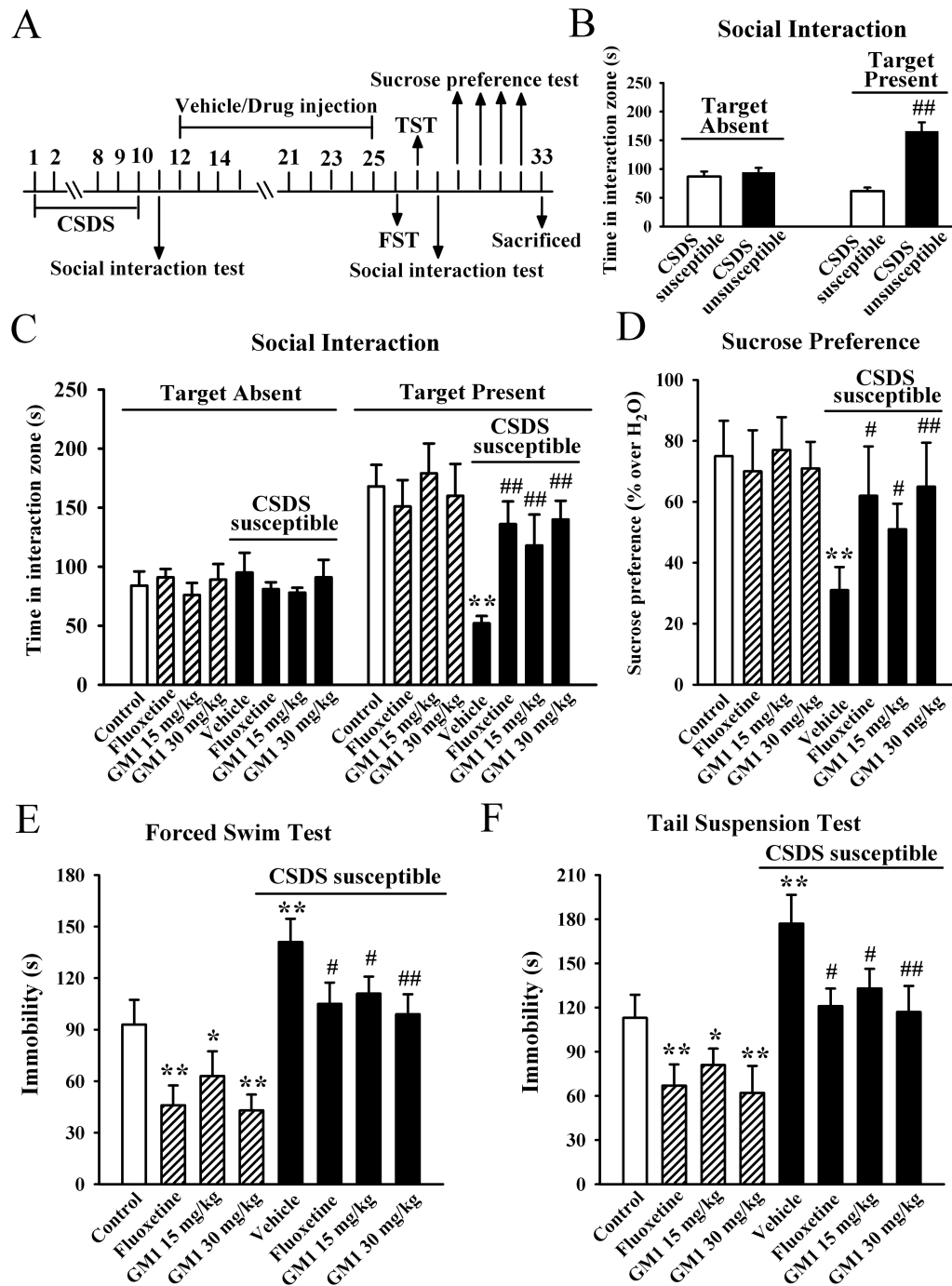


Figure 1. Monosialotetrahexosylganglioside (GM1) produces robust antidepressant-like effects in the chronic social defeat stress (CSDS) model of depression. (A) Schematic timeline of the experimental procedure. A total of 109 C57BL/6j mice were used in this experiment with 61 CSDS-stressed mice and 48 nonstressed mice. After 10 days of CSDS, nonstressed mice and CSDS-susceptible mice received daily injection of vehicle (0.9% saline), fluoxetine (20 mg/kg), or GM1 (15, 30 mg/kg) for another 14 days, after which behavioral tests were conducted. (B) The social interaction results for CSDS-susceptible mice ($n = 48$) and unsusceptible mice ($n = 13$) in this experiment. (C) The antidepressant-like effects of GM1 in the social interaction test. CSDS-susceptible + GM1 mice spent significantly more time engaged in social interaction than CSDS-susceptible + vehicle mice ($n = 12$). (D) GM1 treatment reversed the decrease in sucrose preference induced by CSDS. CSDS-susceptible + GM1 mice displayed higher sucrose preference than CSDS-susceptible + vehicle mice ($n = 12$). (E) The antidepressant-like effects of GM1 in the forced swim test (FST). CSDS-susceptible + GM1 mice displayed significantly lower immobility time than CSDS-susceptible + vehicle mice in the FST ($n = 12$). (F) The antidepressant-like effects of GM1 in the tail suspension test (TST). CSDS-susceptible + GM1 mice displayed significantly lower immobility time than CSDS-susceptible + vehicle mice in the TST ($n = 12$). Data are expressed as the mean \pm SEM; * $P < .05$, ** $P < .01$ vs control; # $P < .05$, ## $P < .01$ vs CSDS-susceptible/CSDS-susceptible + vehicle. Comparison was made by 2-way ANOVA followed by posthoc Bonferroni's test.

