

# Swimming exercise reverses chronic unpredictable mild stress-induced depression-like behaviors and alleviates neuroinflammation and collapsing response mediator protein-2-mediated neuroplasticity injury in adult male mice

Yumeng Xie, Zuotian Wu, Limin Sun, Lin Zhou, Ling Xiao, Huiling Wang and Gaohua Wang

**Objective** Impaired neuroplasticity and neuroinflammation are vital in the mechanisms of depression. Exercise alleviates depressive symptoms and ameliorates body functions. Swimming is one of the most common exercises; however, whether swimming alters depressive behaviors and the underlying mechanism has not been fully elucidated.

**Methods** Male C57/BL6J mice were exposed to chronic unpredictable mild stress (CUMS) for 6 weeks and then were subjected to a 5-week swimming program. Behavioral test, including sucrose preference test (SPT), open field test (OFT), elevated plus-maze (EPM) test, and tail suspension test (TST), was conducted to assess the anxiety-like and depressive behaviors. Western blotting and immunofluorescence staining were carried out after tissue collection.

**Results** This study showed that CUMS-induced depressive behaviors but swimming exercise increased sucrose preference in SPT, increased time and velocity in the center on OFT, decreased time in the closed arm, increased time in the open arm in EPM, and decreased immobility time in TST. We further found swimming exercise increased hippocampal collapsing response

mediator protein-2 (CRMP2) expression and decreased p-CRMP2 expression in CUMS mice. CUMS inhibited the levels of  $\alpha$ -tubulin and CRMP2, and the expression of ionized calcium-binding adaptor molecule 1 and caspase-1, whereas swimming reversed them in CUMS-exercised mice.

**Conclusion** Our study confirmed that swimming exercise reverses CUMS-induced depressive behaviors, and neuroinflammation and CRMP2-mediated neuroplasticity are involved, which may provide a new insight into the antidepressant therapy of exercise. *NeuroReport* 33: 272–280 Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc.

*NeuroReport* 2022, 33:272–280

**Keywords:** chronic unpredicted mild stress, depression, exercise, neuroinflammation, neuroplasticity

Department of Psychiatry, Renmin Hospital of Wuhan University, Hubei, PR China

Correspondence to Gaohua Wang, MD, PhD, Department of Psychiatry, Renmin Hospital of Wuhan University, Jiefang Road No.238, Wuhan 430060, Hubei, PR China

Tel: +86 13607167402; fax: +027 88072022; e-mail: wgh6402@whu.edu.cn

Received 23 February 2022 Accepted 10 March 2022

## Introduction

Depression is now the most serious disease burden among nonfatal neuropsychiatric disorders and is projected to be in the top three of all disease burdens by 2030, according to the Global Burden of Disease Project [1]. Exercise has been shown to alleviate depressive symptoms and ameliorate body functions [2–4]. Swimming is one of the most common exercises. However, whether swimming alters depressive behaviors and the underlying mechanism has not been fully elucidated.

Collapsing response mediator protein-2 (CRMP2) is an important molecule related to neuroplasticity, which functions in modulation of neuroprotection and emotional behaviors [5]. CRMP2 fulfills functions by regulating the dynamics of microtubules [6], which is composed of  $\alpha/\beta$ -tubulin heterodimer [7]. When CRMP2 exists in the form of high phosphorylation, it fails to combine tubulin and decreases the growth of microtubules [8]. Results have shown a great decrease in CRMP2 expression in the hippocampus of depressive patients [9]. In addition, fluoxetine treatment increased the expression of CRMP2 and decreased the expression of p-CRMP2, the inactive form of CRMP2, while increasing the interaction between CRMP2 and  $\alpha$ -tubulin in the rat hippocampus, thereby improving depression-like behaviors [10].

Neuroinflammation is also an important component of the pathogenesis of depression. Stress can cause the activation of microglia and the release of proinflammatory

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, [www.neuroreport.com](http://www.neuroreport.com).

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

factors [11,12]. Ionized calcium-binding adaptor molecule 1 (Iba1) is a biomarker of microglial activation [13]. The activation of microglia under pathological conditions leads to a decrease in neurogenesis [14] and the release of inflammatory factors, causing depression and anxiety [11]. Previous study [15] showed stress-activated hippocampal microglia and increased hippocampal Iba1 expression in depressive rats. Caspase-1 is the effector protein of the nucleotide-binding domain, leucine-rich-containing family, pyrin-domain-containing-3 (NLRP3) inflammasome [16]. After caspase-1 is activated, cleaved caspase-1 is released from the inflammasome [17]. Results have shown that the downregulation of caspase-1 is associated with reduced depression-like behaviors in chronic unpredictable mild stress (CUMS) mice [18].

We hypothesized that swimming alleviated CUMS-induced depressive behaviors, where CRMP2-mediated neuroplasticity and neuroinflammation were involved. To verify our hypothesis, we constructed CUMS depression models with/without exercise intervention to explore the behavioral alterations using behavioral tests and examine biomarkers of the CRMP2-mediated neuroplasticity and neuroinflammation in mice.

## Methods

### Animals

Male C57/BL6J mice ( $n = 48$ ; provided by the animal facility at Renmin Hospital of Wuhan University) at 5 weeks of age were used in these experiments. Animals were housed under standard conditions (room temperature at  $22 \pm 1^\circ\text{C}$ ,  $55 \pm 5\%$  humidity, fed ad libitum) on a 12/12-h light/dark cycle. Adaptive feeding lasted for 1 week. All procedures were conducted following the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China and the approval of the Ethics Committee at Renmin Hospital of Wuhan University.

