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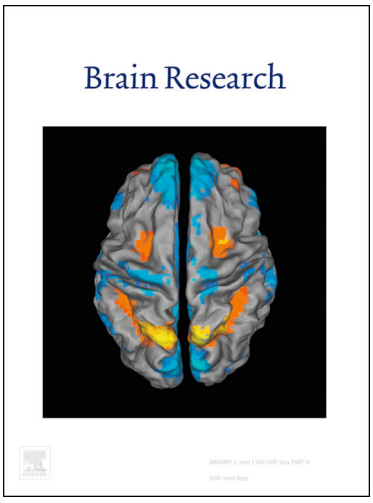

Research report

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PII: S0006-8993(20)30202-X
DOI: https://doi.org/10.1016/j.brainres.2020.146846
Reference: BRES 146846

To appear in: *Brain Research*

Received Date: 14 October 2019
Revised Date: 17 April 2020
Accepted Date: 19 April 2020

Please cite this article as: X. Feng, Y. Fan, C.Y. Chun, Mefenamic Acid can attenuate depressive symptoms by suppressing microglia activation induced upon chronic stress, *Brain Research* (2020), doi: https://doi.org/10.1016/j.brainres.2020.146846

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Abstract: Background: Depression is the most debilitating neuropsychiatric disorder, and psychosocial stressors are major risk factors for the onset of depression. Depression is closely associated with chronic inflammation and microglia are the principal mediators of inflammation in the central nervous system (CNS). Mefenamic acid (MA) and celecoxib are nonselective and selective inhibitors of cyclooxygenase (COX), respectively. COX is a key enzyme in mediating inflammatory response in microglia. In this study, we examine the effects of inhibiting COX by MA on depressive-like behaviors and microglia activation in the hippocampus. Methods: We evaluate the effect of MA on chronic mild stress (CMS) induced depressive-like behavior by sucrose preference and forced swimming tests. Effect of MA on microglia activation in dentate gyrus (DG) of hippocampus was examined by immunohistochemistry. *In vitro* experiments including western blotting and phagocytosis assay were used to investigate the effect of MA on microglia activation. Results: Behavioral assays reveal MA and celecoxib ameliorate CMS-induced depressive-like behavior. Compared to the stressed mice, the number of activated/phagocytic microglia ($Iba1^{+}$/ $CD68^{+}$) in DG of hippocampus significantly decreases in stressed mice treated with MA or celecoxib. MA and celecoxib play a role in inhibiting microglia activation by inhibiting of ERK1/2 and P38 MAPK activation and iNOS expression. MA or celecoxib also reduce the high phagocytic activity of activated microglia. Conclusion: MA inhibits microglia activation/phagocytosis induced upon chronic stress in the hippocampus, which might result in the improvement of depressive symptoms.

Keywords: mefenamic acid, depression, microglia, inflammation, phagocytosis

1. Introduction

The clinical features of depression include significant and lasting depressed mood, anhedonia, pessimism, and can range from the feeling of grief to suicide attempts or acts. The onset of depression may be accompanied by psychotic symptoms such as hallucinations and delusions, neurologic disease symptoms such as impaired ability attention, cognitive impairment, and memory loss, or somatization symptoms such as slow and reducing speech, loss of appetite, weight loss, and flexibility reduction[1-3]. Depression is the result of complex and multifactorial interactions, but many studies indicated that chronic stress plays a crucial role in the development of this disorder [4]. More recent studies show that long-time or severe stress induces both onset and relapse of depressive disorders[5]. According to the preclinical results, chronic stress can affect neuroendocrine or autonomic changes, resulting in the disruption of the ability of the brain to maintain its normal stress response, eventually leading to depression[6]. Unfortunately, the mechanism of depression by chronic stressors has not been fully understood yet, even though it has been studied for decades.

Microglia, the resident tissue macrophages present in the central nervous system, comprise approximately 12% of the brain cells and serve as the brain's primary immune defense in the brain. Microglia have highly branched and ramified morphology in resting state. Upon brain injury, microglia can be activated, resulting in the modification of their shapes into amoeboid form and the production of multiple inflammatory cytokines that can promote neuronal dysfunction and death to aggravate disease progression[7]. Studies have revealed that the number of activated microglia in postmortem brains from depressed patients was significantly greater than those of matched subjects without depressive symptoms, indicating that microglia might be a key player in the onset of neuroinflammation during the progression of depressive disorder[8, 9].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are agents having analgesic and antipyretic effects. NSAIDs inhibit the synthesis of inflammatory mediators prostaglandins (PGs), mainly via the inhibition of cyclooxygenase enzymes (COX)[10, 11]. It has been demonstrated that inflammation is involved in both mood disorders and neurodegenerative diseases, and thus NSAIDs have been reported to have a neuroprotective function in the pathogenesis of both diseases[12, 13]. Previous research also demonstrated that life stressors and social defeat experiences could increase the neuro-inflammatory activity by modifying the levels pro-inflammatory cytokines of the immune system[14]. Moreover, neuroinflammation triggered by stress, in turn, impels the development of depression and causes the initial symptoms of behavioral changes in depression patients[15]. The abnormal expressions of pro-inflammatory cytokines, interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP), play a role in the pathogenesis of depressive disorder[16]. All these imply that inflammation is a significant part of the onset and development of depression, and anti-inflammatory drugs might play a vital role in deferring or preventing the development of depressive disorder via inhibiting the aberrant activation of microglia.

Mefenamic acid (MA), (2',3')-dimethyl-N-phenyl-anthranilic acid, is a fenamate nonsteroidal anti-inflammatory drug. As a nonselective inhibitor of COX, it has lasting antipyretic and anti-inflammatory effects. It also has a potent analgesic effect for mild and moderate pain, such as soft tissue or bone injury pain and inflammation of arthritis. Studies found that COX-2 participates in the processes of neuronal death in cerebral ischemia, and COX inhibitors including MA provide neuroprotective effect and reduce the infarct volume[17, 18]. These suggest that MA might be a new tool for controlling microglia activation/phagocytosis, contributing to attenuate the onset of depression. In this study, we propose to determine whether MA has a suppressive effect on the activation of microglia during

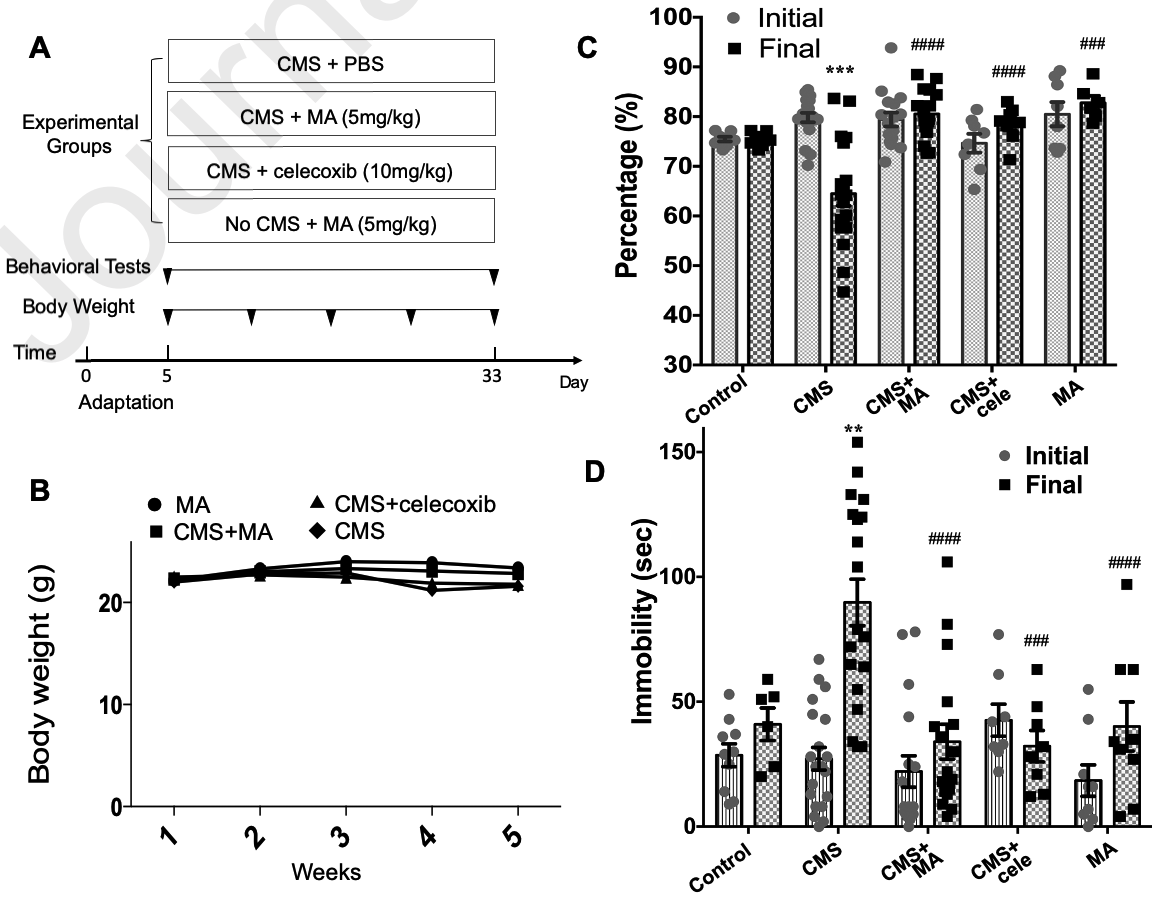
the development of chronic depression induced by chronic stresses and to examine which signaling pathway mediates MA's actions. We utilized celecoxib, one of the NSAIDs, as a positive control to determine whether MA and celecoxib work in the same mode of action by examining a synergistic effect exists between MA and celecoxib. Celecoxib, a selective COX-2 inhibitor, has been demonstrated to decrease the level of both activated p38 MAPK and ERK1/2 in human osteoarthritic chondrocytes previously[19]. Later, studies found that celecoxib decreased LPS-induced increase of IL-1β and TNFα in microglia and attenuated systemic LPS-induced brain inflammation[20]. We found that MA has a significant neuroprotective effect on the onset of depressive-like behavior in the mouse model of chronic depression. MA significantly decreased the number of activated/phagocytic microglia in the DG area of hippocampus. Furthermore, we demonstrated that MA might exert its neuroprotective effect via the inhibition of phagocytosis of activated microglia by downregulating the activation of ERK1/2 and p38MAPK and the phosphorylation of paxillin at Ser[83].