Similarly, the decreased pTrkB [ANOVA: CSDS, $F(1, 22) = 118.655$, $P < .01$; drug treatment, $F(3, 22) = 30.226$, $P < .01$; interaction, $F(3, 22) = 41.583$, $P < .01$], pERK1/2 [ANOVA: CSDS, $F(1, 22) = 143.422$,

$P < .01$; drug treatment, $F(3, 22) = 34.597$, $P < .01$; interaction, $F(3, 22) = 46.472$, $P < .01$], pAKT [ANOVA: CSDS, $F(1, 22) = 104.433$, $P < .01$; drug treatment, $F(3, 22) = 26.166$, $P < .01$; interaction, $F(3,$

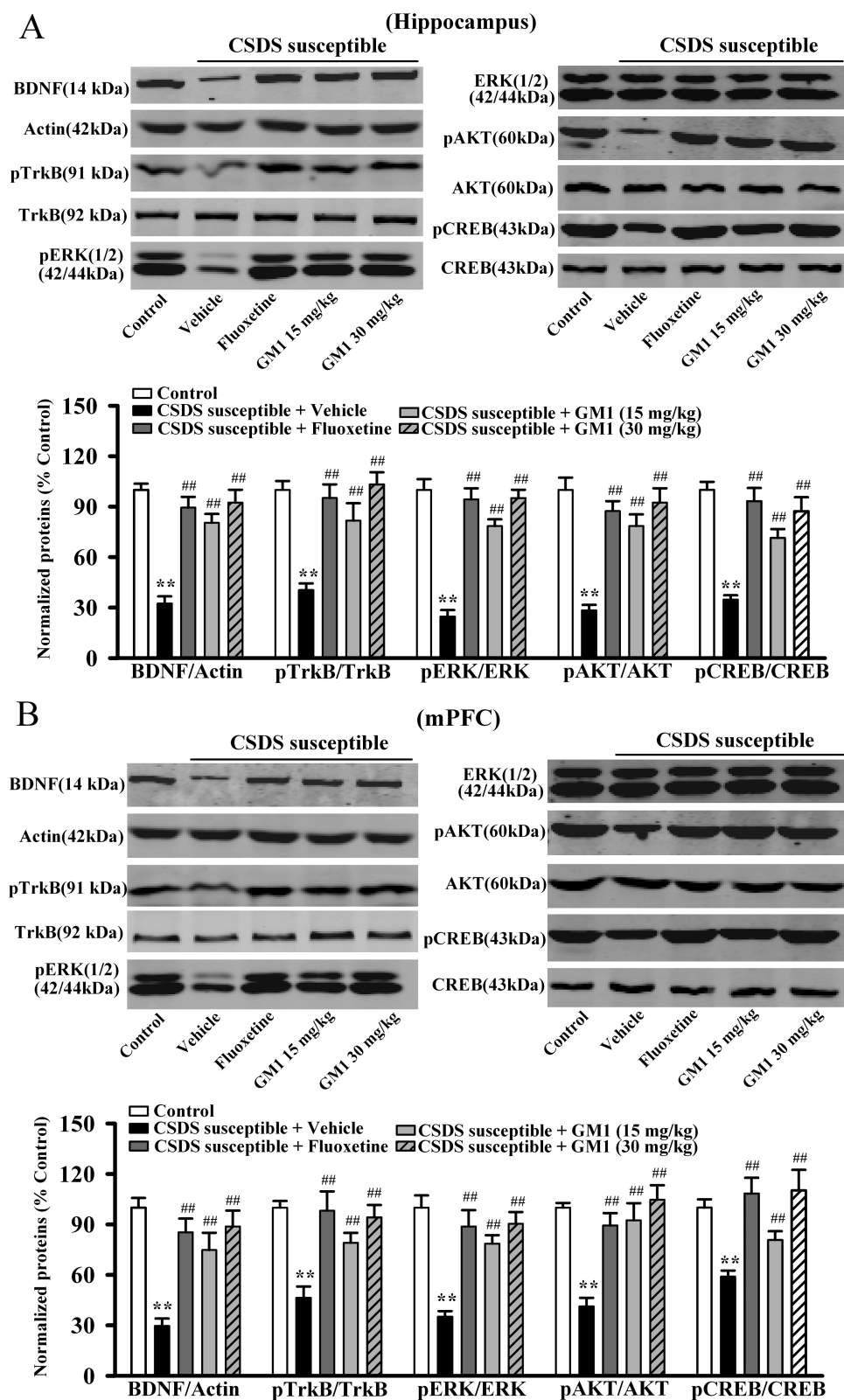


Figure 2. Monosialotetrahexosylganglioside (GM1) treatment reverses the chronic social defeat stress (CSDS)-induced decrease of brain derived neurotrophic factor (BDNF) signaling pathway in the hippocampus and medial prefrontal cortex (mPFC). (A) Western blot results showed that CSDS-susceptible + GM1 mice displayed significantly higher BDNF, pTrkB, pERK1/2, pAKT, and pCREB expression in the hippocampus than CSDS-susceptible + vehicle mice (n=6). (B) Parallel to the hippocampus data, CSDS-susceptible + GM1 mice also had significantly more BDNF, pTrkB, pERK1/2, pAKT, and pCREB expression in the mPFC compared with CSDS-susceptible + vehicle mice (n = 6). Data are expressed as the mean \pm SEM; **P < .01 vs control; ##P < .01 vs CSDS susceptible + vehicle. Comparison was made by 2-way ANOVA followed by posthoc Bonferroni's test.

