*Regular Article*

# Ketamine Alleviates Depressive-Like Behaviors *via* Down-Regulating Inflammatory Cytokines Induced by Chronic Restraint Stress in Mice

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The purpose of the present study was to investigate whether ketamine's rapid antidepressant effects were associated with its anti-inflammatory actions and to explore the underlying molecular mechanism. Depressive-like behaviors was induced in mice using chronic restraint stress (CRS) method. Anti-depressive effects of ketamine were evaluated by forced swimming tests (FST) and sucrose preference test (SPT). Subsequently, brain tissue was harvested to investigate inflammatory response in the hippocampus *via* investigating reactive microglia numbers, serum cytokines levels and the toll-like receptor type 4 (TLR4)/p38 mitogen-activated protein kinase (MAPK) pathway. CRS exposure caused depressive-like behaviors in mice, which was associated with increased pre-inflammatory cytokines (interleukin (IL)-1$\beta$, tumor necrosis factor (TNF)-$\alpha$ and IL-6) levels, reactive microglia numbers and up-regulated regulatory molecules such as TLR4/p38 and P2X7 receptor in hippocampus. Such neurobehavioral and biochemical abnormalities were normalized by ketamine treatment. CRS-induced depression-like behaviours are associated with activation of hippocampal inflammatory response, whereas down-regulation of pro-inflammatory cytokines may contribute to ketamine's antidepressant effects in mice.

**Key words**    ketamine; depression; inflammation; toll-like receptor type 4 (TLR4); P2X7

Major depression is a common psychiatry disease which affects millions of people worldwide.[1] In the United States, the yearly prevalence rate and lifetime prevalence rate of major depression are 8.3 and 19.2%, respectively.[2] Although it is generally accepted that chronic stress plays a critical role in the progression of major depression, the pathogenesis remains incompletely understood. Currently existing antidepressants, such as tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitor (SSRI) are monoamine-based, which unfortunately have delayed effective times and low responding rates, particularly for the subjects with higher suicide rates.[3] Hence, it is of considerable importance to better understand the mechanism of major depression and develop fast-acting and more effective antidepressants.

Recently, there is growing evidence that immune response in the brain may contribute to depression.[4] In clinical practice, increased serum pro-inflammatory cytokines are associated with depression and correlated with the severity of symptoms. In addition, antidepressants have anti-inflammatory consequence because alleviation of depressive symptom is associated with decreased cytokines.[5,6] Microglia, the resident immune cells in the central nervous system (CNS), is usually found to be activated in the brain from depressive subjects who committed to suicide.[7] Moreover, the residential microglia was found to be activated following exposure to restraint stress,[8] which further supports the hypothesis that microglia plays a critical role in modulating the impact of stress and potentially contributed to the progression of depression. Hence, preclinical and clinical data suggest that depression may be an inflammation-related disorder, and inhibition of pro-inflammatory responses is probably beneficial for treatment of depression[9]

Ketamine, an *N*-methyl-D-aspartate (NMDA) receptor an-

tagonist, has recently been found to be a rapid fast-acting anti-depressive agent.[10,11] Although the mechanism that mediated such effects and the full spectrum of ketamine's action are not poorly understood, both the clinic and laboratory investigations demonstrate its immunomodulatory effects. Specifically, Dale *et al.* found that ketamine has anti-inflammatory effects as intraoperative administration of ketamine significantly inhibits the early postoperative IL-6 inflammatory response.[12] Furthermore, several *in vivo* studies found that ketamine inhibits the production and release of pro-inflammatory cytokines, interleukin (IL)-1$\beta$, tumor necrosis factor (TNF)-$\alpha$ and IL-6, in microglial cells, macrophage and astrocytes, raising the possibility that these activities contributed to ketamine's rapid antidepressant effects.[13–15] However, little attention has been paid to the role and mechanism of ketamine's anti-inflammatory actions in the antidepressant effects following chronic stress exposure. In the present study, we hypothesized that inhibition of microglia activation and down-regulation of pro-inflammatory cytokines contribute to ketamine's rapid antidepressant effects. We establish the depression model in mice using chronic restraint stress (CRS) to investigate whether the alleviation of depressive symptoms is associated with the inhibition of inflammatory reaction intrigued by CRS and further explored the underlying signaling pathway.

## MATERIALS AND METHODS

**Animals and Drug Administration**    All animal experiments were approved by the Animal Experimentation Ethics Committee (AEEC) of the University of South China, which in accordance with the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health, U.S.A. Kunming mice weighing 25–30 g were provided by the experi-

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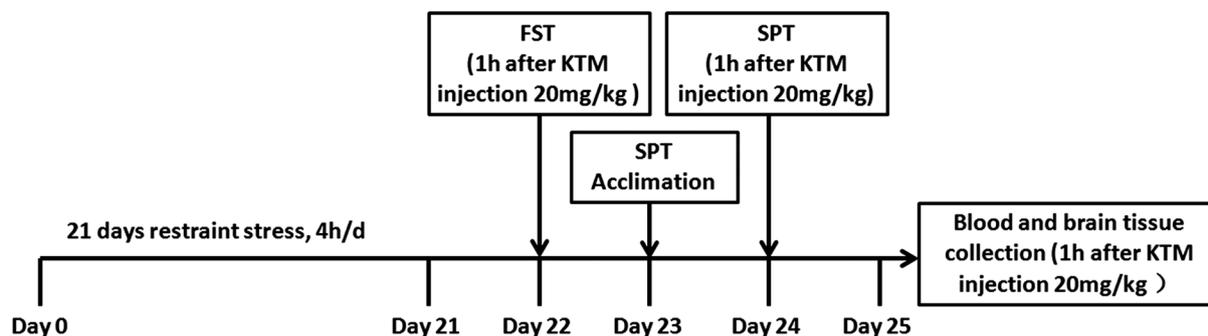
Fig. 1.   Experimental Timeline