2. Results

2.1. MA attenuated the depressive symptoms caused by chronic mild stresses

To determine if MA has any neuroprotective role on the onset of the depression in the chronic mild unpredictable stress mouse model (CMS), we employed two behavioral assays, sucrose preference test and forced swimming test (Fig. 1). There was no difference in mice body weight among the four experimental groups of stress, stress+MA, stress+celecoxib, and MA, which means the CMS did not affect the body weight (Fig. 1B). In the sucrose preference test, the sucrose solution accounted for 74.65%-80.49% (mass ratio) of daily water intake of mice in all four groups in the initial week (Fig. 1C). Exposure to CMS for four weeks decreased sucrose intake to 64.47% in stressed mice, and there was a significant difference between the stress group and the other three groups ($p<0.05$). While mice in the groups of stress+MA, stress+celecoxib, and MA still held the percentage from 78.53% to 80.56%. This result indicates chronic stressors might cause anhedonia for sweet taste in mice, and both MA and celecoxib could ameliorate the degree of anhedonia efficiently.

In the forced swimming test (FST), a significant increase of immobility time was observed in mice of the stress group after four-week CMS. FST with another cohort of mice without



sucrose preference test showed very similar results (data not shown). In contrast, mice in the groups of stress+MA, stress+celecoxib, and MA did not show a significant difference in the immobility time between the initial week and fourth week (Fig. 1D). These results indicate that COX inhibitors significantly reduce the behavioral despair induced by CMS.

Figure 1. (A) Schedule scheme of chronic mild stress, behavioral tests, and body weight measurements. Behavioral tests include sucrose preference test and forced swimming test. (B) Body weight was measured weekly during the 28-day CMS procedure. No significant difference in body weight was observed among different groups following the CMS procedure. (C) Sucrose preference was tested in the first and final (4^{th}) week during the CMS procedure. MA and celecoxib could attenuate anhedonia symptom caused by CMS. Two-way ANOVA CMS Initial/Final week comparision, $F(1, 106) = 1.734$, $p = .19$, and different treatment comparisions, $F(4, 106) = 10.15$, $p < .0001$, and a significant interaction (treatment and CMS), $F(4, 106) = 12.82$, $p < .0001$. *** $p<0.001$ vs. Control, ###$p<0.001$, ####$p<0.0001$ vs. CMS. (D) Forced swimming test was conducted in the initial and final week during the CMS procedure. Duration of immobility were measured and shown. MA and celecoxib could ameliorate desperate-like symptom induced by CMS. Results shown are mean ± SEM (n=8 in control group, n=21 in CMS group, n=17 in CMS+MA group, n=8 in CMS+celecoxib or MA group). Two-way ANOVA CMS Initial/Final week comparision, $F(1, 113) = 14.42$, $p = .0002$, and Different treatment comparisions, $F(4, 113) = 7.426$, $p < .0001$, and a significant interaction (treatment and CMS), $F(2, 145) = 7.541$, $p < .0001$. ** $p<0.01$ vs. Control; ###$p<0.001$, ####$p<0.0001$ vs. CMS.

2.2. MA decreases microglia activation induced by CMS in DG of the hippocampus

The hippocampus is a crucial area of the brain for many brain functions such as regulations of scenario, spatial memory, and orientation[21]. The *dentate gyrus* (DG) region of the hippocampus, is related to a series of functions, such as memory formation, exploration, stress, and depression. The behavioral effects of antidepressants impart modulation of DG function[22], including the regulation of neurogenesis, and microglia activation, which leads to an increased resilience of animals to unpredictable stress[23]. Therefore, we tested the hypothesis that MA acts via the attenuation of microglia activation in the DG of the hippocampus. To visualize and quantify activated and phagocytic microglia, stereotaxic immunohistochemistry of brain sections from control, CMS, CMS+MA, CMS+celecoxib, and MA mice were performed with Iba1 and CD68 primary antibodies. Iba1, ionized calcium binding adapter molecular 1, is the protein that is expressed specifically in microglia of the central nervous system and CD68 is a transmembrane glycoprotein that is highly expressed in activated and phagocytic (M1) microglia. The number of activated microglia was significantly increased in the DG of stressed mice regardless of the location within the dorso-ventral axis, effects that were normalized by both MA and celecoxib dosing ($p<0.001$) (Fig. 2). There is no significant difference between CMS+MA and CMS+celecoxib mice in the number of CD68 positive microglia, indicating that both are effective in attenuating microglia activation. No significant change of Arg-1 positive microglia (M2) in DG area in CMS mice was observed (data not shown)

2.3. MA has a suppressive effect on inflammatory activation caused by LPS

LPS is the classical inflammatory trigger resulting in microglia activation, brain injury, and alterations in cytokine expression levels *in vitro* and *in vivo*[24]. To determine if MA influences the activation of the mitogen-activated protein kinases (MAPK) p44/42 (ERK1/2), which have been reported as biomarkers of activated microglia, resting BV2 cells were treated with 1μM, 5μM, and 10μM MA in the presence of LPS (100ng/mL) for 6 hours. The phosphorylation state of ERK1/2 and p38 were determined by western blotting assay. Results showed that LPS caused increased phosphorylation of both ERK1/2 and p38 MAPK, effects

that were prevented by co-treatment with MA in a concentrate-dependent way (Fig. 3). Furthermore, the expression of iNOS, which is controlled by the p38 MAPK signaling pathway, was also inhibited by MA in a dose-dependent manner (Fig. 3B and C). These data suggest that MA decreased the microglial activation and inflammatory responses induced by LPS. In another study, we found that the activation of p38 MAPK, ERK1/2, and iNOS upon LPS stimulation can be recapitulated in primary microglia (Yang et al., unpublished data, 2019).

During inflammation, COX-2 plays a vital role in synthesizing PGs from arachidonic acid, which is released to extracellular fluid and then binds to the G-protein coupled receptors (GPCR) EP1-4 to amplify inflammation cascades[25]. Among several subtypes of PGs, PGE2 is the most extensively studied for its role in inflammation[26]. To check if the activation of PGE_2 receptor was necessary for the induction of iNOS and to rule out possible off-target effects of MA, we tested if antagonists of PGE2 receptors have additional inhibitory effects on microglia activation. Resting BV2 cells were treated with 1μM ONO-8130, 1μM PF-04418948, and 1μM MF498 (selective antagonists of EP1, 2, and 4 respectively) in the presence of 5μM MA and 100ng/mL LPS for 6 hours. All EP1, EP2, and EP4 antagonists significantly blocked the activation of ERK1/2, p38 MAPK, and iNOS induced by LPS, but no synergistic effects between MA and antagonists have been observed (Fig. 4), suggesting that MA is exerting an inhibitory effect via blocking PGE_2 production.