22) = 35.659, $P < .01$], and pCREB [ANOVA: CSDS, $F(1, 22) = 97.228$, $P < .01$; drug treatment, $F(3, 22) = 22.863$, $P < .01$; interaction, $F(3, 22) = 31.779$, $P < .01$] levels in the mPFC of CSDS-susceptible mice were also restored by GM1 exposure ($n = 6$, $P < .01$ vs CSDS susceptible + vehicle) (Figure 2B). Moreover, GM1 administration also enhanced the pTrkB, pERK1/2, pAKT, and pCREB expression in the hippocampus [ANOVA: pTrkB, $F(3, 20) = 39.344$, $P < .01$; pERK1/2, $F(3, 20) = 42.507$, $P < .01$; pAKT, $F(3, 20) = 26.488$, $P < .01$; pCREB, $F(3, 20) = 33.637$, $P < .01$] and mPFC [ANOVA: pTrkB, $F(3, 20) = 20.491$, $P < .01$; pERK1/2, $F(3, 20) = 16.524$, $P < .01$; pAKT, $F(3, 20) = 15.884$, $P < .01$; pCREB, $F(3, 20) = 19.775$, $P < .01$] of naive mice ($n = 6$, $P < .01$ vs control) (supplemental Figure 2). By contrast, the total ERK1/2, AKT, and CREB levels were unchanged among all experimental groups.

We also examined the BDNF system in NAc following CSDS and GM1 treatment. As shown in supplemental Figure 3, ANOVA indicated significant effects for CSDS [$F(1, 30) = 80.223$, $P < .01$] and GM1 [$F(2, 30) = 19.856$, $P < .01$] but with no interaction [$F(2, 30) = 0.935$, $P = .0274$]. While GM1 administration promoted the levels of BDNF and pCREB in the NAc of naive mice, consistent with Valdomero et al. (2015), GM1 had no effects on the enhanced expression of accumbal BDNF and pCREB in CSDS susceptible mice ($n = 6$). Collectively, the GM1-induced effects on the BDNF signaling cascade in hippocampus and mPFC may be involved in its antidepressant-like effects.

GM1 Produced Antidepressant-Like Effects through the BDNF Signaling Pathway

To further determine whether BDNF system is necessary for the effects of GM1, K252a was applied as a potent pharmacological inhibitor of the BDNF receptor TrkB (Tapley et al., 1992; Yan et al., 2010). CSDS-susceptible mice were co-treated with GM1 (30 mg/kg) and K252a (25 μ g/kg) for 14 days, and behavioral tests were then performed. Data are summarized in Figure 3. Co-treatment of K252a with GM1 significantly blocked the antidepressant-like effects of GM1 in the social interaction test [ANOVA: GM1, $F(1, 40) = 55.421$, $P < .01$; K252a, $F(1, 40) = 81.879$, $P < .01$; interaction, $F(1, 40) = 47.438$, $P < .01$; $n = 11$, Figure 3C], sucrose preference test [ANOVA: GM1, $F(1, 40) = 46.406$, $P < .01$; K252a, $F(1, 40) = 70.182$, $P < .01$; interaction, $F(1, 40) = 44.636$, $P < .01$; $n = 11$, Figure 3D], FST test [ANOVA: GM1, $F(1, 40) = 48.707$, $P < .01$; K252a, $F(1, 40) = 71.588$, $P < .01$; interaction, $F(1, 40) = 42.273$, $P < .01$; $n = 11$, Figure 3E], and TST test [ANOVA: GM1, $F(1, 40) = 59.476$, $P < .01$; K252a, $F(1, 40) = 86.101$, $P < .01$; interaction, $F(1, 40) = 52.772$, $P < .01$; $n = 11$] (Figure 3F). Similarly, K252a injection prevented the effects of GM1 on BDNF signaling pathway in depressed mice, as the mature BDNF, pTrkB, pERK1/2, pAKT, and pCREB levels in the hippocampus and mPFC of CSDS-susceptible + GM1 + K252a mice were significantly lower than that of CSDS-susceptible + GM1 mice, respectively (Figure 4). Moreover, naive mice were also co-treated with GM1 (30 mg/kg) and K252a (25 μ g/kg) for 14 days, and behavioral tests showed that while K252a treatment produced no effects on the social interaction ($n = 11$, supplemental Figure 4A) and sucrose preference ($n = 11$, supplemental Figure 4B) of animals, it blocked the GM1-reduced immobility of naive mice in the FST [ANOVA: GM1, $F(1, 40) = 37.346$, $P < .01$; K252a, $F(1, 40) = 72.851$, $P < .01$; interaction, $F(1, 40) = 32.906$, $P < .01$; $n = 11$, Figure S4C] and TST [ANOVA: GM1, $F(1, 39) = 47.362$, $P < .01$; K252a, $F(1, 39) = 84.723$, $P < .01$; interaction, $F(1, 39) = 42.477$, $P < .01$; $n = 10-11$] (Figure supplemental 4D). Further western blotting results showed that K252a also prevented the effects of GM1 on BDNF signaling pathway in naive mice ($n = 6$) (supplemental Figure 5).