Animals were randomly divided into two groups: control mice ( $n = 24$ ) and CUMS mice ( $n = 24$ ). After the CUMS procedure, we segregated the mice into four groups: control nonexercised mice (Con+NE,  $n = 12$ ), CUMS nonexercised mice (CUMS+NE,  $n = 12$ ), CUMS-exercised mice (CUMS+E,  $n = 12$ ) and control exercised mice (Con+E,  $n = 12$ ).

### Chronic unpredictable mild stress

The CUMS procedure was established as previously described [19]. Briefly, a randomized schedule consisted of various stressors: water deprivation (24 h), food deprivation (24 h), tail clamping (1 min), ice water swimming (5 min at  $5^\circ\text{C}$ ), hot water swimming (5 min at  $45^\circ\text{C}$ ), inversion of the 12/12 h light/dark cycle for 24 h, damp bedding (24 h, 200 ml water per cage), and cage tilting ( $45^\circ$ , 24 h). Mice were exposed to one of these stresses daily, and the same stress was not applied for 2 consecutive days to ensure the unpredictability of the experiments.

### Exercise protocol

The swimming program [20] included two phases: adaptation and training. During the first week for adaptation, the training was graded beginning with 15 min on the first day until 60 min on the last day. Then, the training period began with the intensity of 60 min/day and 5 days/week, for 4 weeks. Daily swimming exercise was performed in a large glass water tank (100 cm length  $\times$  60 cm width  $\times$  80 cm height) at  $32 \pm 1^\circ\text{C}$ . The animals were swum as a group of six to eight mice and were continuously supervised. The exercise was performed at the same time every day (between 9:00 and 11:00 a.m.). After swimming, mice were towed dry and kept warm.

### Behavioral tests

After CUMS procedure and swimming exercise protocol, mice were subjected to behavioral tests, respectively. All tests were conducted from 8:00 a.m. to 12:00 p.m. in a quiet room and the temperature and humidity of the testing room remained unchanged. Sucrose preference test (SPT), open field test (OFT), elevated plus-maze (EPM) test, and tail suspension test (TST) were conducted to assess the anxiety-like behavior and depression-like behavior. For more details, please refer to supplementary materials, Supplemental digital content 1, <http://links.lww.com/WNR/A654>. Moreover, body weight is measured weekly.

### Tissue collection

Mice were anesthetized with 1% pentobarbital and then decapitated. The brains were carefully removed and immediately placed on ice. The hippocampi were removed meticulously.

### Western blotting

The total amount of protein was detected by the bicinchoninic acid method (Beyotime, Shanghai, China). Proteins (20  $\mu\text{g}$ ) were separated by SDS-PAGE on 10% polyacrylamide gels and transferred electrophoretically to polyvinylidene fluoride membranes with the TGX Stain-Free FastCast Acrylamide Kit (Bio-Rad, California, USA). The membranes were blocked with 5% nonfat dry milk in TBS + 0.1% Tween-20 for 1 h and incubated overnight at  $4^\circ\text{C}$  with the primary antibodies in bond primary antibody diluent (see Supplementary Table 2, Supplemental digital content 1, <http://links.lww.com/WNR/A654>, for antibody information), followed by secondary antibodies [horseradish peroxidase (HRP)-labeled goat antimouse immunoglobulin (Ig)G, 1:5000, and HRP-labeled goat antirabbit IgG, 1:10 000, Abcam, Cambridge, UK] at room temperature for 1 h. The proteins were detected by the Bio-Rad ChemiDoc Touch Image System (Bio-Rad).

### Immunofluorescence analysis

Half of the mice were anesthetized and perfused transcardially with PBS, followed by 4% paraformaldehyde. The brain was then postfixed in 4% paraformaldehyde

overnight. Brain sections were embedded in paraffin, cut to 4- $\mu$ m thickness. The paraffin-embedded tissue cross-sections were dewaxed in xylol, rehydrated, antigen retrieval, washed with PBS, and blocked with 1% BSA (Roche, Switzerland) in PBS for 2 h at room temperature. The sections were incubated at 4 °C overnight with primary antibodies against rabbit anti-CRMP2 (1:500, Abcam), rabbit anti-Iba1 (1:400, CST) and mouse anti- $\alpha$ -tubulin (1:1000, Abcam), followed by incubation with Alexa Fluor 594-conjugated goat antirabbit IgG secondary antibody (1:250, Abcam) and Alex Fluor 488-conjugated goat antimouse IgG secondary antibody (1:200, Abcam) for 120 min. 4',6-diamidino-2'-phenylindole (Thermo Fisher, Massachusetts, USA) was used as a nuclear stain. The images were collected under an inverted fluorescence microscope (IX53, Olympus, Tokyo, Japan). Image J was used to analyze the integral optical density of the target protein.

### Statistical analysis

Statistical analyses were performed using SPSS (Chicago, Illinois, USA). Data are presented as the mean  $\pm$  SD. The results were analyzed using a one-way analysis of variance (ANOVA) to compare differences and a least significant difference test for post hoc comparisons. The level of confidence was set at 95% ( $P < 0.05$ ).