   Mice received restraint stress from days 0 to 21, 4 h/d for 21 consecutive days. Forced swimming tests (FST), sucrose preference test (SPT) and sample collection were performed at days 22, 24 and 25, respectively. Thirty minutes before the behavioral tests and sample collection, mice in the CRS+KTM group received ketamine (20 mg/kg) *via* intraperitoneal (i.p.) injection, while mice in CON and CRS group received equal amount of 0.9% saline.

mental animal center of the University of South China. Before starting the experiments, all of the mice were acclimated to the novel experimental environment (12 h light dark cycle, 22±2°C of room temperature) for one week with free access to food and water. The mice were randomly assigned to the three groups: Control (CON) group, chronic restraint stress (CRS) group and the ketamine treatment (CRS+KTM) group. For the CRS and CRS+KTM group, mice received 21 d of restraint stress as performed previously reported to induce the depressive behavior. Mice were placed inside a plastic tube (internal diameter 26 mm) for 4 h/d for 21 consecutive days. Forced swimming test, sucrose preference test and sample collection were performed at days 22, 24 and 25, respectively. One hour before the behavioral tests and sample collection, mice in the CRS+KTM group received ketamine (20 mg/kg) *via* intraperitoneal (i.p.) injection, while mice in CON and CRS group received equal amount of 0.9% saline (Fig. 1).

**Forced Swimming Tests (FST)**   Cylinders for forced swimming tests were 65 cm tall, 30 cm in diameter and filled with water (22–23°C) to a depth of 40 cm. When the test started, mice were placed into the cylinder gently for 6 min. The immobility time (in seconds) was recorded during the last 5 min of the 6-min session by two observers blinded to the group assignment. Immobility was defined as floating in the water without struggling and only making necessary movements to keep the head above water. Water in the cylinder was changed after every test. All the procedures were conducted between 09:00 and 15:00.

**Sucrose Preference Test (SPT)**   Sucrose preference test was performed as previously described.[16] On the day before the test, two bottles containing 1% sucrose solution (w/v) were placed on each cage and the mice had free access to drink from these bottles for 24 h. On the next 24 h, one bottle was filled with tap water while another continued to contain 1% sucrose solution. The positions of the two bottles were placed randomly and exchanged after 12 h to avoid the influence of bottle position. The amount of water and sucrose solution consumption was recorded, and the sucrose preference rate was calculated as consumed sucrose solution/(consumed water+consumed sucrose solution).

**Enzyme-Linked Immunosorbent Assay (ELISA)**   Following the behavioral test, mice were killed by decapitation. Serum and brain tissues was collected from each animal and stored at −80°C for further analysis. Corticosterone in serum was determined as previously reported[17] using commercial

ELISA kits (Cayman Chemical Company, Ann Arbor, MI, U.S.A.). Levels of pro-inflammatory cytokines, IL-1$\beta$, TNF-$\alpha$ and IL-6, were measured using a radioimmunoassay (RIA) (North China Institute of Biotechnology, Nanjing, China) following the manufacturer's instructions. All the steps were performed in accordance with the manufacturer's recommendations. Concentrations were calculated by referring to a standard curve, according to the manufacturer's instructions.

**Immunohistochemistry**   For immunostainning, samples were fixed in 4% paraformaldehyde, dehydrated in graded concentrations of ethanol, cleared in xylene, embedded in paraffinwax and sectioned 5 $\mu$m in a rotary microtome. Then the sections were deparaffinized, rehydrated and the endogenous peroxidase were blocked with 10% hydrogen peroxide ($H_2O_2$) in 100% methanol for 30 min. Sections were permeabilized (antigen retrieval) with 1×phosphate buffered saline (PBS) supplemented with 0.1% Triton-X and 0.05% Tween 20 for 10 min. After three rinses in 1×PBS, non-specific binding was suppressed by 1.5% normal blocking serum for 30 min. Thereafter, the sections were incubated at 4°C overnight with rabbit anti-IBA-1 (Proteintech, Chicago, IL, U.S.A.) diluted 1:100 in blocking solution. On the next day, sections were washed three times with 1×PBS (5 min each) and incubated with biotinylated secondary antibody (1:1000 diluted in blocking solution) for 1 h at room temperature. Subsequently, sections were washed three times in 1×PBS (5 min each) again and the sections were incubated with streptavidin–horseradish peroxidase (HRP) (1:1000 diluted in PBS) for 1 h. After three times of rinse with 1×PBS, the colors were developed by dimethylaminoazobenzene (DAB). Sections were dehydrated and mounted and the pictures were captured using digital camera at 40×lens.

**Western Blotting**   Frozen hippocampi ($n$=6) were thawed and homogenized in 300 $\mu$L RIPA lysis buffer supplemented with protease inhibitor cocktail (Millipore Inc., Billerica, MA, U.S.A.). Lysates were centrifuged at 12000×$g$ at 4°C for 30 min and the supernatants were collected and stored at −20°C until use. Protein concentration was determined by the Bio-Rad DC protein assay (Bio-Rad Laboratories Inc., Hercules, CA, U.S.A.). Each sample of 50 $\mu$g protein was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis using 100 Volt for 2 h and then transferred to a polyvinylidene difluoride (PVDF) membrane at 200 mA for 60 min. The membrane was blocked with 5% non-fat dry milk for 1 h at room temperature and then

incubated overnight at 4°C with primary antibodies diluted as followed: toll-like receptor type 4 (TLR4) (1 : 1000) (Santa Cruz Biotechnologies, CA, U.S.A.), p-P38 (1 : 1000) (Cell Signaling, MA, U.S.A.), P2X7 (1 : 1000) (Proteintech, Chicago, IL, U.S.A.), $\beta$-actin (1 : 20000) (MAB1501, Millipore, Billerica, MA, U.S.A.). On the next day, after 3 times of washing with 0.05% Tween-20 and PBS, the membrane was incubated with the corresponding HRP conjugated secondary antibody for 1 h at room temperature. Blots then developed using an ECL Plus Kit on Fuji Medical X-ray film and scanned using a Bio-Rad 6500 scanner. Optical density was quantified with Image J software.