In a parallel series, we used an anti-BDNF antibody to specifically block the BDNF system, as utilized previously (Jiang et al., 2015a; Jiang et al., 2015b). CSDS-susceptible mice were co-treated with GM1 (30 mg/kg) and anti-BDNF antibody (20 μ g/mL) for 14 days, and behavioral tests were then performed. Data are summarized in Figure 5. Anti-BDNF infusion fully abolished the antidepressant-like effects of GM1 in the social interaction test [ANOVA: GM1, $F(1, 38) = 79.366$, $P < .01$; Anti-BDNF, $F(1, 38) = 97.451$, $P < .01$; interaction, $F(1, 38) = 70.115$, $P < .01$; $n = 10-11$, Figure 5C], sucrose preference test [ANOVA: GM1, $F(1, 38) = 60.268$, $P < .01$; Anti-BDNF, $F(1, 38) = 81.603$, $P < .01$; interaction, $F(1, 38) = 56.121$, $P < .01$; $n = 10-11$, Figure 5D], FST test [ANOVA: GM1, $F(1, 38) = 60.448$, $P < .01$; Anti-BDNF, $F(1, 38) = 78.927$, $P < .01$; interaction, $F(1, 38) = 54.879$, $P < .01$; $n = 10-11$, Figure 5E] and TST test [ANOVA: GM1, $F(1, 38) = 64.629$, $P < .01$; Anti-BDNF, $F(1, 38) = 85.025$, $P < .01$; interaction, $F(1, 38) = 58.864$, $P < .01$; $n = 10-11$] (Figure 5F). Furthermore, parallel to the behavioral data, anti-BDNF infusion also blocked the GM1-induced effects on BDNF signaling pathway. We found that the mature BDNF, pTrkB, pERK1/2, pAKT, and pCREB levels in both the hippocampus and mPFC regions of CSDS-susceptible + GM1 + anti-BDNF mice were significantly lower than that of CSDS-susceptible + GM1 mice, respectively ($n = 5$) (Figure 6). Thus, these results indicate that the antidepressant-like effects of GM1 require BDNF system.

Serotonin Depletion Does Not Alter the Antidepressant-Like Effects of GM1

Selective serotonin reuptake inhibitors are the most widely used antidepressants; therefore we wanted to test whether the GM1-induced antidepressant-like effects depend on serotonin system. The tryptophan hydroxylase inhibitor PCPA was used to deplete serotonin (Heurteaux et al., 2006; Coryell et al., 2009). CSDS-susceptible mice were co-treated with GM1 (30 mg/kg) and PCPA (300 mg/kg) for 14 days, and behavioral tests were then performed. Data are summarized in supplemental Figure 6. It was found that PCPA co-treatment did not block the antidepressant-like effects of GM1 in the social interaction test [ANOVA: GM1, $F(1, 39) = 67.213$, $P < .01$; PCPA, $F(1, 39) = 0.956$, $P = .0257$; $n = 10-11$, supplemental Figure 6C], sucrose preference test [ANOVA: GM1, $F(1, 39) = 49.677$, $P < .01$; PCPA, $F(1, 39) = 1.348$, $P = .141$; $n = 10-11$, supplemental Figure 6D], FST test [ANOVA: GM1, $F(1, 39) = 53.014$, $P < .01$; PCPA, $F(1, 39) = 1.088$, $P = .179$; $n = 10-11$, supplemental Figure 6E] and TST test [ANOVA: GM1, $F(1, 38) = 64.296$, $P < .01$; PCPA, $F(1, 38) = 0.951$, $P = .261$; $n = 10-11$, supplemental Figure 6F], while PCPA co-treatment significantly prevented the effects of fluoxetine.

Discussion

The major findings of this study are as follows. First, GM1 produces significant antidepressant-like effects in the CSDS model of depression, confirmed by different behavioral tests, including the social interaction, sucrose preference, FST, and TST tests. Second, the antidepressant-like effects of GM1 require BDNF signaling pathway, since these effects were blocked by selective inhibition of BDNF system in the brain. Together, these data indicate that GM1 could be developed as a novel antidepressant.

GM1 has several important physiological properties such as impacting neuronal plasticity, releasing neurotrophins, and repairing damaged neurons in the brain (Mocchetti, 2005; Lim et al., 2011; Valdomero et al., 2015; Zhang et al., 2015). In this study, the most important reason for assuming that GM1 may possess antidepressant-like effects came from several reports