## Results

### Chronic unpredictable mild stress-induced depression-like behaviors in male mice

Figure 1a and b shows the experiment groups and timeline of our study. Figure 1c shows that the mice body weight of CUMS group was lower than the Con group from the second week. No significance was observed throughout the stress exposure except for the third week of the CUMS procedure [week 3:  $F(1,46) = 7.367$ ;  $P = 0.009$ ]. Figure 1d shows the bodyweight of the four groups throughout the experiment. In behavioral tests, results indicated that CUMS induced depression-like behaviors, including decreased time in the center in OFT [ $F(1,46) = 15.953$ ;  $P < 0.001$ ; Fig. 1e], increased time in closed arm and decreased time in open arm in EPM [ $F(1,46) = 12.302$ ;  $P = 0.001$ ;  $F(1,46) = 17.515$ ;  $P < 0.001$ ; Fig. 1f and g], and increased immobility time in TST [ $F(1,46) = 4.798$ ;  $P = 0.034$ ; Fig. 1h], compared with the Con mice. In addition, in SPT, the CUMS group showed no significance in sucrose preference, compared with the Con group [ $F(1,46) = 3.833$ ;  $P = 0.056$ ; Fig. 1i].

### Swimming exercise reversed depression-like behavioral changes

As shown in Fig. 2, one-way ANOVA suggested a significant effect of treatment on OFT [ $F(3,44) = 6.407$ ;  $P = 0.001$ ;  $F(3,44) = 9.314$ ;  $P < 0.001$ ], EPM [ $F(3,44) = 16.214$ ;  $P < 0.001$ ;  $F(3,44) = 14.018$ ;  $P < 0.001$ ], TST ( $F(3,44) = 6.565$ ;  $P = 0.001$ ), and SPT [ $F(3,44) = 5.803$ ;  $P = 0.002$ ]. Post hoc analysis indicated

that swimming ameliorated depression-like behaviors induced by CUMS, including increased time and velocity in the center on OFT ( $P = 0.005$ , Fig. 2a;  $P < 0.001$ , Fig. 2b), decreased time in the closed arm, and increased time in the open arm in EPM ( $P < 0.001$ , Fig. 2c;  $P < 0.001$ , Fig. 2d), as well as decreased immobility time in TST ( $P = 0.010$ ; Fig. 2e), compared with the CUMS+NE group. In SPT, the CUMS+E group showed significantly higher sucrose preference than the CUMS+NE group ( $P = 0.020$ ; Fig. 2f).

There was no significant difference in depression-like behaviors between the Con+NE and CUMS+E groups, suggesting that swimming exercise reversed depression-like behaviors in CUMS mice. There was significance in behaviors between the Con+E and Con+NE groups ( $P = 0.003$ ; Fig. 2f), suggesting that swimming exercise may enhance behavioral changes in healthy mice also.

### Swimming exercise increased hippocampal collapsible response mediator protein-2-induced neuroplasticity

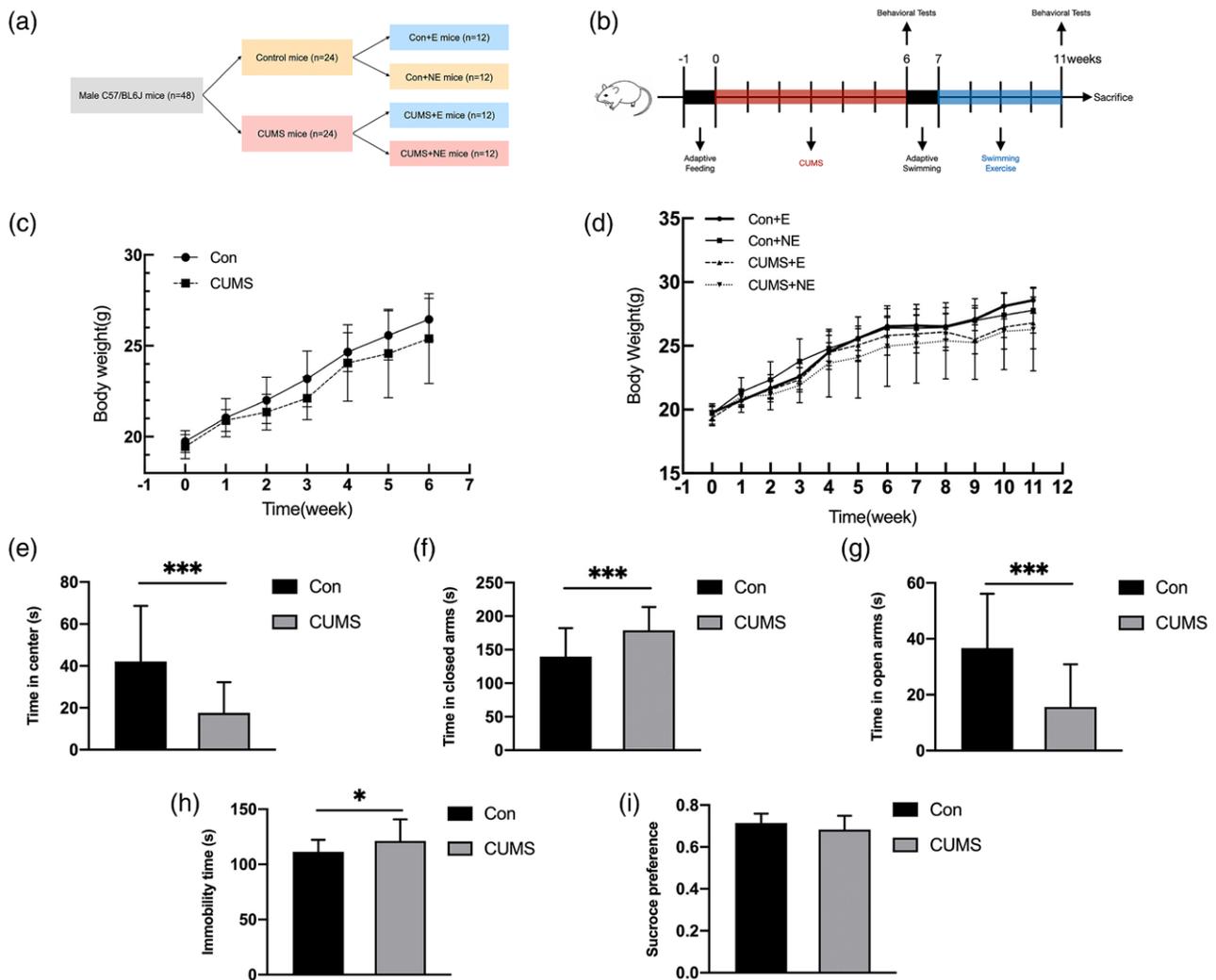
Western blotting was used to examine the neuroplasticity-related gene expression (Fig. 3). One-way ANOVA revealed a significant effect of treatment on the protein level of CRMP2 [ $F(3,20) = 3.541$ ;  $P = 0.033$ ] and p-CRMP2 [ $F(3,20) = 4.154$ ;  $P = 0.019$ ]. Post hoc analysis indicated that swimming exercise increased hippocampal CRMP2 ( $P = 0.046$ ; Fig. 3b) protein expression and decreased p-CRMP2 protein expression ( $P = 0.010$ ; Fig. 3c) in CUMS mice. Between the Con and CUMS+E groups, no significant differences were found in the gene expressions above, suggesting that swimming exercise reversed the consequences of CUMS exposure. In the two Con groups, the protein level of CRMP2 and p-CRMP2 showed no significant difference.