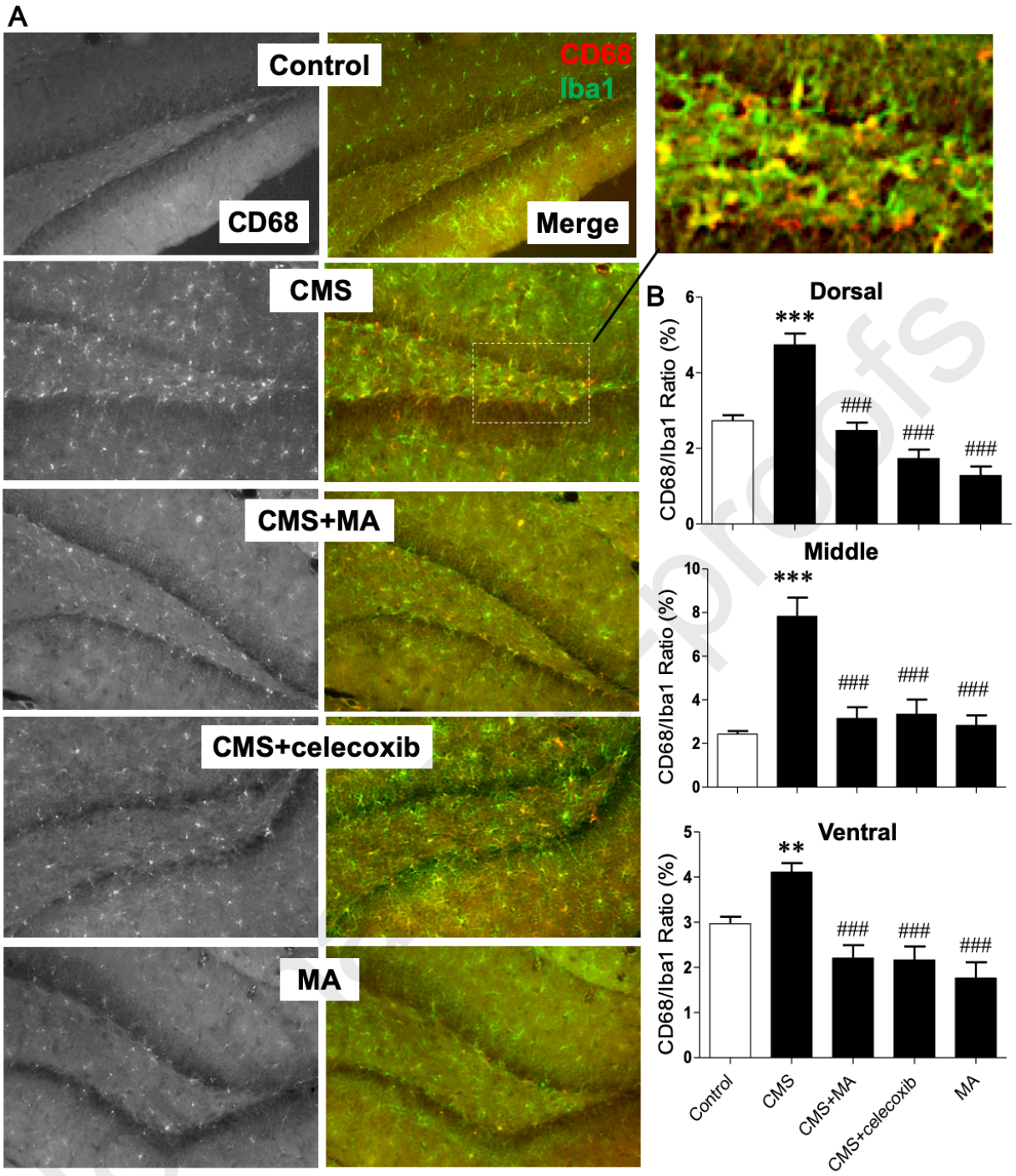


Figure 2. MA or celecoxib treatment decreases the CMS-induced microglial activation in DG region of dorsal, middle, and ventral parts in the hippocampus. Left panels show images of immunohistochemistry using CD68 antibody in the dorsal hippocampus. Right panels show merged images of Iba1 (green) and CD68 (red). MA or celecoxib could decrease the number of activated/phagocytic ($CD68^{+}$) microglia cells in the DG part of the dorsal, middle, and ventral hippocampus. Results shown are mean ± SEM (n=10 in control group, n=14 in CMS group, n=10 in CMS+MA group, n=9 in CMS+celecoxib or MA group). **p<0.01, *** p<0.001 vs. Control; ###p<0.001 vs. CMS.

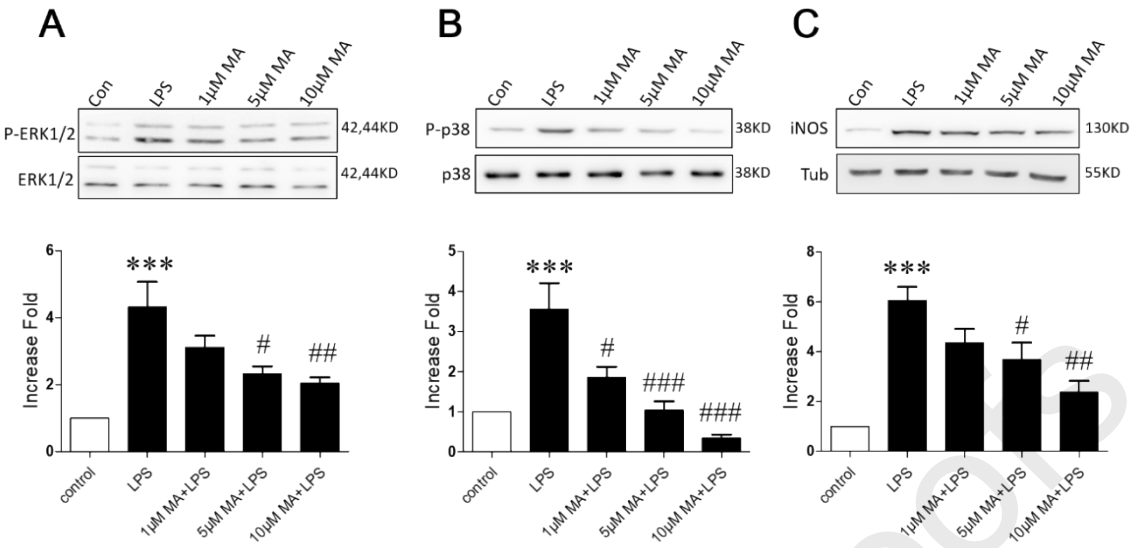


Figure 3. MA treatment inhibits the LPS-induced increase of (A) phospho-ERK1/2 and (B) phospho-p38 and (C) iNOS expression in the BV2 microglia cells in a dose-dependent manner. The intensity value of the control group was set arbitrarily to 1. Ratios of other groups to the control group were then calculated and plotted as fold increase. Results shown are mean ± SEM (n=3 per group). *** p<0.001 vs. Control; #p<0.05, ##p<0.01, ###p<0.001 vs. LPS.

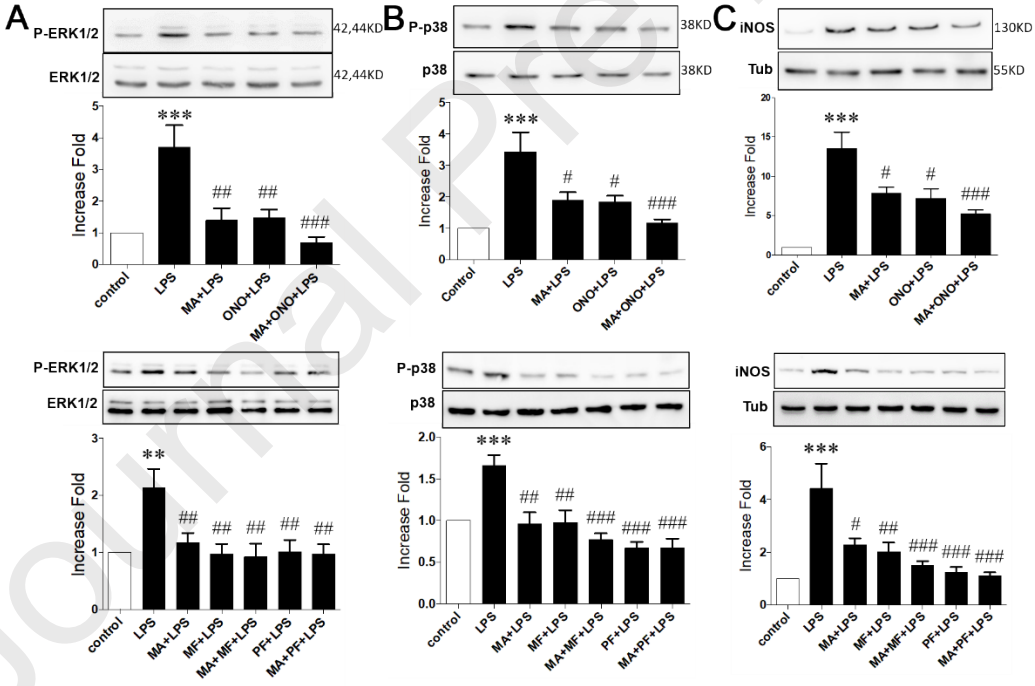


Figure 4. Effects of antagonists of PGE2 receptors on LPS-induced inflammatory responses. All EP1, EP2, and EP4 antagonists had significantly blocked the activation of ERK1/2 (A), p38 MAPK (B), and iNOS expression (C) induced by LPS, but no synergistic effects between MA and antagonists have been observed. Results shown are the mean ± SEM (n=3 per group). **p<0.01, *** p<0.001 vs. Control; #p<0.05, ##p<0.01, ###p<0.001 vs. LPS.

2.4. MA and celecoxib decrease LPS-induced inflammatory responses via blocking the same pathway

In order to examine whether MA and celecoxib are blocking the same pathway to decrease LPS-induced inflammatory responses, BV2 cells were treated with 5μM MA and 5μM celecoxib separately or together in the presence of LPS for 6 hours (Fig. 5). MA and celecoxib decreased LPS-induced microglia activation, respectively, but no synergistic effect was observed when cells were treated with both, suggesting that MA and celecoxib inhibit in the same pathway. Blocking of p38 MAPK activation by the knock-down of COX-2 using siRNA also confirms the action mode of MA (Fig. 5D). To examine if similar molecular effects such as activation of ERK1/2 or p38 MAPK happen in the hippocampus of stressed animals, brain sections were stained with antibodies against phospho-p38MAPK and CD68. Consistent with in vitro results, many activated/phagocytic microglia (CD68+) showed high levels of p38MAPK phosphorylaton in the DG of stressed mice, when compared to matched controls (Fig 6). MA treatment significantly reduced the number of microglia showing high CD68 and p38MAPK activity.