**Statistical Analysis** Significance of differences among the groups was assessed by one-way ANOVA followed by the Bonferroni test for *post-hoc* comparisons. Calculations were performed using SPSS software (version 16.0). Differences were considered significant when $p < 0.05$. Data were presented as mean±standard error of the mean (S.E.M.)

## RESULTS

**Ketamine Reverses Depressive-Like Behaviors Induced by CRS in FST and SPT in Mice** Compared with the controls, mice subjected to CRS exposure exhibited a significantly increased immobility time (Fig. 2A) ($p < 0.05$), which indicated that CRS exposure caused depressive-like behavior in mice. To investigate the effects of ketamine on the depressive-like behaviors in mice, we injected ketamine to mice that subjected to CRS. The results showed that infusion of ketamine significantly decreased immobility time in FST (Fig. 2A) ($p < 0.05$). We also employed another commonly used behavioral test, namely SPT, to confirm our findings about the antidepressant effects of ketamine in mice. It was found that CRS exposure significantly decreased the percentage of sucrose preference compared with controls ($p < 0.05$) while infusion of ketamine increased the percentage of sucrose preference ($p < 0.05$) (Fig. 2B). Both the *FST* and *SPT* showed that ketamine reversed depressive-like behaviors caused by CRS in mice.
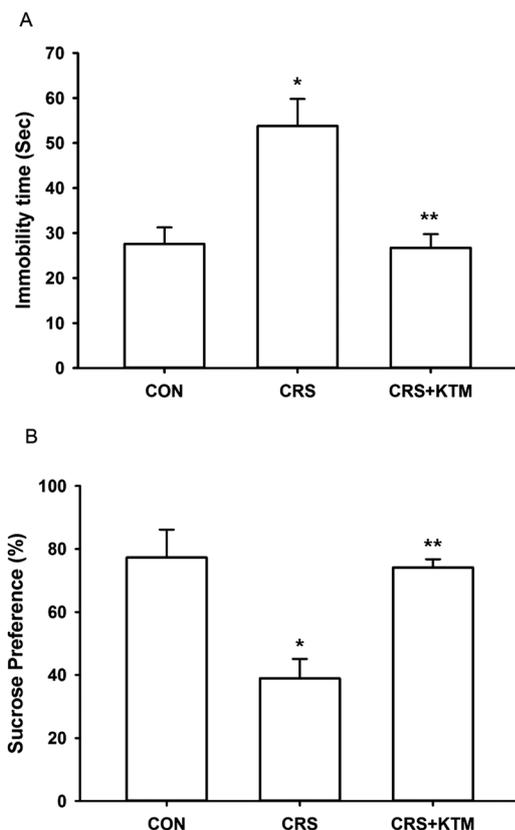
**Effects of Ketamine on Plasma Corticosterone Levels in Mice** Corticosterone is the major stress hormone increased in depressive subjects. We further determine the levels of serum corticosterone in mice using ELISA. As expected, CRS exposure significantly increased serum corticosterone levels in mice when compared with controls ($p < 0.05$) (Fig. 3). Following ketamine infusion, serum corticosterone levels in the CRS+KTM group decreased significantly when compared with the CRS group ($p < 0.05$). These results further confirmed our findings about the anti-depressive effects of ketamine in the behavioral tests.

**Suppressive Effects of Ketamine on Microglia Activation in the Hippocampus** Immuohistochemistry was performed using a cell-specific marker (IBA-1) for microglia to investigate effects of ketamine on microglia activation in the hippocampus. As shown in Figs. 4A–C, the "resting" form of microglia cells were composed of long branching processes and a small cellular body while reactive microglia presented large cell body with devoid of branching processes (arrow). As shown in Fig. 4D, CRS exposure significantly increased the number of activated microglia cells compared with the controls ($p < 0.05$). Ketamine decreased the number of activated microglia cells in the hippocampus when compared with the CRS group ($p < 0.05$).

**Ketamine Suppressed CRS-Induced Inflammatory Cytokine Levels in Mice** To further investigate whether ketamine's antidepressant effects is associated with its inflammatory suppression action, we further determined the levels of inflammatory cytokines in mice serum. Compared with the control group, levels of pro-inflammatory factors IL-1$\beta$, TNF-$\alpha$ and IL-6, increased significantly in the CRS exposed

Fig. 2. Effects of Ketamine on Depressive-Like Behaviors Induced by CRS in FST and SPT in Mice

Mice were subjected to CRS and treated with ketamine (i.p. 20 mg/kg). Depressive-like behaviors changes were measured by FST and SPT. (A) Immobility time in FST. (B) Percentage of sucrose preference. Data were presented as mean±S.E.M. ($n$=12). * $p < 0.05$, compared to the CON group, ** $p < 0.05$, compared to the CRS group.

Fig. 3. Effects of Ketamine on Corticosterone Levels in Mice

Mice were subjected to CRS and treated with ketamine (i.p. 20 mg/kg), blood corticosterone levels were measured by ELISA. Data were presented as mean±S.E.M. ($n$=6). * $p < 0.05$, compared to the Con group, ** $p < 0.05$, compared to the CRS group.

group ($p<0.05$) (Fig. 5). Infusion of ketamine significantly decreased levels of pro-inflammatory factors in mice ($p<0.05$).