### Swimming exercise increased hippocampal microtubule-related neuroplasticity

Figure 3h shows that the protein expression of  $\alpha$ -tubulin was decreased in CUMS+NE mice but recovered in the CUMS+E mice [ $F(3,20) = 3.694$ ,  $P = 0.029$ , post hoc  $P = 0.023$  for CUMS+NE compared with Con+NE,  $P = 0.026$  for CUMS+E compared with CUMS+NE, Fig. 3d].

We further analyzed the expression of CRMP2 and  $\alpha$ -tubulin hippocampus utilizing immunofluorescence staining and colocalization analysis (Fig. 4). Figure 4a shows that the hippocampal sections were double-stained for  $\alpha$ -tubulin and CRMP2 to localize and assess microtubule dynamics. Quantification by immunofluorescence analysis revealed a significant effect of treatment on the expression of  $\alpha$ -tubulin [ $F(3,20) = 7.177$ ;  $P = 0.002$ ] and CRMP2 [ $F(3,20) = 6.366$ ;  $P = 0.003$ ]. Post hoc analysis indicated that CUMS exposure inhibited the expression of  $\alpha$ -tubulin ( $P = 0.001$ ; Fig. 4b) and CRMP2 ( $P = 0.003$ ; Fig. 4c), whereas swimming exercise normalized the decrease ( $\alpha$ -tubulin:  $P = 0.002$ ; CRMP2:  $P = 0.005$ ).

Fig. 1.



Experimental procedures and effects of CUMS on depression-like behaviors. (a) Experiment groups; (b) timeline. (c) Changes in body weight during 6-week exposure to CUMS, (d) changes in body weight throughout the experiment, (e) time in the center in OFT, (f) time in closed arms in elevated plus-maze (EPM), (g) time in open arms in EPM, (h) immobility time in tail suspension test (TST), and (i) sucrose preference in sucrose preference test (SPT). Data are presented as means  $\pm$  SD [ $n = 24$  per group in (c–g)]. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . CUMS, chronic unpredictable mild stress; OFT, open field test.

There was no difference in the Con+E, Con+NE, and CUMS+E groups.

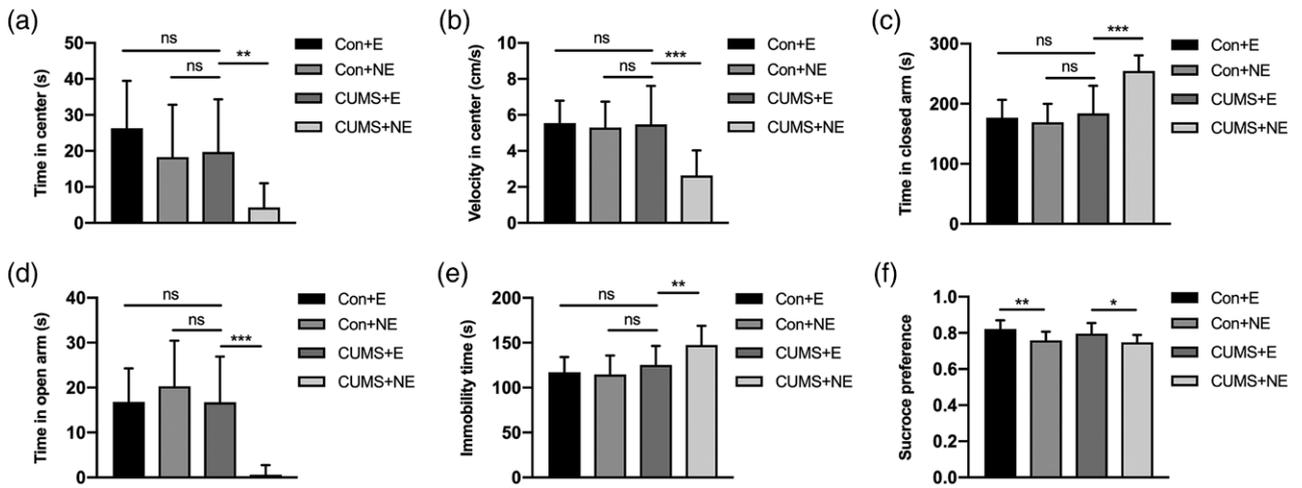
### Swimming exercise inhibited hippocampal neuroinflammation