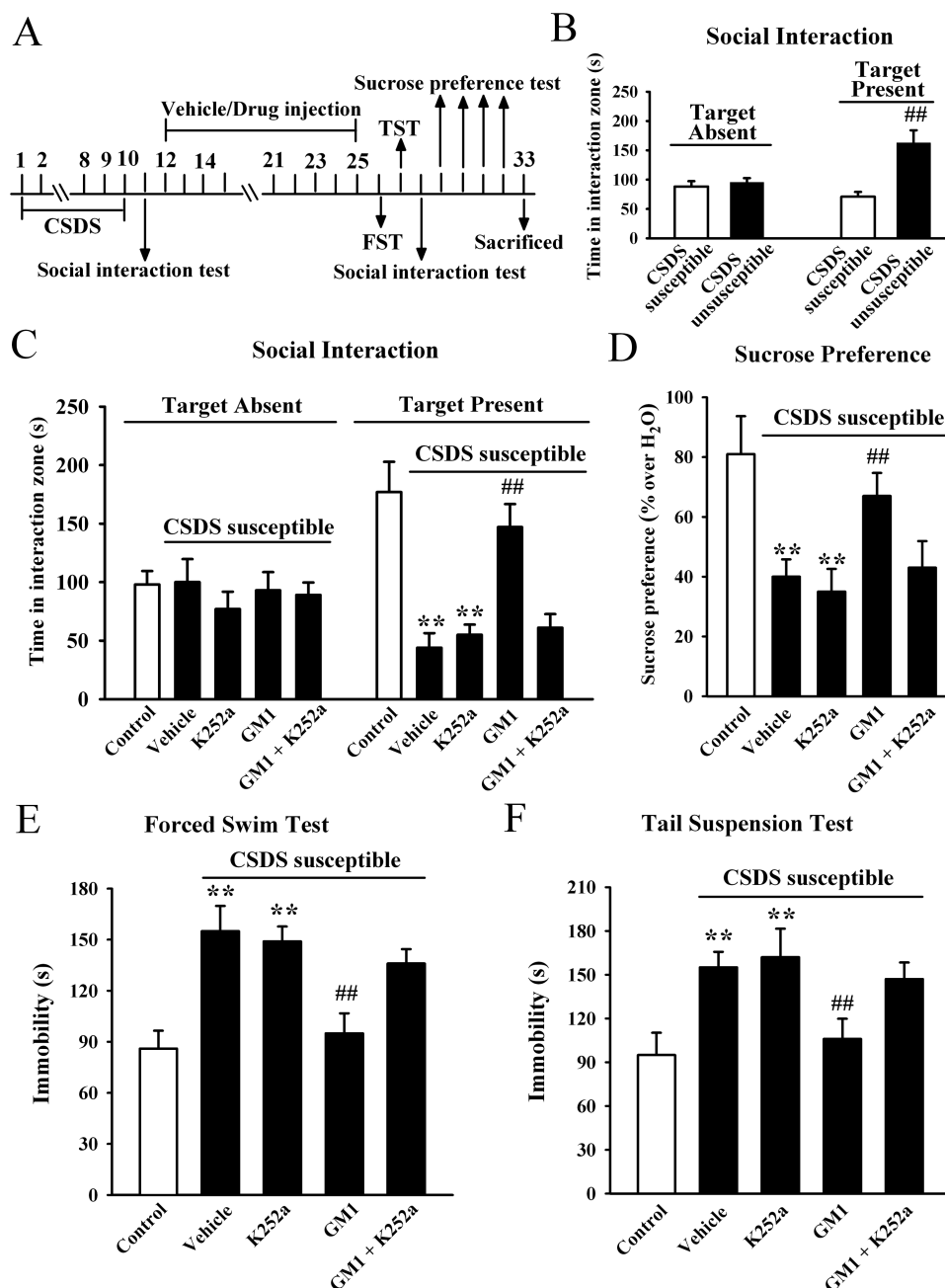
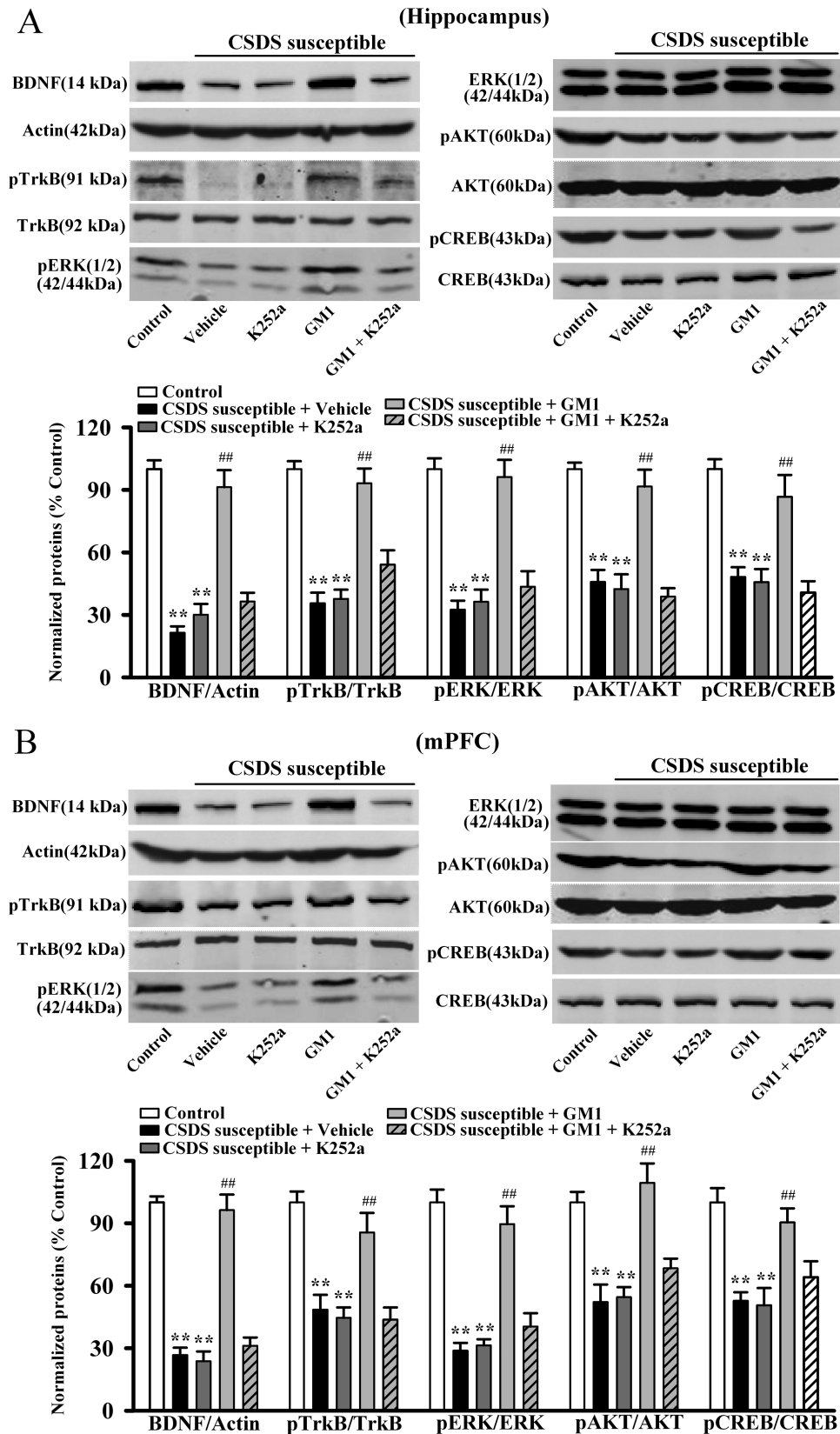