**Ketamine Down-Regulated the TLR4/p38 Pathway in the Hippocampus**   To investigate the mechanism of microglia suppression and cytokine down-regulation following infusion of ketamine in CRS-exposed mice, we employed Western blot to determine protein levels of TLR4 and p-p38 in the hippocampus. As shown in Fig. 6, CRS exposure activated the TLR4/p38 pathway through up-regulating these proteins in hippocampus. Following ketamine infusion, TLR4/p38 signal-
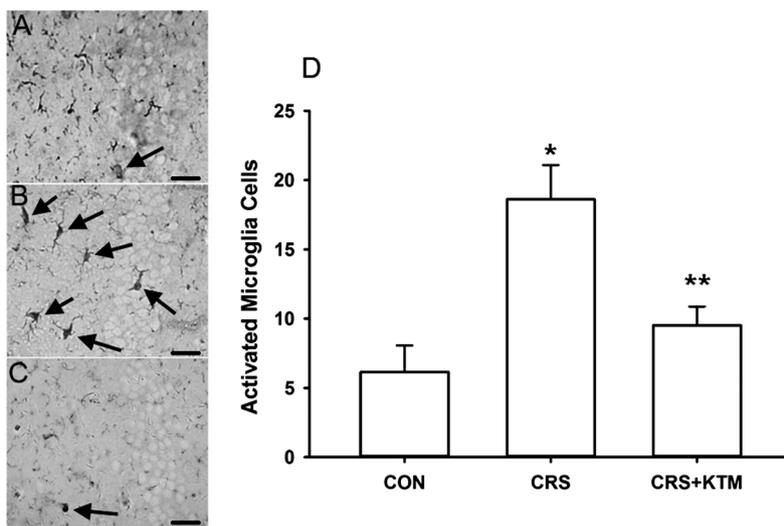


**Fig. 4.   Effects of Ketamine on Microglia Activation in the Hippocampus**

Mice were subjected to CRS and treated with ketamine (i.p. 20 mg/kg), reactive microglia cells in hippocampus were labeled with IBA-1. (A) Representative image from the CON group. (B) Representative image from the CRS group. (C) Representative image from the CRS+KTM group. (D) Statistics of the numbers of reactive microglia cells in three groups. Data were presented as mean±S.E.M. ($n=6$), *$p<0.05$, compared to the Con group, **$p<0.05$, compared to the CRS group. Arrow indicates the reactive microglia cells. Scale bar, 20 μm.
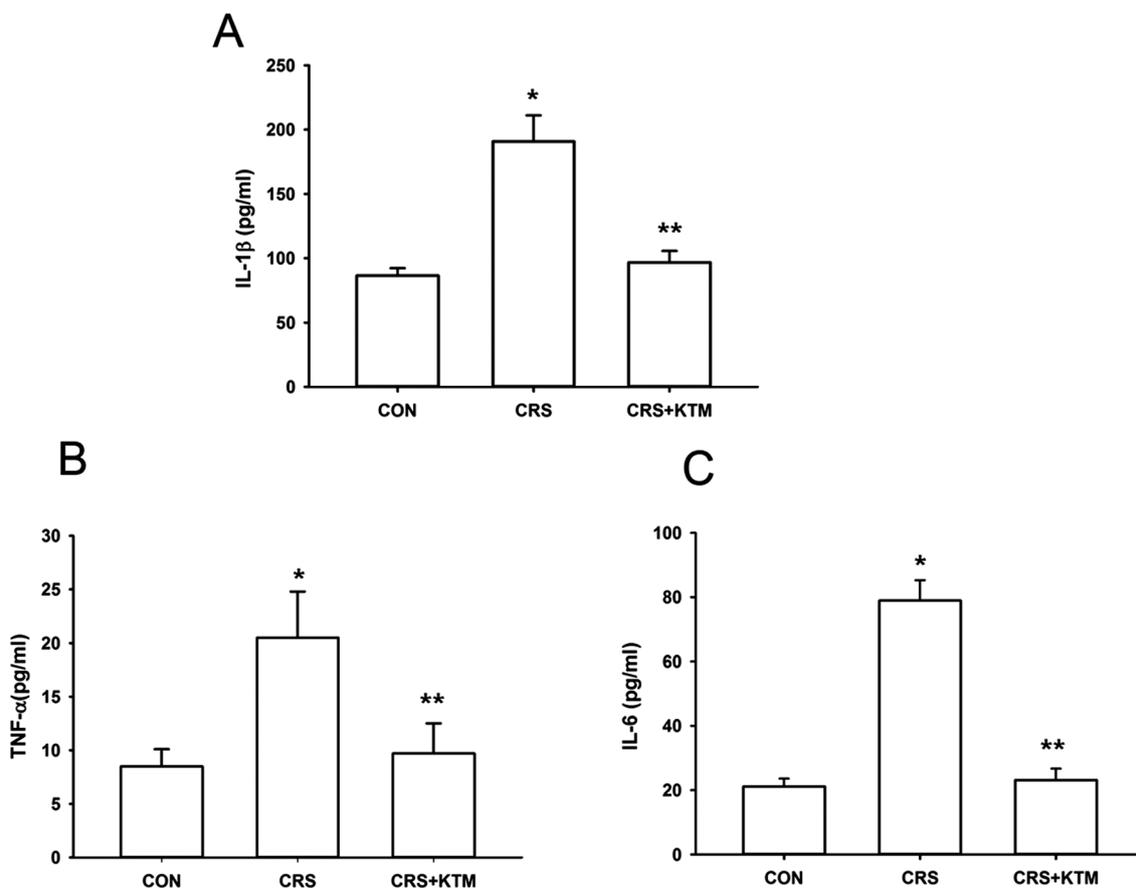