We explored the expression of Iba1 and caspase-1 (pro-caspase-1 and cleaved caspase-1) to investigate whether antineuroinflammation was involved in the antidepressant effect of swimming. The hippocampal protein level of Iba1 was activated by CUMS and then decreased by swimming exercise [ $F(3,20) = 6.497$ ,  $P = 0.003$ , post hoc  $P = 0.013$  for CUMS+NE compared with Con+NE,  $P = 0.002$  for CUMS+E compared with CUMS+NE; Fig. 3c], as well as the protein level of pro-caspase-1 [ $F(3,20) = 8.199$ ,  $P = 0.001$ , post hoc  $P = 0.001$

for CUMS+NE compared with Con+NE,  $P = 0.008$  for CUMS+E compared with CUMS+NE, Fig. 3f]. In addition, the protein expression of cleaved caspase-1 was ameliorated by swimming in CUMS mice [ $F(3,20) = 4.320$ ,  $P = 0.017$ , post hoc  $P = 0.006$  for CUMS+NE compared with Con+NE,  $P = 0.040$  for CUMS+E compared with CUMS+NE; Fig. 3g].

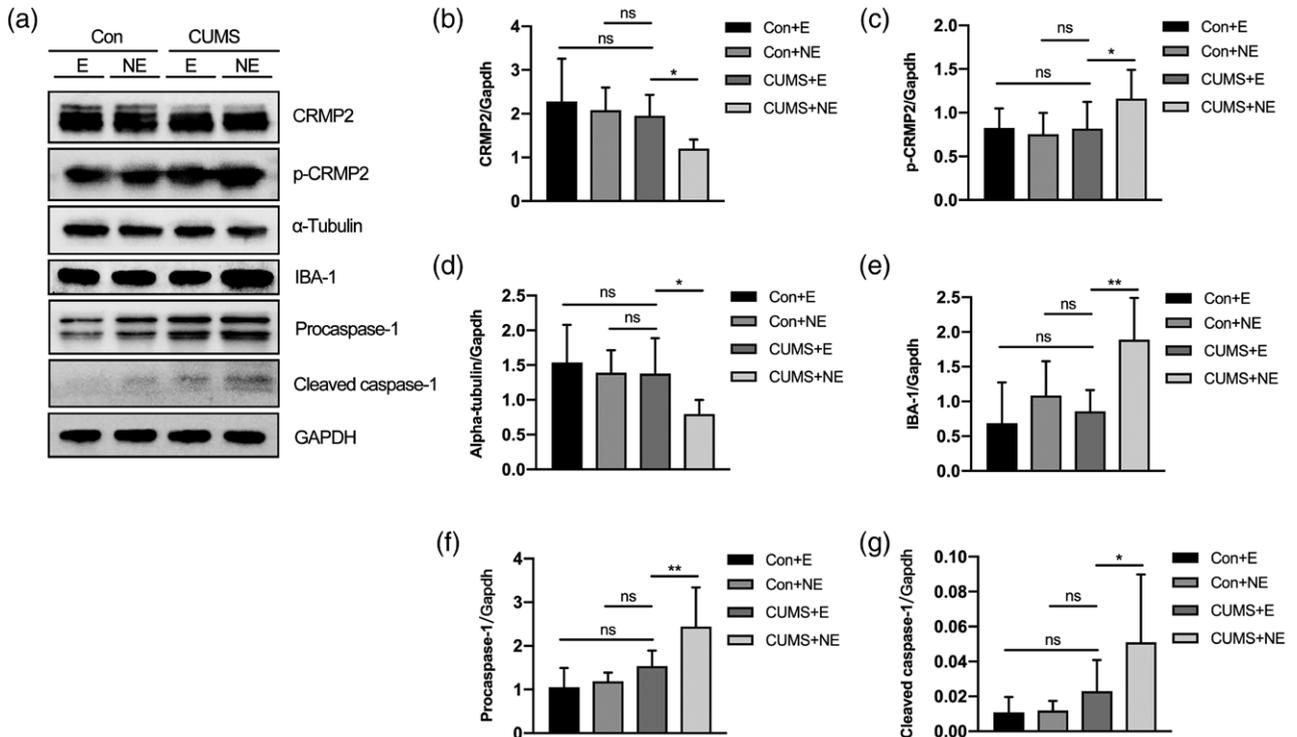
To investigate swimming's effect on Iba1 in microglia, immunofluorescence staining for hippocampal sections was performed (Fig. 5). The Iba1 level in the hippocampus was dramatically increased by CUMS treatment, whereas exercise ameliorated the Iba1 level in the hippocampal region [ $F(3,20) = 10.211$ ,  $P < 0.001$ , post hoc  $P < 0.001$  for CUMS+NE compared with Con+NE,  $P = 0.001$  for CUMS+E compared with CUMS+NE; Fig. 5b].

Fig. 2.