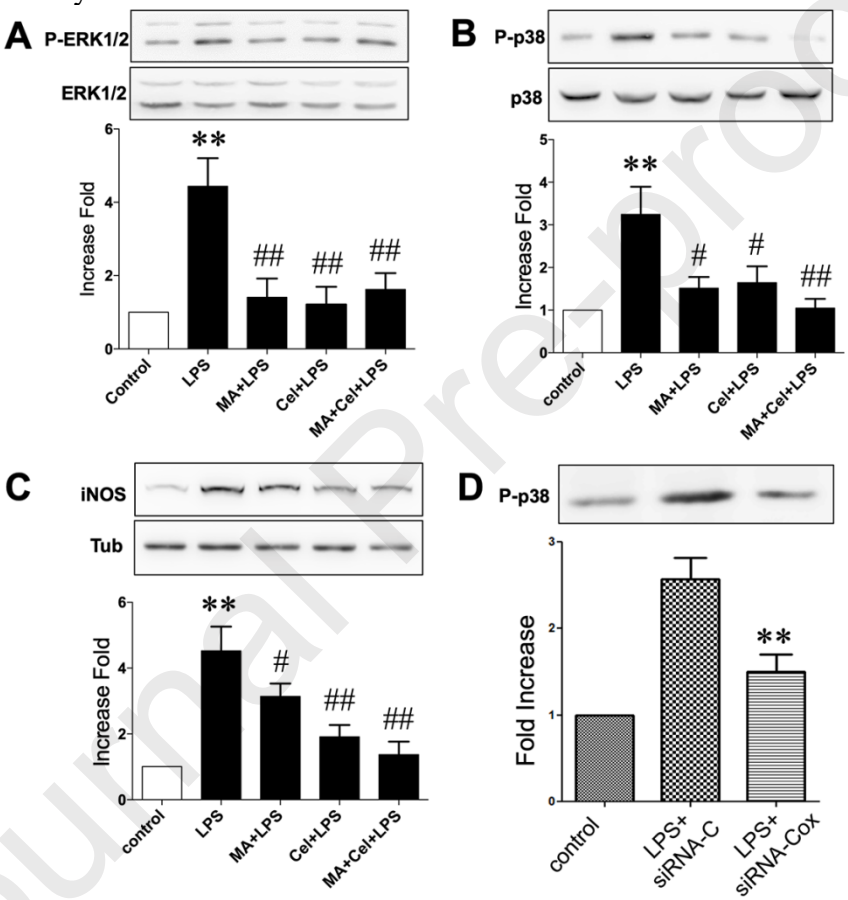


Figure 5. MA and celecoxib show the equal efficacy in inhibiting the LPS-induced increase of (A) P-ERK1/2 and (B) P-p38 MAPK and (C) iNOS expression in the BV2 microglia cells. When cells were treated with both MA and celecoxib, no synergistic increase of inhibition was observed between MA+LPS group and celecoxib+LPS group, suggesting they are working in the same pathway. Results shown are mean ± SEM (n=4 per group). **p<0.01 vs. Control; #p<0.05, ##p<0.01 vs. LPS. (D) Knock-down of COX-2 by siRNA blocks the activation of p38 MAPK induced by LPS. **p<0.01 vs scrambled siRNA (siRNA-C).

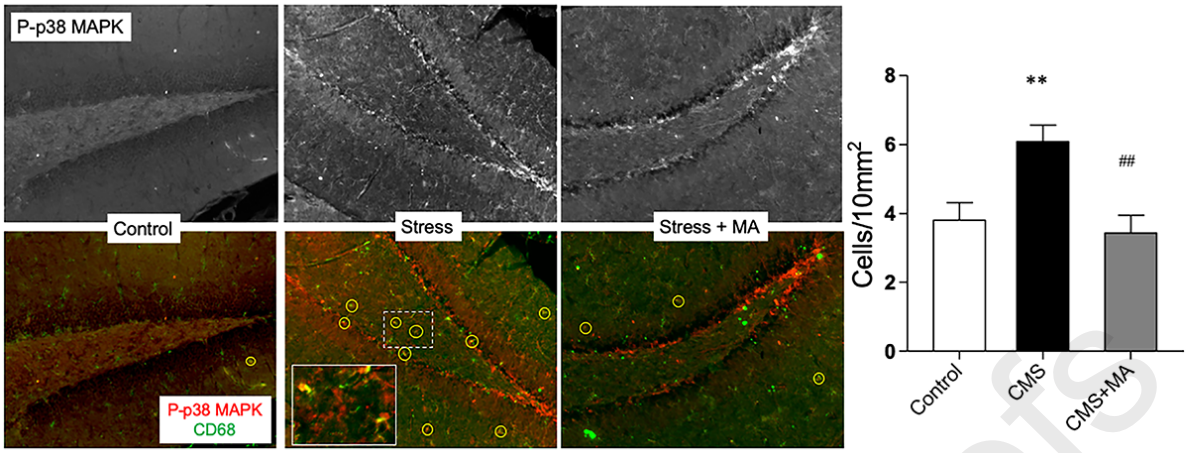


Figure 6. MA treatment decreases the CMS-induced p38MAPK activation in activated microglia in DG region of hippocampus in stressed mice. Immunohistochemical staining of brain sections from stressed mice revealed many activated microglia ($CD68^+$) showed high level of p38MAPK activity (marked by yellow circles), which can be blocked by MA treatment. Results shown are mean ± SEM (n=8 in control or CMS+MA group, n=9 in CMS group). **p<0.01 vs. Control; ##p<0.01 vs. CMS.

2.5. MA blocks microglia phagocytosis

Phagocytosis is the first and fundamental innate immune defense against foreign pathogens. It includes a series of complex processes, such as rearrangement of the cytoskeleton and signaling transduction. p38 MAPK activation controls phagocytosis via the phosphorylation of paxillin, which influences the dynamic changes in filamentous actin[27] formation, and thus participates in controlling cytoskeleton functions. Phosphorylation of paxillin on Ser83 by p38MAPK was reported to have a significant impact on the phagocytic activity of microglia[28], and therefore, we examined the level of paxillin Ser^{83} phosphorylation in primary microglia. Primary microglia cells were treated with 10μM MA or 5μM celecoxib in the presence of LPS (100ng/mL) for 6h and paxillin Ser^{83} phosphorylation levels were examined by western blot (Fig. 7A). Both MA and celecoxib inhibited Ser^{83} phosphorylation induced by LPS. Then we examined the extent by which the phagocytic activity of the primary microglia was affected by MA and celecoxib in cells activated by LPS using fluorescent beads. Resting primary microglia were treated with 10μM MA or 5μM celecoxib in the existence of LPS (100ng/mL) for 6h, and then phagocytic engulfment of fluorescent beads was examined by confocal microscopy (Fig. 7B). Primary microglia showed enhanced phagocytic activity upon LPS treatment, while MA and celecoxib attenuated the LPS-induced phagocytic activity. This result is consistent with previous results indicating that MA and celecoxib share anti-inflammatory effects on LPS induced microglia activation. In conclusion, MA has the neuroprotective potential for controlling microglia activation/phagocytosis upon chronic stress and improving depression symptoms.

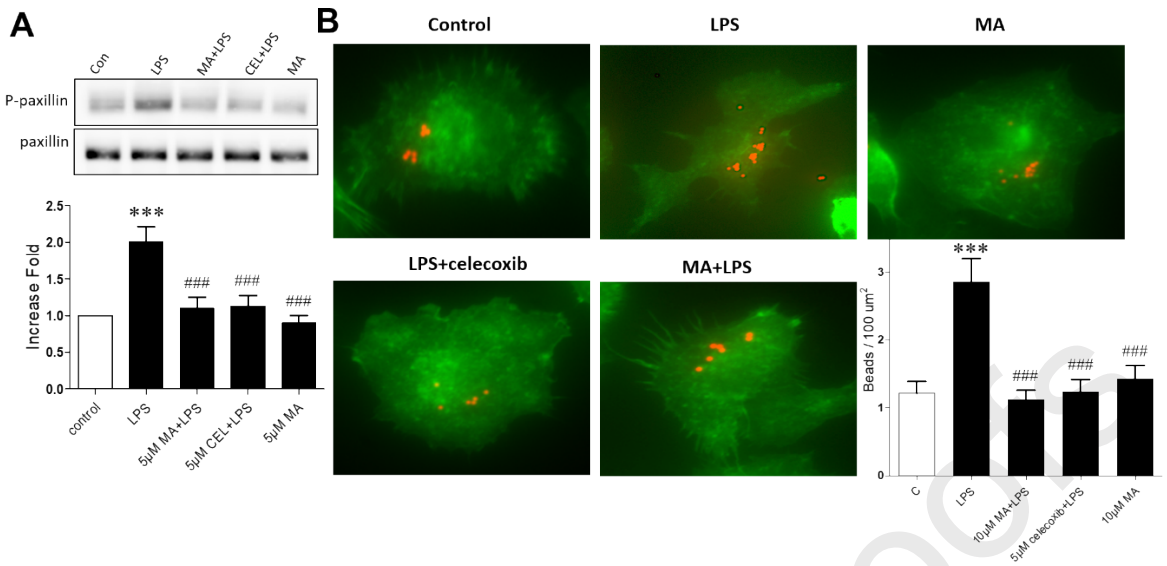


Figure 7. (A) MA or celecoxib treatment inhibits the increase of paxillin phosphorylation at Ser[83] induced by LPS. (B) MA or celecoxib blocks microglia phagocytosis caused by LPS in primary microglia. Phagocytic activity of microglia was measured by examining the phagocytosis of beads labeled with Alexa 594 (1 μm diameter) by cells for 30 min, followed by phalloidin staining. Results shown are the mean ± SEM (n=3 per group for western blot, n=10 cells per group for phagocytosis assay). *** p<0.001 vs. Control; ###p<0.001 vs. LPS. Scale bar = 10 μm.

Activated microglia in the hippocampus has been suggested to suppress neurogenesis presumably by inhibiting proliferation or survival of neural progenitor cells[29-31]. Adult animals continue to produce new neurons in the dentate gyrus of hippocampus and Ki-67, a cellular marker for proliferation, can be used as an endogenous marker for neurogenesis[32]. To quantify Ki-67 labeling in the proliferative zone of DG, brain sections were labeled with Ki-67 antibody and the numbers of labeled cells in DG area were counted. Compared to mice treated with MA only, a significant reduction of the number of Ki-67-labelled cells was observed in the DG of stressed mice (Fig. 8). The reduction of neurogenesis in stressed mice can be reversed to the control level by the treatment of MA.