**Fig. 5.   Effects of Ketamine on Cytokine Levels in Mice**

(A) IL-1β, (B) TNF-α, (C) IL-6. Data were presented as mean±S.E.M. ($n=6$), *$p<0.05$, compared to the Con group, **$p<0.05$, compared to the CRS group.
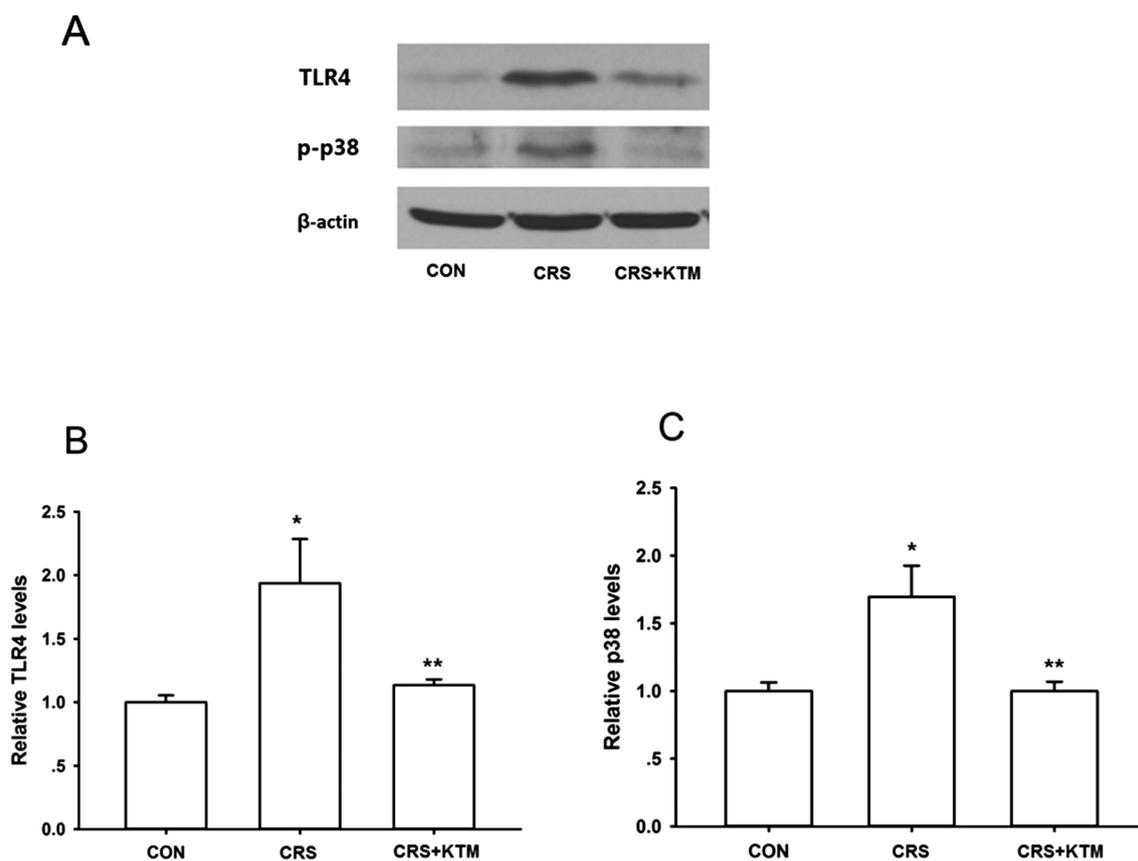
Fig. 6.　Effects of Ketamine on the TLR4/p38 Pathway in the Hippocampus

(A) Representative image from each group, (B) statistics of TLR4 levels from each group, (C) statistics of p-p38 levels from each group. Data were presented as mean±S.E.M. ($n=6$), *$p<0.05$, compared to the Con group, **$p<0.05$, compared to the CRS group.
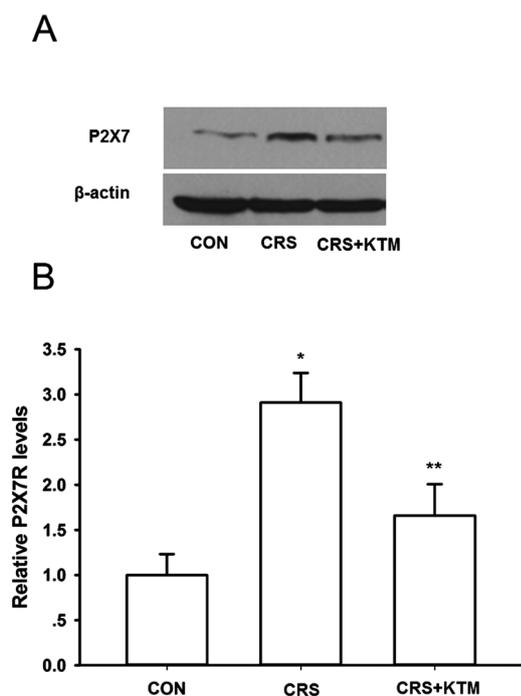


Fig. 7.　Effects of Ketamine on P2X7 Levels in the Hippocampus

(A) Representative image from each group, (B) statistics of P2X7 levels from each group. Data were presented as mean±S.E.M. ($n=6$), *$p<0.05$, compared to the Con group, **$p<0.05$, compared to the CRS group.

ing pathway was down-regulated significantly ($p<0.05$).

**Effects of Ketamine on P2X7 Receptor in the Hippocampus**　P2X7 receptor also played a pivotal role in the microglia activation and expression of P2X7 receptor in the hippocampus was determined using Western blot. It was found that CRS exposure also significantly increased the protein levels of P2X7 receptor in the hippocampus ($p<0.05$), while ketamine infusion significantly down-regulated P2X7 receptor levels ($p<0.05$) (Fig. 7).