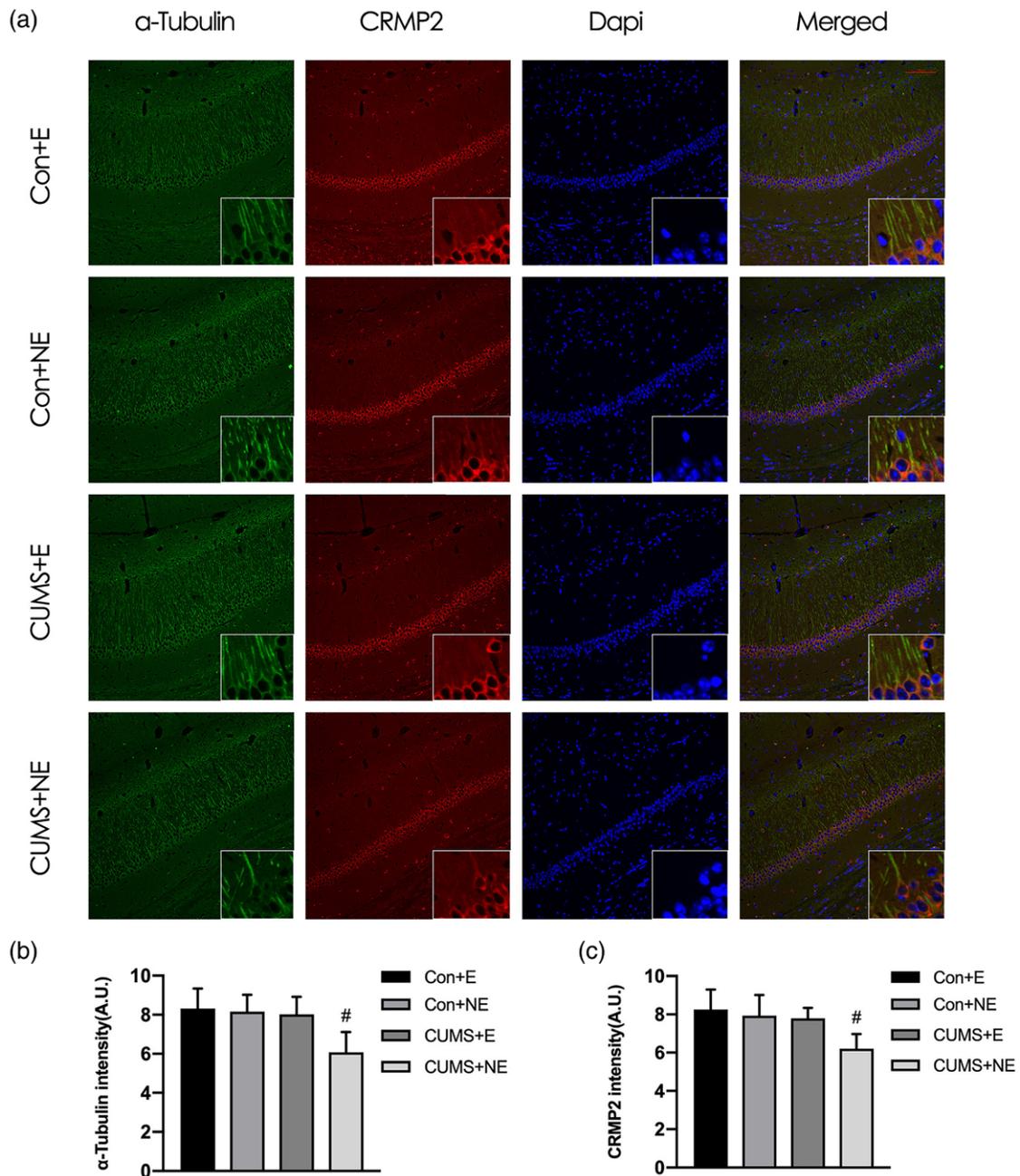
Effects of swimming exercise on CUMS-induced behavioral changes. (a) Time in the center in OFT, (b) velocity in the center in OFT, (c) time in closed arms in elevated plus-maze, (d) time in open arms in elevated plus-maze, (e) immobility time in tail suspension test (TST), and (f) sucrose preference in sucrose preference test (SPT). Data are presented as means  $\pm$  SD ( $n = 12$  per group). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . CUMS, chronic unpredictable mild stress; OFT, open field test.

Fig. 3.



The antidepressant effect of swimming exercise via enhancing neuroplasticity and antiinflammation. (a) Representative protein bands of CRMP2, p-CRMP2,  $\alpha$ -tubulin, Iba1, pro-caspase-1, cleaved caspase-1, and GAPDH in the hippocampus. (b) CRMP2 protein level, (c) p-CRMP2 protein level, (d)  $\alpha$ -Tubulin protein level, (e) Iba1 protein level, (f) Pro-caspase-1 protein level, and (g) cleaved caspase-1 protein level. Data are presented as means  $\pm$  SD ( $n = 6$  per group). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . CRMP2, collapsing response mediator protein-2; Iba1, ionized calcium-binding adaptor molecule 1.

Fig. 4.