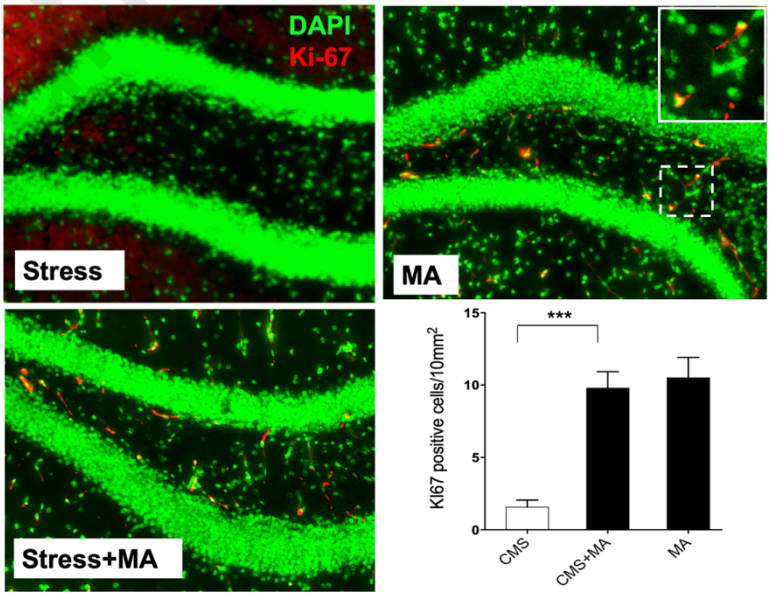


Figure 8. Effect of MA on cell proliferation in the dentate gyrus of chronically stressed mice. Brain sections were stained with anti-Ki-67 antibody (left panels). Merged images of Ki-67 (red) and DAPI (green) are shown in right panels. Inside panel shows a high magnification Histogram of the density (cells/mm2) of Ki-67–expressing cells in the granule cell layer of the dentate gyrus is shown. Results shown are the mean ± SEM (n=10). *** $p<0.001$ vs. CMS.

3. Discussion

In the present study, we demonstrate that mefenamic acid treatment ameliorated depression-like behaviors induced by unpredictable mild chronic stress in mice and attenuated the pro-inflammatory state of activated BV2 microglia cells by decreasing the activation of ERK1/2, p38 MAPK, and iNOS. We also provide a link between these results by showing the effects of mefenamic acid on a reduced number of activated and phagocytic microglia ($CD68^+$) in DG area of the hippocampus. These effects of mefenamic acid presumably resulted from its inhibition of COX as celecoxib, another COX inhibitor, showed almost identical results.

Depression, a common psychiatric disorder, is characterized by symptoms of anhedonia, tiredness, sleep disorders, and persistent feeling of sadness. It is also major causes of disability and death by suicide. Depressive disorders would be the second leading cause of disease burden by 2020[33]. Based on its prevalence, it has been an enormous burden for patients, families, and the whole society. Depression has been often described as a stress-related disorder[5] as people who are suffering from stressful experiences have more possibilities for the onset of depression. Therefore, our protocol of using unpredictable mild chronic stresses mimics the environmental stressors that human beings face in their lives. Four-week exposure to the unpredictable mild chronic stress was reported to be suitable to cause a series of depressive behavioral phenotypes of mice[5, 34]. Two behavioral tests, sucrose preference test and forced swimming test, have been well defined to examine depression-like symptoms[35]. Our results are consistent with previous studies, suggesting that exposure to CMS resulted in a significant decrease in sucrose preference and a more extended immobility period in the forced swimming test compared to the control groups[36-38]. However, it should be noted that modeling of human neuropsychiatric disorders in animals has some limitations due to subjective nature of many key symptoms and the lack of biomarkers and objective diagnostic tests[39]. It is challenging to rule out the possibility that experimentally induced defeat or despair (which might be physiological (i.e., adaptive) rather than pathological) can be extrapolated as being depression-like. It is also difficult to differentiate depression-like behaviors from anxiety-like behaviors which can also be induced by stress[40].

Conventional clinical treatment including selective serotonin reuptake inhibitors and trycyclic antidepressants are ineffective to a large number of patients, suggesting that additional factors play a role in the deterioration of this disorder and cause treatment resistance and recurrence. Many studies have found the elevation of pro-inflammatory biomarkers and a larger number of activated microglia cells in the brains during the development of animal model of depression[41]. These suggested that neuroinflammation might be a critical factor that plays a role in the pathogenesis of the depressive disorder. NSAIDs, the most widely used anti-inflammatory drugs, have the potential to attenuate depressive symptoms because they can pass through the blood-brain barrier.

Celecoxib might be the one most intensively investigated NSAIDs in attenuating the symptoms of depression in animal models. Studies suggest that, in an olfactory bulbectomized rat model of depression, celecoxib dosing attenuates higher locomotor activity in the open field test and decreases the levels of IL-1, TNF-α and IL-10 in both in hippocampus and hypothalamus[42]. Furthermore, in a 21-day CMS-induced depressive disorder rat model, celecoxib could alleviate the depressive-like behaviors and inhibit the increasing levels of COX-2 and PGE2 in the rat brain[43], which is consistent with our results. In the clinical trials,

adding celecoxib to the regular medication of patients could improve the depressive symptoms rapidly[44], also having significant synergistic effects on the long-term amelioration of depressive symptom[12, 45-47]. Our findings not only broaden the effectiveness of MA exerting a neuroprotective function by decreasing microglia activation in CMS mice model of depression but also illustrated, for the first time, that MA and celecoxib almost share an identical mechanism for modifying the progression and exacerbation of microglia activation. Both compounds could ameliorate the activation of ERK1/2 and p38 MAPK, and the expression of iNOS induced by LPS in BV2 microglia cells.

A previous study suggested that depressive-like behavior caused by acute stresses in mice can be reversed by COX-1 inhibitors, rather than selective COX-2 inhibitors. Both nonselective COX inhibitors (indomethacin and ibuprofen) and selective COX-1 inhibitors (piroxicam and sulindac) can attenuate acute depression-induced behaviors while selective COX-2 inhibitors (nimesulide and nuflimic acid) have no effect in the same experimental conditions[48]. However, in our study, MA, a nonselective COX inhibitor, and celecoxib, a selective COX-2 inhibitor, share the same effect of attenuating depression-like behaviors. This apparent discrepancy could be explained by the difference between the stress models. We chose the chronic stress model by treating mice with unpredictable psychological stressors, while previous results mentioned above were from systemic inflammation model by injecting LPS or the acute stress model. Our result of COX-2 knock-down experiment also suggests that COX-2 plays a role in the activation of microglia. The similarity between MA and celecoxib in inhibiting CMS-induced neuro-inflammation might result from the inhibition of COX-2, which blocks ERK1/2 and p38 MAPK activation and the expression of iNOS induced by inflammation trigger. Both ERK1/2 and p38 MAPK are subgroups of mitogen-activated protein kinases, which transport the signals from the cell surface to the nucleus and respond to stress or inflammation reaction. Our data is consistent with the hypothesis that increases in the activated forms of ERK1/2 and p38 MAPK are a hallmark of activated microglia in the CNS, which is typically observed with LPS-induced stimulation[26, 49, 50]. Furthermore, both activated ERK1/2, and p38 MAPK are the upstream kinases for the phosphorylation of paxillin-Ser[38], causing cytoskeleton changes and microglia phagocytosis[28]. And LPS induced microglia chemotactic migration could be blocked by inhibiting the activation of ERK1/2[51]. Our result is also consistent with a previous report that pharmacological inhibition and siRNA knock-down of p38MAPK abolished pressure-stimulated phagocytosis of THP-1 macrophages[52], suggesting that activation of MAPK signaling pathways is essential to the phagocytic phenotype observed in activated microglia, and that this is a common signaling mechanism underlying immune activation in the brain following chronic stress exposure. It has been demonstrated that microglial PGE2 receptor was necessary for lipopolysaccharide (LPS)-activated microglia-mediated neurotoxicity, as well as induction of iNOS[53]. Our data also indicate that MA is exerting an inhibitory effect on iNOS induction via blocking PGE_2 production. However, we can't rule out the possibility that mefenamic acid could inhibit binding of PGE2 to its specific receptor in a dose-dependent manner as reported[54].

Hippocampus is the region of the brain that is associated with long term and spatial memory. Studies have demonstrated that dorsal hippocampus is involved in the cognitive and spatial memory associated with exploration and locomotion and the ventral hippocampus takes charge of motivational and emotional behaviors related to anxiety, frustration, and depression, while the middle hippocampus encodes the cognitive and spatial knowledge into motivation and movement which is critical for survival[55-57]. DG region serves as the first place to converge the inputs from the environment into the hippocampus to moderate neuroplasticity [58]. Preclinical evidence suggested that neuroinflammation characterized by glial cell activation occurred in the hippocampus after CMS exposure[59]. Our study also demonstrated that the effects of MA on depressive-like behavior induced by CMS parallel the reductions in

phagocytic/activated microglia in all three zones of the DG. These data are consistent with previous suggestions that the behavioral changes of locomotion and emotion induced by CMS are associated with dorsal and ventral DG regions of the hippocampus. In contrast, one study found that the level of microglia activation was unchanged after chronic stress exposure[60]. The discrepancy might be due to differences in CMS procedure and duration, which trigger adaptation responses.

One of the most sensitive phenotypes observed in response to the neurotoxic effects of stress in mice is the reduction in hippocampal neurogenesis in depression, which has been supported by many studies [61-63]. Neurogenesis is a key process of neurons generating from neural stem cells and progenitor cells, and relates to neuronal plasticity, emotional behaviors, and cognitive function. Studies demonstrated that reduced neurogenesis is associated with depressive disorder[61-63] and the anxiety-like or depressive-like behavior and cognitive inflexibility in mice[64-66]. Reduced neurogenesis could decrease the activity of stress-responsive cells, silence the adult-born neurons that are essential for resilience mechanisms, and increase vulnerability to stressors[23, 67, 68]. Microglia activation has been proposed to be the link between stress and reduced neurogenesis. Activated microglia in the hippocampus has been suggested to suppress neurogenesis presumably by inhibiting proliferation or survival of neural progenitor cells or causing neural stem cell dysfunction[29-31]. Our study showed that, when exposed to stresses, the number of activated/phagocytic microglia increases in the DG subregion of the hippocampus. Our study further provided an evidence for the a significant reduction of neurogenesis marker in DG region of stressed mice and the rescue of neurogenesis by the treatment of MA, suggesting a possibility that phagocytic microglia might engulf new-born neurons. In conclusion, MA inhibits microglia activation/phagocytosis induced upon chronic stress in the hippocampus, resulting in the improvement of depressive symptoms.