## DISCUSSION

The major findings of the present study were that CRS exposure caused depressive-like behaviors in mice, which was associated with increased pre-inflammatory cytokine (IL-1$\beta$, TNF-$\alpha$ and IL-6) levels and reactive microglia numbers and up-regulated regulatory molecules such as TLR4/p38 and p2x7 receptor in hippocampus. These neurobehavioral and biochemical abnormalities were reversed by infusion of ketamine, a promising antidepressant. These results suggested that the rapid antidepressive effects of ketamine are related to its actions on down-regulation of inflammatory response to the CRS exposure in hippocampus.

Depression is a major mental disorder characterized by despair and anhedonia, which has high mortality caused by suicide. Although the mechanisms of the depression are not fully understood, several lines of evidence have demonstrated that depression is closely related to activation of inflammatory reaction in the CNS following chronic psychological

stress. CRS has been widely used to establish animal model for depression.[18] As expected, our results showed that 21 d of restraint stress increased despair and anhedonia behaviors in the FST and SPT, respectively. The hypothalamic–pituitary–adrenal (HPA) axis is the master sensor of stress and secreting stress hormone (such as corticsoterone, CORT) is the main indication of stress adaptation. Circulating CORT exhibited negative feedback effects on HPA axis through acting on the limbic system such as hippocampus.[19] Unfortunately, chronic elevation of CORT and persistent activation of this negative feedback pathway may cause damage in the hippocampus thus exacerbate the stress reaction. In the present study, we found that circulating CORT levels has been increased dramatically following CRS. Together with the behavioral changes, elevation of serum CORT indicates that the depression animal model has been established in mice using CRS in our study.

It has been widely demonstrated that cytokines, especially the pro-inflammatory factors including IL-1$\beta$, TNF-$\alpha$ and IL-6, are closely associated with depression in clinical and animal studies as well.[20,21] In clinical practice, many cases of comorbidity between autoimmune disease (*e.g.* rheumatoid arthritis and asthma) and depression were reported.[22,23] A recent meta-analysis also demonstrated elevated serum IL-6 levels in depressed subjects compared with controls. Moreover, several lines of evidence indicate that both acute and chronic stress lead to increase in serum pro-inflammatory cytokine levels in animal studies with unclear mechanisms.[24,25] Interestingly, Voorhees *et al.* also found that IL-10 has been decreased following a stress paradigm, indicating that stress may not only alter the pro-inflammatory cytokines but also the anti-inflammatory cytokines. It should be mentioned that pro-inflammatory cytokines may activate the HPA axis directly to augment the stress response.[26,27] CRS lead to the depressive behaviors in mice, which were associated with increase in pro-inflammatory cytokines as demonstrated in our results. One possible pathway by which inflammatory stimuli contribute to depression is to deplete tryptophan synthesis and reduce 5-hydroxytryptamine (5-HT) *via* activating indoleamine 2,3-dioxygenase (IDO).[28] Hence, cytokines played a critical role in the pathogenesis of depression and would contribute vitally to the therapeutic interventions of anti-depressive agents. As the resident immune cells, microglia is sensitive to environmental changes in the brain. Following stress, resting form of microglia converted to the reactive form to synthesize and secret cytokines. Microglia activation was found in prefrontal cortex, anterior cingulate cortex and hippocampus of depression subjects who committed suicide.[29,30] Interestingly, we also found that elevated pro-inflammatory cytokines were associated with increased numbers of reactive microglia cells in the brain, indicating that CRS exposure activated this brain innate immune cells during the pathogenesis of depression.

Recently, studies have shown that ketamine, a NMDA antagonist, has rapid, robust, and long-lasting antidepressant properties.[10,11] The antidepressant effects of ketamine were also well demonstrated in human subjects.[31] In agreement with the previous studies, we show that neurobehavioral abnormalities induced by CRS exposure were reversed by infusion of ketamine. It should be mentioned that ketamine has long be reported to have functions in down-regulating inflammatory responses and has been recommended for use in surgery of sepsis subjects due to its anti-inflammatory

effects.[12,32] Previous studies reporting inhibitory effects of ketamine on lipopolysaccharide (LPS)-induced microglia activation suggested ketamine's inflammatory modulating effects may partly contributed to the beneficial effects of ketamine on depressive symptoms, which is consistent with our findings in animal models.[12,13] As shown in our results, infusion of ketamine significantly decreased the numbers of reactive microglia cells in hippocampus, which is associated with the down-regulation of the pro-inflammatory cytokines.

It has been shown that Toll-like receptors (TLRs) played a pivotal role in the initiation of innate immune responses. Among the identified TLRs, TLR4 is expressed on microglia and participates in activation of these cells induced by various stressful events in the brain.[33,34] Several studies have reported that TLR4-dependent activation of microglia is involved in depressive disorder.[35,36] TLR4 may be activated by the cytokines such as IL-1$\beta$ secreted by microglia cells, then further induces the inflammatory process by increasing phosphorylation of P38 mitogen activated protein kinases (MAPK). Activation of the TLR4/p38 MAPK signaling pathway enhances the transcription of the pro-inflammatory cytokines.[37] Interestingly, it has been widely demonstrated that ketamine has inhibitory effects on the TLR4 mediated inflammatory signaling pathways in LPS induced astrocytes and microglia cell activation.[13,38] In the present study, we show that the CRS-induced activation of TLR4/p38 MAPK signaling pathway is suppressed by ketamine treatment, which further support our hypothesis that ketamine's anti-inflammatory effects contributed to its antidepressive action.