Figure 3. Blockade of brain derived neurotrophic factor (BDNF) signaling cascade by K252a abolishes the antidepressant-like actions of monosialotetrahexosylganglioside (GM1). (A) Schematic timeline of the experimental procedure. Total 73 C57BL/6J mice were used in this experiment with 62 chronic social defeat stress (CSDS)-stressed mice and 11 nonstressed mice. CSDS-susceptible mice were co-injected with GM1 and K252a for 14 days, and behavioral tests were then performed. The vehicle refers to 0.1% DMSO in 0.9% saline. (B) The social interaction results for CSDS-susceptible mice ($n = 44$) and unsusceptible mice ($n = 18$) in this experiment. (C) Co-treatment GM1 with K252a blocked the antidepressant-like effects of GM1 in the social interaction test. CSDS-susceptible + GM1 + K252a mice displayed significantly lower social interaction than CSDS-susceptible + GM1 mice ($n = 11$). (D) CSDS susceptible + GM1 + K252a mice displayed significantly lower sucrose preference than CSDS susceptible + GM1 mice ($n = 11$). (E) CSDS susceptible + GM1 + K252a mice displayed significantly higher immobility time than CSDS susceptible + GM1 mice in the forced swim test (FST) ($n = 11$). (F) CSDS-susceptible + GM1 + K252a mice also displayed significantly higher immobility time than CSDS-susceptible + GM1 mice in the tail suspension test (TST) ($n = 11$). Data are expressed as the mean \pm SEM; ^{**} $P < .01$ vs control; ^{##} $P < .01$ vs CSDS-susceptible/CSDS-susceptible + vehicle. Comparison was made by 2-way ANOVA followed by posthoc Bonferroni's test.

demonstrating that GM1 could affect the function of BDNF signaling pathway in the brain, since BDNF is critical for the pathophysiology of depression. For example, [Valdomero et al. \(2015\)](#) reported that GM1 administration induced a significant increase in BDNF protein levels in the NAC. Similarly, [Lim et al. \(2011\)](#) found that exogenous GM1 could evoke the release of mature BDNF from hippocampal neurons. Furthermore, [Duchemin](#)

[et al. \(2002\)](#) reported that GM1 can induce the phosphorylation and activation of tyrosine kinase receptors for neurotrophins, TrkA, TrkB, and TrkC in the striatum, hippocampus, and frontal cortex. [Zakharova et al. \(2014\)](#) also reported that GM1 has protective effects on PC12 cells exposed to hydrogen peroxide depending on the activation of Trk receptor tyrosine kinase and downstream activation of AKT and ERK1/2 signaling. Here, we