4. Materials and Methods

4.1. Animals and housing

Male C57BL/6 mice with initial weights of 20-25 g (7 weeks old) were purchased from Beijing Huafukang Bio Co. (Beijing, China). All mice were housed singly at an average room temperature of 22±1℃ and humidity of 50–60% with a 12-h light/dark cycle (lights on from 8:30 am to 8:30 pm) and given access to food and water. The animals were acclimatized for at least 5 days before use in the experiments. Body weight measurements were taken weekly in all groups. All experiments were conducted in accordance with the guidelines of Animal Care and Use Committee of Tianjin Medical University. Every effort was made to minimize the number of animals used and their suffering.

4.2. Chemicals and reagents

The mefenamic acid and celecoxib were purchased from the Selleck Co. (Shanghai, China). The ONO-8130 was purchased from the Cayman Chemical Co. (Ann Arbor, Michigan, USA). The MF498 and PF-04418948 were purchased from the MCE Co. (NJ, USA). All the chemicals were dissolved in dimethyl sulfoxide (DMSO) and diluted in phosphate buffered saline (PBS). For animal experiments, the final concentration of DMSO used was less than 1% (v/v). For cell experiments, the final concentration of DMSO used was less than 0.1% (v/v).

4.3. CMS procedure and drug treatments

The CMS-exposed and control groups were housed in separate cages under similar conditions. For our CMS procedure, we used various stressors, of which the sequence was intentionally designed to maximize unpredictability. All CMS mice were exposed to one-two

stressor each day for 28 days. Briefly, CMS consisted of exposure to a variety of the following unpredictable stressors: food deprivation for 24 h, water deprivation for 24 h, wet mattress for 24 h, placing the animal in a 50mL centrifuge tube for half an hour, shaking home cages for 15 min, placing the animal in a dirty cage full of excretory products for 24 h and pair-housing for 24 h[38, 69]. These stressors were randomly scheduled and repeated throughout the 4-week experiment. Different groups of animals were administered with vehicle (0.5% DMSO in PBS 100 μL/20 g), mefenamic acid (5mg/kg), and celecoxib (10 mg/kg) respectively. Mefenamic acid and celecoxib were administrated by means of intraperitoneal injection once daily during the whole CMS procedure. The behavioral testing was performed at least 12 h after the last dose to avoid the acute effects of drug treatment. The experimental design is described in Fig. 1.

4.4. Sucrose preference test

After five days of initial adaption to the laboratory, mice were trained to drink a sucrose solution. Mice were exposed to two standard drinking bottles with random positions, one containing 1% sucrose and the other tap water for three days. After this phase, mice were divided into four experimental groups: CMS group, CMS + MA group, CMS + celecoxib group, and MA group. Then all mice were exposed to the sucrose solution and water for four days. After a 4-week duration of CMS exposure, repeat the 7-day test, including the training phase and test phase, as described above. The position of the two bottles (right/left) was varied randomly from trial to trial and bottles were rotated every day to prevent place-preference by the animals. The relative sucrose intake preference was calculated by the percentage of sucrose intake of total intake.

4.5. Forced swimming test

Forced swimming test (FST) is one of the most commonly used assays for the study of depressive-like behavior in rodents and the main advantages of FST are that it is relatively easy to perform and that results can be easily and quickly analyzed. Furthermore, a wealth of data from many studies allows researchers to compare and contrast results. It has been shown that FST can manifest factors that are influenced by depression in humans, including changes in food consumption, sleep abnormalities, and anhedonia[70]. Despite of its usefulness, some scientists claimed that FST does not provide sufficient mechanistic specificity required for understanding mechanisms underlying human depression. However, FST can indicate sensitivity to a broad range of antidepressant drugs or experimental manipulations[70]. FST can be crucial to answering scientific questions if it is adequately used by adhering to certain procedural details and minimizing unwarranted stress to the mice. The results from FSTs can also be considered alongside those from various other methods, including the sucrose consumption test. The procedure employed here was based on the previous description[71]. Mice were dropped gently and individually into glass cylinders (height 22 cm, diameter 13 cm), which contains 12 cm of water maintained about 22-25°C. Left mice in the water for 6 min, and the surroundings should be quiet and unmanned during that time. The duration of immobility was recorded during the last 4 min of the 6 min testing period. A mouse could be judged to be immobile when it floated in an upright position and made only small movements to keep its head above water.

4.6. Western blotting

The cell lysate was obtained from the BV2 or primary microglia cells (generous gift from Dr. Imshik Lee at Nankai University, China). When the confluence reached 70%-80%, cells were treated with different drugs for 6 hours. The total cell lysate was separated on 10% SDS–

PAGE, and then the gel was transferred at 12 V to the polyvinylidene fluoride (PVDF) membrane for one hour. The membranes were blocked with Tris-buffered saline with 0.1% Tween 20 (TBST, v/v) containing 5% non-fat dried milk for one hour at room temperature followed by incubation with primary antibodies at 4℃ overnight. The primary antibodies used were rabbit anti-phospho-p42/44 MAPK, rabbit anti-p42/44 MAPK (1:2500, Cell Signaling Technology, USA), rabbit anti-phospho-p38 MAPK, rabbit anti-p38 MAPK (1:5000, Cell Signaling Technology, USA), rabbit anti-iNOS (1:2500, Cell Signaling Technology, USA), mouse anti-α Tubulin, (1:2500, Cell Signaling Technology, USA). The membranes were washed extensively with TBST for 5 min *three times. Then incubated with secondary antibodies in TBST containing 5% non-fat dried milk for 1 h at room temperature. After washing procedure, the signal was detected using an enhanced chemiluminescence method (ECL kit, USA). The result images were imaged and analyzed using the Image J Analysis Software.

4.7. Immunofluorescence microscopy

The animals were anesthetized by inhaling 2% isoflurane (RWD life science, Shenzhen, China) and perfused transcardially with PBS, then with a fixative containing 4% paraformaldehyde (Solarbio, Beijing, China) in the PBS. Took the brain out and soaked in the fixative containing 4% paraformaldehyde for 12 h. After fixing procedure, brains were dehydrated in 30% sucrose (BODIchem, Tianjin, China) solution (m/v). Coronal sections, including the hippocampus, were cut at 30 μm in a cryostat (CM 1950; Leica, Germany). Selected sections from mouse hippocampus of different groups were soaked in the Tris Buffered Saline with 0.2% Triton X-100 (v/v) for half an hour at room temperature followed by blocked in TBST containing 5% goat serum at 37℃ for 30 min. Then, incubated in primary antibodies of rabbit anti-Iba1 (Proteintech, USA), rabbit anti p-P38 (Cell Signaling Technology, USA), and rat anti-CD68 (Cell Signaling Technology, USA) at 4℃ overnight. Subsequently, brain slices were washed extensively with TBST and incubated in a mixture of the appropriate secondary antibodies: mouse anti-rat (1:500) and donkey anti-rabbit (1:500) at 4℃ for two hours. After washing with TBST extensively for three times, sections were mounted in Fluorsave mounting medium (Calbiochem, USA) and examined using a $A1^{+}$ confocal microscope (Nikon, Japan). The result images were imaged and analyzed using the Image J Analysis Software.

4.8. Phagocytosis assay

Primary microglia cells were incubated with latex beads labeled with Alexa 594 (1 μm diameter) for 30 min. Then Cells were fixed, washed, and permeabilized with 0.1% Triton X-100 then fixed, and further stained with Alexa 488- labeled phalloidin to visualize F-actin. To exclude beads bound to the surface of the cells, cell bottoms were focused on using F-actin. Engulfed beads and F-actin were imaged with fluorescence microscopy and analyzed by Image J Software.

4.9. Data analysis

The data are presented as mean ± SEM and analyzed with the GraphPad Prism 5.0 software. The significance of the difference between the controls and samples treated with various compounds was determined by a one-way ANOVA followed by Tukey test, compare all pairs of columns. Data from sucrose preference test and forced swim test were analyzed by two-way ANOVA followed by Tukey test. The p-value, $p<0.05$, was considered statistically significant.

Acknowledgements: We thank members of the Chung lab for useful discussions and critical reading of the manuscript. We are indebted to Dr. Ana MD Carneiro for helpful discussions. This work is partially supported by a grant from the National Science Foundation of China (31671450).

Conflict of interest: The authors declare no competing or financial interests.

Author contributions: X.F., Y.F., and C.C. performed experiments and analyzed the data. X.F. and C.C. conceived the experiments, analyzed the data and wrote the paper.