The P2X7 receptor is a purinergic, ATP binding receptor, and has been reported to be involved in the progression of depressive disorders. Mutation in the gene coding for P2X7 was significantly associated with depression, especially the recurrent major depression.[39] Deletion of P2X7 exerted antidepressive effects as evidenced by longer swim times and increased mobility in the forced swim and tail suspension tests in mice.[40] It should be mentioned that P2X7 receptor was identified as a key player in the process of microglia activation. In the quiescent microglia, P2X7 is permeable to small cations while in the reactive microglia P2X7 was converted to the pore status, which facilitates the release of pro-inflammatory cytokines TNF-$\alpha$ and IL-1$\beta$ from microglia.[41] Recently, P2X7 was reported as a novel target of depression treatment in mice.[42] We showed here that keamine down-regulated the P2X7 protein levels in hippocampus following CRS, indicated that P2X7 could be also involved in ketamine's antidepressant effects. Future studies to test whether using P2X7 inhibitors brilliant blue G could have the same result with the anti-depressive effects of ketatmine could further confirm the critical role of P2X7 in the action of ketamine.

In conclusion, microglia cells, the key innate immune cells of the brain, have been extensively involved in the progression of depression because of the regulatory roles in stress response. The evidence presented here revealed that the rapid anti-depressive effects of ketamine are related to its actions on down-regulation of inflammatory response to the CRS exposure in hippocampus. Furthermore, findings in the current study indicated that ketamine could not only down-regulate cytokine synthesis through the TLR4/p38 signaling pathway but also inhibit cytokine release from microglia by down-regulation of P2X7.

**Conflict of Interest** The authors declare no conflict of interest.

## REFERENCES

1) Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med.*, **3**, e442 (2006).

2) Hirschfeld RM. The epidemiology of depression and the evolution of treatment. *J. Clin. Psychiatry*, **73** (Suppl. 1), 5–9 (2012).

3) Nakajima S, Suzuki T, Watanabe K, Kashima H, Uchida H. Accelerating response to antidepressant treatment in depression: a review and clinical suggestions. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **34**, 259–264 (2010).

4) Raedler TJ. Inflammatory mechanisms in major depressive disorder. *Curr. Opin. Psychiatry*, **24**, 519–525 (2011).

5) Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol. Psychiatry*, **65**, 732–741 (2009).

6) Zunszain PA, Hepgul N, Pariante CM. Inflammation and depression. *Curr. Top. Behav. Neurosci.*, **14**, 135–151 (2013).

7) Steiner J, Bielau H, Brisch R, Danos P, Ullrich O, Mawrin C, Bernstein HG, Bogerts B. Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. *J. Psychiatr. Res.*, **42**, 151–157 (2008).

8) Sugama S, Takenouchi T, Fujita M, Kitani H, Hashimoto M. Cold stress induced morphological microglial activation and increased IL-1$\beta$ expression in astroglial cells in rat brain. *J. Neuroimmunol.*, **233**, 29–36 (2011).

9) Schmidt FM, Kirkby KC, Lichtblau N. Inflammation and immune regulation as potential drug targets in antidepressant treatment. *Curr. Neuropharmacol.*, **14**, 674–687 (2016).

10) Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, Li XY, Aghajanian G, Duman RS. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science*, **329**, 959–964 (2010).

11) Li N, Liu RJ, Dwyer JM, Banasr M, Lee B, Son H, Li XY, Aghajanian G, Duman RS. Glutamate *N*-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biol. Psychiatry*, **69**, 754–761 (2011).

12) Dale O, Somogyi AA, Li Y, Sullivan T, Shavit Y. Does intraoperative ketamine attenuate inflammatory reactivity following surgery? A systematic review and meta-analysis. *Anesth. Analg.*, **115**, 934–943 (2012).

13) Chang Y, Lee JJ, Hsieh CY, Hsiao G, Chou DS, Sheu JR. Inhibitory effects of ketamine on lipopolysaccharide-induced microglial activation. *Mediators Inflamm.*, **2009**, 705379 (2009).

14) Chang HC, Lin KH, Tai YT, Chen JT, Chen RM. Lipoteichoic acid-induced TNF-$\alpha$ and IL-6 gene expressions oxidative stress production in macrophages are suppressed by ketamine through downregulating Toll-like receptor 2-mediated activation of ERK1/2 and NF$\kappa$B. *Shock*, **33**, 485–492 (2010).

15) Yuhas Y, Ashkenazi S, Berent E, Weizman A. Immunomodulatory activity of ketamine in human astroglial A172 cells: Possible relevance to its rapid antidepressant activity. *J. Neuroimmunol.*, **282**, 33–38 (2015).

16) Willner P, Towell A, Sampson D, Sophokleous S, Muscat R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology*, **93**, 358–364 (1987).

17) Tan S, Xu C, Zhu W, Willis J, Seubert CN, Gravenstein N, Sumners C, Martynyuk AE. Endocrine and neurobehavioral abnormalities induced by propofol administered to neonatal rats. *Anesthesiology*, **121**, 1010–1017 (2014).

18) Chiba S, Numakawa T, Ninomiya M, Richards MC, Wakabayashi C, Kunugi H. Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **39**, 112–119 (2012).

19) Herman JP, McKlveen JM, Ghosal S, Kopp B, Wulsin A, Makinson R, Scheimann J, Myers B. Regulation of the hypothalamic–pituitary–adrenocortical stress response. *Compr. Physiol.*, **6**, 603–621 (2016).

20) Kim JW, Szigethy EM, Melhem NM, Saghafi EM, Brent DA. Inflammatory markers and the pathogenesis of pediatric depression and suicide: a systematic review of the literature. *J. Clin. Psychiatry*, **75**, 1242–1253 (2014).

21) Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol.*, **27**, 24–31 (2006).

22) Jacob L, Rockel T, Kostev K. Depression risk in patients with rheumatoid arthritis in the United Kingdom. *Rheumatol. Ther.*, **4**, 195–200 (2017).