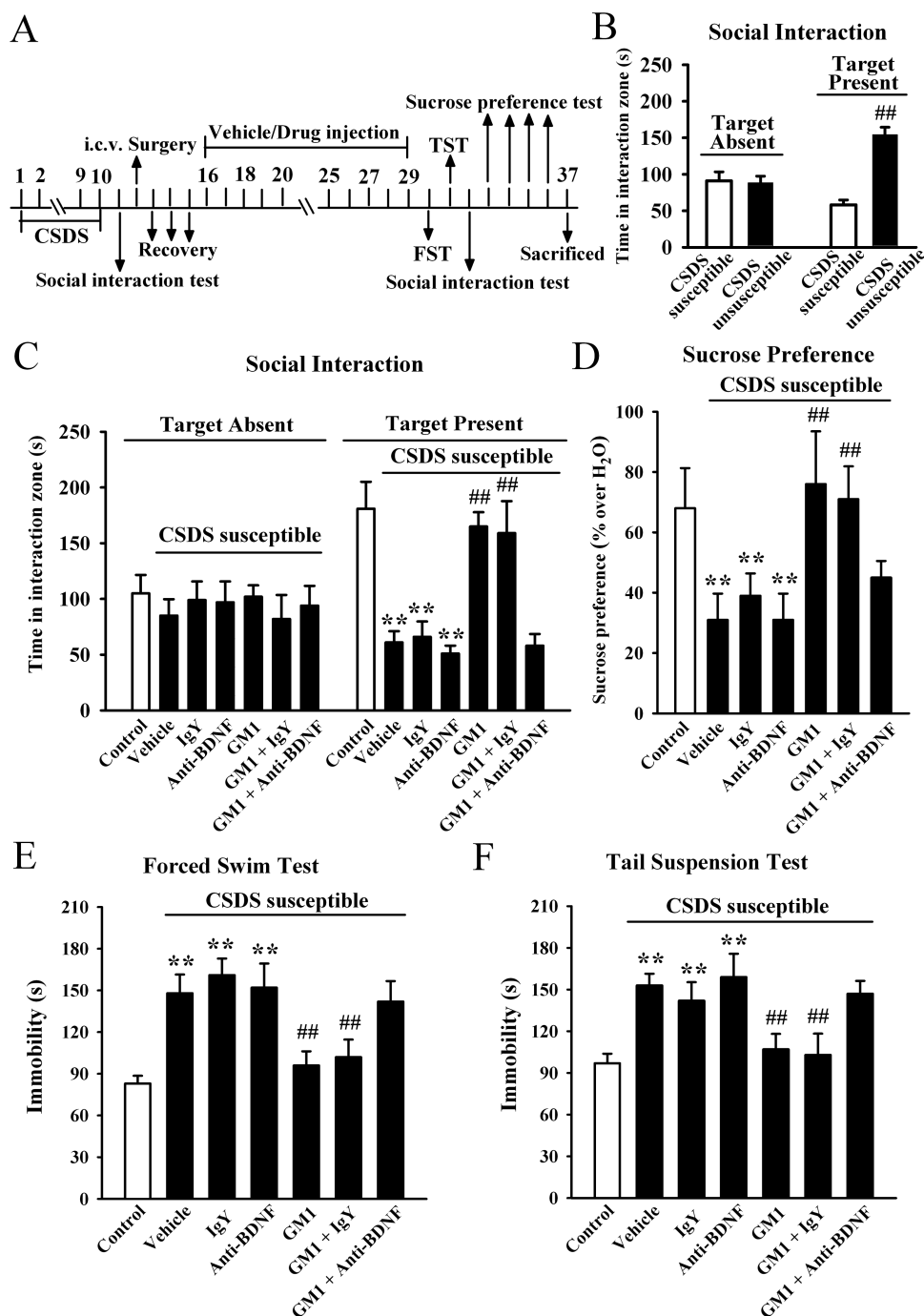


Figure 5. Blockade of brain derived neurotrophic factor (BDNF) signaling cascade by anti-BDNF infusion abolishes the antidepressant-like effects of monosialotetrahexosylganglioside (GM1). (A) Schematic timeline of the experimental procedure. Total 103 C57BL/6j mice were used in this experiment with 92 chronic social defeat stress (CSDS)-stressed mice and 11 nonstressed mice. CSDS-susceptible mice were co-treated with GM1 and anti-BDNF antibody for 14 days, with behavioral tests then performed. The vehicle refers to 0.9% saline. (B) The social interaction results for CSDS-susceptible mice ($n = 63$) and unsusceptible mice ($n = 29$) in this experiment. (C) Co-treatment with GM1 and anti-BDNF blocked the antidepressant-like effects of GM1 in the social interaction test. CSDS-susceptible + GM1 + anti-BDNF mice displayed significantly lower social interaction than CSDS-susceptible + GM1 mice ($n = 10-11$). (D) CSDS-susceptible + GM1 + anti-BDNF mice displayed significantly lower sucrose preference than CSDS-susceptible + GM1 mice ($n = 10-11$). (E) CSDS-susceptible + GM1 + anti-BDNF mice displayed significantly higher immobility time than CSDS-susceptible + GM1 mice in the forced swim test (FST) ($n = 10-11$). (F) CSDS-susceptible + GM1 + anti-BDNF mice also displayed significantly higher immobility time than CSDS-susceptible + GM1 mice in the tail suspension test (TST) ($n = 10-11$). Results are expressed as the mean \pm SEM; ^{**} $P < .01$ vs control; ^{##} $P < .01$ vs CSDS-susceptible/CSDS-susceptible + vehicle. Comparison was made by 2-way ANOVA followed by posthoc Bonferroni's test

found that GM1 indeed produced significant antidepressant-like activities in the CSDS model of depression, and more importantly, GM1 blocked the CSDS-induced changes on BDNF signaling pathway in the hippocampus and mPFC, in accordance with these previous reports.

GM1 has multiple pharmacological effects on the central nervous system. Previous reports have shown that GM1 produced improvements in several neurological disorders, including Parkinson's disease, Alzheimer's disease, Huntington's disease, and cerebral ischemia-reperfusion (Oikawa et al., 2009;