References

1. Lam, R. W.; McIntosh, D.; Wang, J.; Enns, M. W.; Kolivakis, T.; Michalak, E. E.; Sareen, J.; Song, W. Y.; Kennedy, S. H.; MacQueen, G. M.; Milev, R. V.; Parikh, S. V.; Ravindran, A. V.; Group, C. D. W., Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 Clinical Guidelines for the Management of Adults with Major Depressive Disorder: Section 1. Disease Burden and Principles of Care. *Can J Psychiatry* **2016,** *61* (9), 510-23.
2. Lam, R. W.; Kennedy, S. H.; Parikh, S. V.; MacQueen, G. M.; Milev, R. V.; Ravindran, A. V.; Group, C. D. W., Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 Clinical Guidelines for the Management of Adults with Major Depressive Disorder: Introduction and Methods. *Can J Psychiatry* **2016,** *61* (9), 506-9.
3. Nestler, E. J.; Barrot, M.; DiLeone, R. J.; Eisch, A. J.; Gold, S. J.; Monteggia, L. M., Neurobiology of depression. *Neuron* **2002,** *34* (1), 13-25.
4. Krishnan, V.; Nestler, E. J., The molecular neurobiology of depression. *Nature* **2008,** *455* (7215), 894-902.
5. Elizalde, N.; Gil-Bea, F. J.; Ramirez, M. J.; Aisa, B.; Lasheras, B.; Del Rio, J.; Tordera, R. M., Long-lasting behavioral effects and recognition memory deficit induced by chronic mild stress in mice: effect of antidepressant treatment. *Psychopharmacology (Berl)* **2008,** *199* (1), 1-14.
6. Radley, J.; Morilak, D.; Viau, V.; Campeau, S., Chronic stress and brain plasticity: Mechanisms underlying adaptive and maladaptive changes and implications for stress-related CNS disorders. *Neurosci Biobehav Rev* **2015,** *58*, 79-91.
7. Frank-Cannon, T. C.; Alto, L. T.; McAlpine, F. E.; Tansey, M. G., Does neuroinflammation fan the flame in neurodegenerative diseases? *Molecular neurodegeneration* **2009,** *4*, 47.
8. Singhal, G.; Baune, B. T., Microglia: An Interface between the Loss of Neuroplasticity and Depression. *Frontiers in cellular neuroscience* **2017,** *11*, 270.
9. Shibata, M.; Suzuki, N., Exploring the role of microglia in cortical spreading depression in neurological disease. *J Cereb Blood Flow Metab* **2017,** *37* (4), 1182-1191.
10. Vellani, V.; Moschetti, G.; Franchi, S.; Giacomoni, C.; Sacerdote, P.; Amodeo, G., Effects of NSAIDs on the Release of Calcitonin Gene-Related Peptide and Prostaglandin E2 from Rat Trigeminal Ganglia. *Mediators Inflamm* **2017,** *2017*, 9547056.
11. Yoshitake, R.; Saeki, K.; Watanabe, M.; Nakaoka, N.; Ong, S. M.; Hanafusa, M.; Choisunirachon, N.; Fujita, N.; Nishimura, R.; Nakagawa, T., Molecular investigation of the direct anti-tumour effects of nonsteroidal anti-inflammatory drugs in a panel of canine cancer cell lines. *Vet J* **2017,** *221*, 38-47.
12. Na, K. S.; Lee, K. J.; Lee, J. S.; Cho, Y. S.; Jung, H. Y., Efficacy of adjunctive celecoxib treatment for patients with major depressive disorder: a meta-analysis. *Prog Neuropsychopharmacol Biol Psychiatry* **2014,** *48*, 79-85.
13. Khansari, P. S.; Halliwell, R. F., Evidence for neuroprotection by the fenamate NSAID,

mefenamic acid. *Neurochem Int* **2009,** *55* (7), 683-8.
14. Glaser, R.; Kiecolt-Glaser, J. K., Stress-induced immune dysfunction: implications for health. *Nat Rev Immunol* **2005,** *5* (3), 243-51.
15. Slavich, G. M.; Irwin, M. R., From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. *Psychol Bull* **2014,** *140* (3), 774-815.
16. Bufalino, C.; Hepgul, N.; Aguglia, E.; Pariante, C. M., The role of immune genes in the association between depression and inflammation: a review of recent clinical studies. *Brain Behav Immun* **2013,** *31*, 31-47.
17. Nogawa, S.; Zhang, F.; Ross, M. E.; Iadecola, C., Cyclo-oxygenase-2 gene expression in neurons contributes to ischemic brain damage. *J Neurosci* **1997,** *17* (8), 2746-55.
18. Khansari, P. S.; Halliwell, R. F., Mechanisms Underlying Neuroprotection by the NSAID Mefenamic Acid in an Experimental Model of Stroke. *Front Neurosci* **2019,** *13*, 64.
19. Takahashi, T.; Ogawa, Y.; Kitaoka, K.; Tani, T.; Uemura, Y.; Taguchi, H.; Kobayashi, T.; Seguchi, H.; Yamamoto, H.; Yoshida, S., Selective COX-2 inhibitor regulates the MAP kinase signaling pathway in human osteoarthritic chondrocytes after induction of nitric oxide. *Int J Mol Med* **2005,** *15* (2), 213-9.
20. Fan, L. W.; Kaizaki, A.; Tien, L. T.; Pang, Y.; Tanaka, S.; Numazawa, S.; Bhatt, A. J.; Cai, Z., Celecoxib attenuates systemic lipopolysaccharide-induced brain inflammation and white matter injury in the neonatal rats. *Neuroscience* **2013,** *240*, 27-38.
21. Lee, C. H.; Ryu, J.; Lee, S. H.; Kim, H.; Lee, I., Functional cross-hemispheric shift between object-place paired associate memory and spatial memory in the human hippocampus. *Hippocampus* **2016,** *26* (8), 1061-77.
22. Sahay, A.; Hen, R., Adult hippocampal neurogenesis in depression. *Nat Neurosci* **2007,** *10* (9), 1110-5.
23. Anacker, C.; Luna, V. M.; Stevens, G. S.; Millette, A.; Shores, R.; Jimenez, J. C.; Chen, B.; Hen, R., Hippocampal neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus. *Nature* **2018,** *559* (7712), 98-102.
24. Lee, J. Y.; Nam, J. H.; Nam, Y.; Nam, H. Y.; Yoon, G.; Ko, E.; Kim, S. B.; Bautista, M. R.; Capule, C. C.; Koyanagi, T.; Leriche, G.; Choi, H. G.; Yang, J.; Kim, J.; Hoe, H. S., The small molecule CA140 inhibits the neuroinflammatory response in wild-type mice and a mouse model of AD. *Journal of neuroinflammation* **2018,** *15* (1), 286.
25. De Keijzer, S.; Meddens, M. B.; Torensma, R.; Cambi, A., The multiple faces of prostaglandin E2 G-protein coupled receptor signaling during the dendritic cell life cycle. *Int J Mol Sci* **2013,** *14* (4), 6542-55.
26. Xia, Q.; Hu, Q.; Wang, H.; Yang, H.; Gao, F.; Ren, H.; Chen, D.; Fu, C.; Zheng, L.; Zhen, X.; Ying, Z.; Wang, G., Induction of COX-2-PGE2 synthesis by activation of the MAPK/ERK pathway contributes to neuronal death triggered by TDP-43-depleted microglia. *Cell Death Dis* **2015,** *6*, e1702.
27. Yamamori, T.; Inanami, O.; Nagahata, H.; Cui, Y.; Kuwabara, M., Roles of p38 MAPK, PKC and PI3-K in the signaling pathways of NADPH oxidase activation and phagocytosis in bovine polymorphonuclear leukocytes. *FEBS Lett* **2000,** *467* (2-3), 253-8.
28. Fan, Y.; Chen, Z.; Pathak, J. L.; Carneiro, A. M. D.; Chung, C. Y., Differential Regulation of Adhesion and Phagocytosis of Resting and Activated Microglia by Dopamine. *Frontiers in cellular neuroscience* **2018,** *12*, 309.
29. Bachstetter, A. D.; Morganti, J. M.; Jernberg, J.; Schlunk, A.; Mitchell, S. H.; Brewster, K. W.; Hudson, C. E.; Cole, M. J.; Harrison, J. K.; Bickford, P. C.; Gemma, C., Fractalkine and CX 3 CR1 regulate hippocampal neurogenesis in adult and aged rats. *Neurobiol Aging* **2011,** *32* (11), 2030-44.
30. Iosif, R. E.; Ekdahl, C. T.; Ahlenius, H.; Pronk, C. J.; Bonde, S.; Kokaia, Z.;

Jacobsen, S. E.; Lindvall, O., Tumor necrosis factor receptor 1 is a negative regulator of progenitor proliferation in adult hippocampal neurogenesis. *J Neurosci* **2006,** *26* (38), 9703-12.
31. Monje, M. L.; Toda, H.; Palmer, T. D., Inflammatory blockade restores adult hippocampal neurogenesis. *Science* **2003,** *302* (5651), 1760-5.
32. Kee, N.; Sivalingam, S.; Boonstra, R.; Wojtowicz, J. M., The utility of Ki-67 and BrdU as proliferative markers of adult neurogenesis. *J Neurosci Methods* **2002,** *115* (1), 97-105.
33. Menken, M.; Munsat, T. L.; Toole, J. F., The global burden of disease study: implications for neurology. *Arch Neurol* **2000,** *57* (3), 418-20.
34. Willner, P.; Moreau, J. L.; Nielsen, C. K.; Papp, M.; Sluzewska, A., Decreased hedonic responsiveness following chronic mild stress is not secondary to loss of body weight. *Physiol Behav* **1996,** *60* (1), 129-34.
35. Willner, P., Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology* **2005,** *52* (2), 90-110.
36. Chu, C.; Wei, H.; Zhu, W.; Shen, Y.; Xu, Q., Decreased Prostaglandin D2 Levels in Major Depressive Disorder Are Associated with Depression-Like Behaviors. *Int J Neuropsychopharmacol* **2017,** *20* (9), 731-739.
37. Xu, A.; Cui, S.; Wang, J. H., Incoordination among Subcellular Compartments Is Associated with Depression-Like Behavior Induced by Chronic Mild Stress. *Int J Neuropsychopharmacol* **2016,** *19* (5).
38. Willner, P., The chronic mild stress (CMS) model of depression: History, evaluation and usage. *Neurobiol Stress* **2017,** *6*, 78-93.
39. Nestler, E. J.; Hyman, S. E., Animal models of neuropsychiatric disorders. *Nat Neurosci* **2010,** *13* (10), 1161-9.
40. Krishnan, V.; Nestler, E. J., Animal models of depression: molecular perspectives. *Curr Top Behav Neurosci* **2011,** *7*, 121-47.
41. Wohleb, E. S.; Hanke, M. L.; Corona, A. W.; Powell, N. D.; Stiner, L. M.; Bailey, M. T.; Nelson, R. J.; Godbout, J. P.; Sheridan, J. F., beta-Adrenergic receptor antagonism prevents anxiety-like behavior and microglial reactivity induced by repeated social defeat. *J Neurosci* **2011,** *31* (17), 6277-88.
42. Myint, A. M.; Steinbusch, H. W.; Goeghegan, L.; Luchtman, D.; Kim, Y. K.; Leonard, B. E., Effect of the COX-2 inhibitor celecoxib on behavioural and immune changes in an olfactory bulbectomised rat model of depression. *Neuroimmunomodulation* **2007,** *14* (2), 65-71.
43. Guo, J. Y.; Li, C. Y.; Ruan, Y. P.; Sun, M.; Qi, X. L.; Zhao, B. S.; Luo, F., Chronic treatment with celecoxib reverses chronic unpredictable stress-induced depressive-like behavior via reducing cyclooxygenase-2 expression in rat brain. *Eur J Pharmacol* **2009,** *612* (1-3), 54-60.
44. Nery, F. G.; Monkul, E. S.; Hatch, J. P.; Fonseca, M.; Zunta-Soares, G. B.; Frey, B. N.; Bowden, C. L.; Soares, J. C., Celecoxib as an adjunct in the treatment of depressive or mixed episodes of bipolar disorder: a double-blind, randomized, placebo-controlled study. *Hum Psychopharmacol* **2008,** *23* (2), 87-94.
45. Fourrier, C.; Sampson, E.; Mills, N. T.; Baune, B. T., Anti-inflammatory treatment of depression: study protocol for a randomised controlled trial of vortioxetine augmented with celecoxib or placebo. *Trials* **2018,** *19* (1), 447.
46. Krause, D.; Myint, A. M.; Schuett, C.; Musil, R.; Dehning, S.; Cerovecki, A.; Riedel, M.; Arolt, V.; Schwarz, M. J.; Muller, N., High Kynurenine (a Tryptophan Metabolite) Predicts Remission in Patients with Major Depression to Add-on Treatment with Celecoxib. *Front Psychiatry* **2017,** *8*, 16.