23) Shen TC, Lin CL, Liao CH, Wei CC, Sung FC, Kao CH. Major depressive disorder is associated with subsequent adult-onset asthma: a population-based cohort study. *Epidemiol. Psychiatr. Sci.*, **19**, 1–8 (2016).

24) Voorhees JL, Tarr AJ, Wohleb ES, Godbout JP, Mo X, Sheridan JF, Eubank TD, Marsh CB. Prolonged restraint stress increases IL-6, reduces IL-10, and causes persistent depressive-like behavior that is reversed by recombinant IL-10. *PLOS ONE*, **8**, e58488 (2013).

25) Himmerich H, Fischer J, Bauer K, Kirkby KC, Sack U, Krügel U. Stress-induced cytokine changes in rats. *Eur. Cytokine Netw.*, **24**, 97–103 (2013).

26) Dunn AJ. Cytokine activation of the HPA axis. *Ann. N. Y. Acad. Sci.*, **917**, 608–617 (2000).

27) Matsuwaki T, Eskilsson A, Kugelberg U, Jönsson JI, Blomqvist A. Interleukin-1$\beta$ induced activation of the hypothalamus–pituitary–adrenal axis is dependent on interleukin-1 receptors on non-hematopoietic cells. *Brain Behav. Immun.*, **40**, 166–173 (2014).

28) Sublette ME, Postolache TT. Neuroinflammation and depression: the role of indoleamine 2,3-dioxygenase (IDO) as a molecular pathway. *Psychosom. Med.*, **74**, 668–672 (2012).

29) Schnieder TP, Trencevska I, Rosoklija G, Stankov A, Mann JJ, Smiley J, Dwork AJ. Microglia of prefrontal white matter in suicide. *J. Neuropathol. Exp. Neurol.*, **73**, 880–890 (2014).

30) Steiner J, Bielau H, Brisch R, Danos P, Ullrich O, Mawrin C, Bernstein HG, Bogerts B. Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. *J. Psychiatr. Res.*, **42**, 151–157 (2008).

31) Zarate CA Jr, Brutsche NE, Ibrahim L, Franco-Chaves J, Diazgranados N, Cravchik A, Selter J, Marquardt CA, Liberty V, Luckenbaugh DA. Replication of ketamine's antidepressant efficacy in bipolar depression: a randomized controlled add-on trial. *Biol. Psychiatry*, **71**, 939–946 (2012).

32) Ward JL, Harting MT, Cox CS Jr, Mercer DW. Effects of ketamine on endotoxin and traumatic brain injury induced cytokine production in the rat. *J. Trauma*, **70**, 1471–1479 (2011).

33) Yao L, Kan EM, Lu J, Hao A, Dheen ST, Kaur C, Ling EA. Toll-like receptor 4 mediates microglial activation and production of inflammatory mediators in neonatal rat brain following hypoxia: role of TLR4 in hypoxic microglia. *J. Neuroinflammation*, **10**, 23 (2013).

34) Zeng KW, Zhang T, Fu H, Liu GX, Wang XM. Schisandrin B

exerts anti-neuroinflammatory activity by inhibiting the Toll-like receptor 4-dependent MyD88/IKK/NF-kappaB signaling pathway in lipopolysaccharide-induced microglia. *Eur. J. Pharmacol.*, **692**, 29–37 (2012).

35) Cheng Y, Pardo M, Armini Rde S, Martinez A, Mouhsine H, Zagury JF, Jope RS, Beurel E. Stress-induced neuroinflammation is mediated by GSK3-dependent TLR4 signaling that promotes susceptibility to depression-like behavior. *Brain Behav. Immun.*, **53**, 207–222 (2016).

36) Xu Y, Sheng H, Tang Z, Lu J, Ni X. Inflammation and increased IDO in hippocampus contribute to depression-like behavior induced by estrogen deficiency. *Behav. Brain Res.*, **288**, 71–78 (2015).

37) Swaroop S, Sengupta N, Suryawanshi AR, Adlakha YK, Basu A. HSP60 plays a regulatory role in IL-1β-induced microglial inflammation *via* TLR4-p38 MAPK axis. *J. Neuroinflammation*, **13**, 27 (2016).

38) Chen TL, Chang CC, Lin YL, Ueng YF, Chen RM. Signal-transducing mechanisms of ketamine-caused inhibition of interleukin-1 beta gene expression in lipopolysaccharide-stimulated murine macrophage-like RAW264.7 cells. *Toxicol. Appl. Pharmacol.*, **240**, 15–25 (2009).

39) McQuillin A, Bass NJ, Choudhury K, Puri V, Kosmin M, Lawrence J, Curtis D, Gurling HM. Case-control studies show that a non-conservative amino-acid change from a glutamine to arginine in the P2RX7 purinergic receptor protein is associated with both bipolar- and unipolar-affective disorders. *Mol. Psychiatry*, **14**, 614–620 (2009).

40) Boucher AA, Arnold JC, Hunt GE, Spiro A, Spencer J, Brown C, McGregor IS, Bennett MR, Kassiou M. Resilience and reduced c-Fos expression in P2X7 receptor knockout mice exposed to repeated forced swim test. *Neuroscience*, **189**, 170–177 (2011).

41) Monif M, Burnstock G, Williams DA. Microglia: proliferation and activation driven by the P2X7 receptor. *Int. J. Biochem. Cell Biol.*, **42**, 1753–1756 (2010).

42) Zhang K, Liu J, You X, Kong P, Song Y, Cao L, Yang S, Wang W, Fu Q, Ma Z. P2X7 as a new target for chrysophanol to treat lipopolysaccharide-induced depression in mice. *Neurosci. Lett.*, **613**, 60–65 (2016).