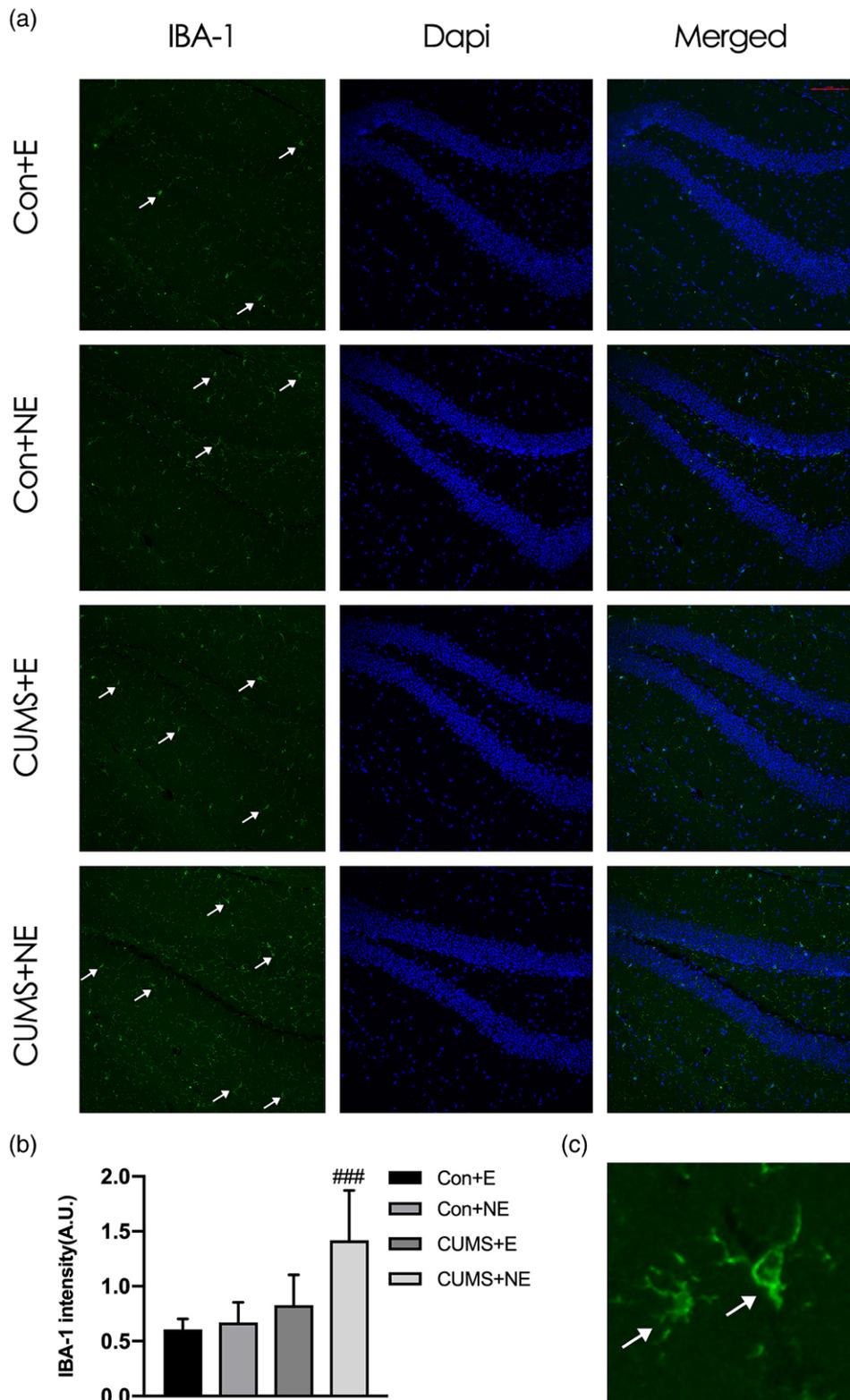
CUMS inhibited microtubule-related neuroplasticity, whereas swimming exercise normalized the alteration. (a) Hippocampal sections were double-stained for  $\alpha$ -tubulin (green) and CRMP2 (red) to localize and assess microtubule dynamics. Nuclei were stained with DAPI (blue). (b) Qualification of  $\alpha$ -tubulin immunofluorescence density. (c) Qualification of CRMP2 immunofluorescence density. Red scale bar, 100  $\mu$ m. White arrows indicate the cells positive for Iba1. Data are presented as means  $\pm$  SD ( $n = 6$  per group).  $\#P < 0.05$ ,  $\#\#P < 0.01$ ,  $\#\#\#P < 0.001$ , compared with the other three groups. CRMP2, collapsing response mediator protein-2; CUMS, chronic unpredictable mild stress; DAPI, 4',6-diamidino-2'-phenylindole; Iba1, ionized calcium-binding adaptor molecule 1.

## Discussion

This study explored the antidepressive effect of swimming exercise on CRMP2-mediated neuroplasticity and neuroinflammation in the hippocampus. CUMS-induced depressive behaviors, but swimming exercise increased sucrose preference in SPT, increased time and velocity

in the center on OFT, decreased time in the closed arm, increased time in the open arm in EPM, and decreased immobility time in TST. We further found swimming exercise increased hippocampal CRMP2 expression and decreased p-CRMP2 expression in CUMS mice. CUMS inhibited the levels of  $\alpha$ -tubulin and CRMP2,

Fig. 5.



CUMS increased microglial activation, whereas swimming exercise improved the alteration. White arrows indicate the cells positive for Iba1. Red scale bar, 100  $\mu$ m. (a) Hippocampal sections were stained with Iba1 (green). Nuclei were stained with DAPI (blue). (b) Qualification of Iba1 immunofluorescence density. (c) The enlarged details of the cells are positive. Data are presented as means  $\pm$  SD ( $n = 6$  per group). # $P < 0.05$ , ## $P < 0.01$ , ### $P < 0.001$ , compared with the other three groups. CUMS, chronic unpredictable mild stress; DAPI, 4',6-diamidino-2'-phenylindole; Iba1, ionized calcium-binding adaptor molecule 1.

the expression of Iba1 and caspase-1, whereas swimming reversed them in CUMS-exercised mice.