47. Adzic, M.; Brkic, Z.; Mitic, M.; Francija, E.; Jovicic, M. J.; Radulovic, J.; Maric, N. P., Therapeutic Strategies for Treatment of Inflammation-related Depression. *Curr Neuropharmacol* **2018,** *16* (2), 176-209.
48. Teeling, J. L.; Cunningham, C.; Newman, T. A.; Perry, V. H., The effect of non-steroidal anti-inflammatory agents on behavioural changes and cytokine production following systemic inflammation: Implications for a role of COX-1. *Brain Behav Immun* **2010,** *24* (3), 409-19.
49. Matsui, T.; Svensson, C. I.; Hirata, Y.; Mizobata, K.; Hua, X. Y.; Yaksh, T. L., Release of prostaglandin E(2) and nitric oxide from spinal microglia is dependent on activation of p38 mitogen-activated protein kinase. *Anesth Analg* **2010,** *111* (2), 554-60.
50. He, G. L.; Luo, Z.; Shen, T. T.; Li, P.; Yang, J.; Luo, X.; Chen, C. H.; Gao, P.; Yang, X. S., Inhibition of STAT3- and MAPK-dependent PGE2 synthesis ameliorates phagocytosis of fibrillar beta-amyloid peptide (1-42) via EP2 receptor in EMF-stimulated N9 microglial cells. *Journal of neuroinflammation* **2016,** *13* (1), 296.
51. Qu, W. S.; Liu, J. L.; Li, C. Y.; Li, X.; Xie, M. J.; Wang, W.; Tian, D. S., Rapidly activated epidermal growth factor receptor mediates lipopolysaccharide-triggered migration of microglia. *Neurochem Int* **2015,** *90*, 85-92.
52. Shiratsuchi, H.; Basson, M. D., Activation of p38 MAPKalpha by extracellular pressure mediates the stimulation of macrophage phagocytosis by pressure. *Am J Physiol Cell Physiol* **2005,** *288* (5), C1083-93.
53. Shie, F. S.; Montine, K. S.; Breyer, R. M.; Montine, T. J., Microglial EP2 is critical to neurotoxicity from activated cerebral innate immunity. *Glia* **2005,** *52* (1), 70-7.
54. Rees, M. C.; Canete-Soler, R.; Lopez Bernal, A.; Turnbull, A. C., Effect of fenamates on prostaglandin E receptor binding. *Lancet* **1988,** *2* (8610), 541-2.
55. Moser, M. B.; Moser, E. I., Functional differentiation in the hippocampus. *Hippocampus* **1998,** *8* (6), 608-19.
56. Fanselow, M. S.; Dong, H. W., Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron* **2010,** *65* (1), 7-19.
57. Bast, T.; Wilson, I. A.; Witter, M. P.; Morris, R. G., From rapid place learning to behavioral performance: a key role for the intermediate hippocampus. *PLoS Biol* **2009,** *7* (4), e1000089.
58. Wright, B. J.; Jackson, M. B., Long-term potentiation in hilar circuitry modulates gating by the dentate gyrus. *J Neurosci* **2014,** *34* (29), 9743-53.
59. Zhao, D.; Xu, X.; Pan, L.; Zhu, W.; Fu, X.; Guo, L.; Lu, Q.; Wang, J., Pharmacologic activation of cholinergic alpha7 nicotinic receptors mitigates depressive-like behavior in a mouse model of chronic stress. *Journal of neuroinflammation* **2017,** *14* (1), 234.
60. Tramullas, M.; Finger, B. C.; Moloney, R. D.; Golubeva, A. V.; Moloney, G.; Dinan, T. G.; Cryan, J. F., Toll-like receptor 4 regulates chronic stress-induced visceral pain in mice. *Biol Psychiatry* **2014,** *76* (4), 340-8.
61. Levone, B. R.; Cryan, J. F.; O'Leary, O. F., Role of adult hippocampal neurogenesis in stress resilience. *Neurobiol Stress* **2015,** *1*, 147-55.
62. Mirescu, C.; Gould, E., Stress and adult neurogenesis. *Hippocampus* **2006,** *16* (3), 233-8.
63. Wu, Y. P.; Gao, H. Y.; Ouyang, S. H.; Kurihara, H.; He, R. R.; Li, Y. F., Predator stress-induced depression is associated with inhibition of hippocampal neurogenesis in adult male mice. *Neural Regen Res* **2019,** *14* (2), 298-305.
64. Santarelli, L.; Saxe, M.; Gross, C.; Surget, A.; Battaglia, F.; Dulawa, S.; Weisstaub, N.; Lee, J.; Duman, R.; Arancio, O.; Belzung, C.; Hen, R., Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **2003,** *301* (5634), 805-9.
65. Hill, A. S.; Sahay, A.; Hen, R., Increasing Adult Hippocampal Neurogenesis is

Sufficient to Reduce Anxiety and Depression-Like Behaviors. *Neuropsychopharmacology* **2015,** *40* (10), 2368-78.
66. Rubin, R. D.; Watson, P. D.; Duff, M. C.; Cohen, N. J., The role of the hippocampus in flexible cognition and social behavior. *Front Hum Neurosci* **2014,** *8*, 742.
67. Snyder, J. S.; Soumier, A.; Brewer, M.; Pickel, J.; Cameron, H. A., Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature* **2011,** *476* (7361), 458-61.
68. Kempermann, G., Regulation of adult hippocampal neurogenesis - implications for novel theories of major depression. *Bipolar Disord* **2002,** *4* (1), 17-33.
69. Willner, P., Reliability of the chronic mild stress model of depression: A user survey. *Neurobiol Stress* **2017,** *6*, 68-77.
70. Cryan, J. F.; Markou, A.; Lucki, I., Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci* **2002,** *23* (5), 238-45.
71. Arndt, D. L.; Peterson, C. J.; Cain, M. E., Differential Rearing Alters Forced Swim Test Behavior, Fluoxetine Efficacy, and Post-Test Weight Gain in Male Rats. *PloS one* **2015,** *10* (7), e0131709.

Chang Chung: Conceptualization, Methodology, Investigation, Visualization, Supervision, Writing- Reviewing and Editing;
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- We examined the effect of Mefenamic Acid on chronic mild stress induced depressive behaviors by sucrose preference and forced swimming tests.
- Behavioral assays revealed Mefenamic Acid and celecoxib ameliorate chronic mild stress-induced depressive behaviors.
- The number of activated microglia in Dentate Gyrus significantly decreases in stressed mice treated with Mefenamic Acid.
- Mefenamic Acid plays an important role in inhibiting microglia activation by inhibiting of P38 MAPK activation.
- Mefenamic Acid blocks increased phagocytic activity of activated microglia, which might protect neurogenesis in stressed mice.

Intitial Week				
Group	Day 1	Day 2	day 3	Day 4
Control	76.1 $\pm$ 4.8	87.5 $\pm$ 3.7	80.4 $\pm$ 10	76.8 $\pm$ 5.0
Stress	75.3 $\pm$ 2.7	76.5 $\pm$ 3.5	73.9 $\pm$ 8.5	77.8 $\pm$ 3.7

Stress + MA	78.2 ± 3.6	80.1 ± 4.1	82.7 ± 5.0	82.8 ± 3.2
Stress + Celecoxib	73.4 ± 4.3	72.5 ± 6.5	73.9 ± 5.9	81.5 ± 4.6
Final Week				
Group	Day 1	Day 2	day 3	Day 4
Control	80.6 ± 3.7	84.1 ± 3.8	83.2 ± 5.2	74.0 ± 4.5
Stress	65.3 ± 4.1	64.3 ± 0.9	66.1 ± 3.8	61.4 ± 3.2
Stress + MA	73.6 ± 1.4	80.3 ± 3.1	75.6 ± 6.8	79.9 ± 4.2
Stress + Celecoxib	79.3 ± 0.9	78.0 ± 1.5	76.2 ± 2.7	80.9 ± 2.1