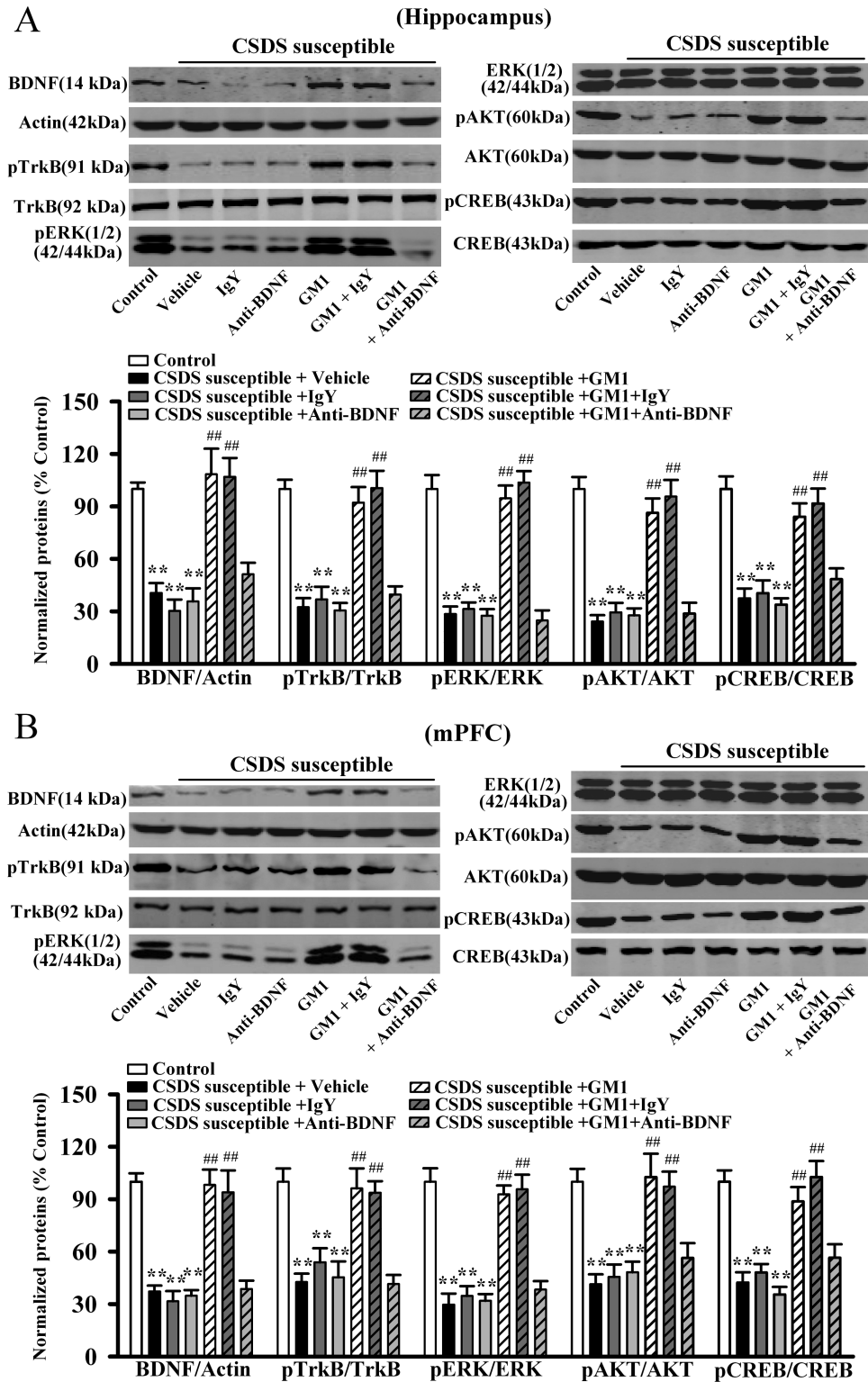


Figure 6. Anti-brain derived neurotrophic factor (BDNF) infusion blocks the effects of monosialotetrahexosylganglioside (GM1) on BDNF signaling pathway in the chronic social defeat stress (CSDS) model. (A) Western blot results showed that CSDS-susceptible + GM1 + anti-BDNF mice displayed significantly less BDNF, pTrkB, pERK1/2, pAKT, and pCREB expression in the hippocampus than CSDS-susceptible + GM1 mice (n = 5). (B) As in the hippocampus, western blot results indicated that the GM1-induced promotion of BDNF, pTrkB, pERK1/2, pAKT, and pCREB levels in the medial prefrontal cortex (mPFC) was also blocked by anti-BDNF antibody (n = 5). Data are expressed as the mean ± SEM; **P < .01 vs control; ##P < .01 vs CSDS-susceptible + vehicle. Comparison was made by 2-way ANOVA followed by posthoc Bonferroni's test.

Maglione et al., 2010; Schneider et al., 2010, 2013; Di Pardo et al., 2012; Yang et al., 2013; Zhang et al., 2015). To our knowledge, our study is the first instance of experimental evidence showing

that GM1 produces beneficial effects against depression, a most burdensome neuropsychiatric disease worldwide. This finding is very interesting and exciting in providing a new potential

antidepressant. In support of this result, there is other evidence implying the antidepressant-like effects of GM1, such as: (1) gangliosides enhance the antidepressant-like and neurochemical effects induced by chronic desipramine treatment (Molina et al., 1989); (2) ganglioside pretreatment enhanced the anti-immobility effect induced in the FST after a chronic treatment with mianserin, clomipramine, nialamide, or repeated electroconvulsive shock in mice (Cordoba et al., 1990); and (3) gangliosides attenuate the stress-induced changes in body weight and motor activity, and attenuate the behavioral response to 5-methoxy-N,N-dimethyltryptamine (Cancela et al., 1996).

Since GM1 produces antidepressant-like effects similar to those of fluoxetine, we must consider the possibility that these effects may be mediated through monoaminergic systems, particularly the serotonergic system. However, we found that depleting serotonin by PCPA did nothing to lessen the antidepressant-like action of GM1, indicating that the molecular mechanisms of GM1 are distinct from the conventional antidepressants.

In summary, GM1 has wide-ranging pharmacologic effects and many reveal positive therapeutic indexes. Our results show that GM1 possesses antidepressant-like activities through the promotion of BDNF signaling pathway proteins, providing a new insight in understanding the pharmacological effects of GM1 and shedding light on the development of new antidepressants with higher efficacy and fewer side effects.

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Statement of Interest

None.

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