CUMS protocol is a robust animal model of depression [21]. Our results in OFT, EPM, TST, and SPT indicated that 6-week CUMS-induced depression-like behaviors, which consistent with previous studies [20,22]. Exercise as a nondrug therapy for depression is gaining momentum nowadays, with more and more studies revealing the its effects and mechanisms on depression [2,23]. For example, running exercise has been shown to be associated with decreased symptoms of depression and protection of hippocampal oligodendrocytes [24], and enhancement on white matter [25]. Swimming exercise is also proved to effect on CUMS-induced depression-like behaviors and hippocampal plasticity-related proteins, mitochondrial motility, etc. [20,26]. Our result that swimming reversed CUMS-induced behavioral alterations also proved that exercise had beneficial effect on depression.

Neuroplasticity is important component of the pathogenesis of depression. In addition, CRMP2 has been well characterized as a target of neuroplasticity and behavioral regulation [27]. CRMP2 overexpression effectively protects against acute axonal degeneration [28], whereas p-CRMP2, the inactive form of CRMP2, leads to the impairment of neuronal plasticity and function [29]. Additionally, CRMP2 fulfills functions by regulating the dynamics of microtubules [6], which have been proven to play a role in the mechanisms of depression. Previous studies found that microtubule dynamics in the hippocampus were decreased in a CUMS depression model [30], whereas fluoxetine enhanced microtubule dynamics, leading to an antidepressant effect [31]. The phosphorylation of CRMP2 suppressed the binding of CRMP2 to  $\alpha$ -tubulin and decreases the microtubule dynamics by regulating the polymerization or depolymerization of microtubule dimers in axonal growth cones [8,32,33]. Our results showed that CRMP2 and  $\alpha$ -tubulin interacted in functions, which was also proven in previous studies [34,35]. The density of CRMP2 and  $\alpha$ -tubulin in the hippocampal region was significantly enhanced jointly in exercise mice, indicating that microtubule-related neuroplasticity is also involved in the mechanism of exercise to improve depression-like behaviors. All these data imply the involvement of the CRMP2-mediated neuroplasticity in the antidepressant effect of swimming exercise. Additionally, CRMP2, the downstream signal factor of protein kinase-B/glycogen synthesis kinase-3 $\beta$  (GSK3 $\beta$ ) signaling, is decreased by the phosphorylation of CRMP2 by GSK3 $\beta$  [36]. Whether there are changes in the expression of the upstream molecules of CRMP2 needs to be further explored.

We also explored alterations in neuroinflammatory molecules. Studies have confirmed that stress can cause the activation of microglia and the release of proinflammatory factors [11,12]. Iba1 is specifically expressed in microglia, playing a role in regulating the function of activated

microglia [13]. The previous study [37] exhibited that chronic stress increased Iba1 expression and also elevated levels of nuclear factor kappa-B, NLRP3, cleaved caspase-1, interleukin-1 $\beta$  (IL-1 $\beta$ ), and IL-18. In this study, we discovered that microglia in the hippocampus was activated by CUMS, as shown by increased Iba1 expression. However, this elevation was reversed by swimming exercise, indicating that the antineuroinflammatory mechanism existed in the antidepressant effect. In addition, we explored the expression level of caspase-1. After exposure to stressors, the assembly of NLRP3 inflammasome increased, leading to the increase in the protein levels of apoptosis-associated speck-like protein containing a CARD and cleaved caspase-1 [38]. NLRP3 continuously cleaves IL-1 $\beta$  and IL-18 precursors into mature IL-1 $\beta$  and IL-18 through activated caspase-1, thereby producing numerous downstream inflammatory mediators [39]. Our results showed that swimming reversed the increase of caspase-1 level induced by CUMS, which further confirmed the antiinflammatory mechanism of exercise antidepressant function.

There have existed some clinical studies on exercise therapy for depression, and some physicians have prescribed exercise as part of clinical treatment though clinical exercise prescriptions have not yet been widely used [40]. Heterogeneity among exercise protocols makes formulating a standardized exercise program challenging. Swimming in this study may provide a new insight into the antidepressant therapy of exercise. However, the underlying mechanism requires further explorations using animal models before translating to clinical application.

## Conclusion

We explored the effects and possible mechanisms of exercise on depressive-like behaviors. CUMS induced depressive-like behaviors, but behavioral tests showed that swimming produced an antidepressant effect. CRMP2-mediated neuroplasticity enhancement and neuroinflammation alleviation may be involved in swimming antidepressant processes. Our study may help provide clues for nondrug treatment of depression and the antidepressant effects of exercise.

## Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 81871072 and No. 82071523) and the Medical Science Advancement Program of Wuhan University (No. TFLC2018001). The design of this study was supported by the Key research and development program of Hubei Province(2020BCA064).

Y.X., Z.W., and G.W. participated in the design of the experiments, carried out the molecular studies, performed the statistical analysis, and drafted the manuscript. L.S. and L.Z. carried out the behavioral tests and helped with the analysis. L.X. and H.W. advised on the experimental design and helped to draft the manuscript. Y.X. and G.W. conceived of the study, participated in its

design and coordination, and edited the manuscript. All authors read, edited, and approved the final manuscript.

### Conflicts of interest

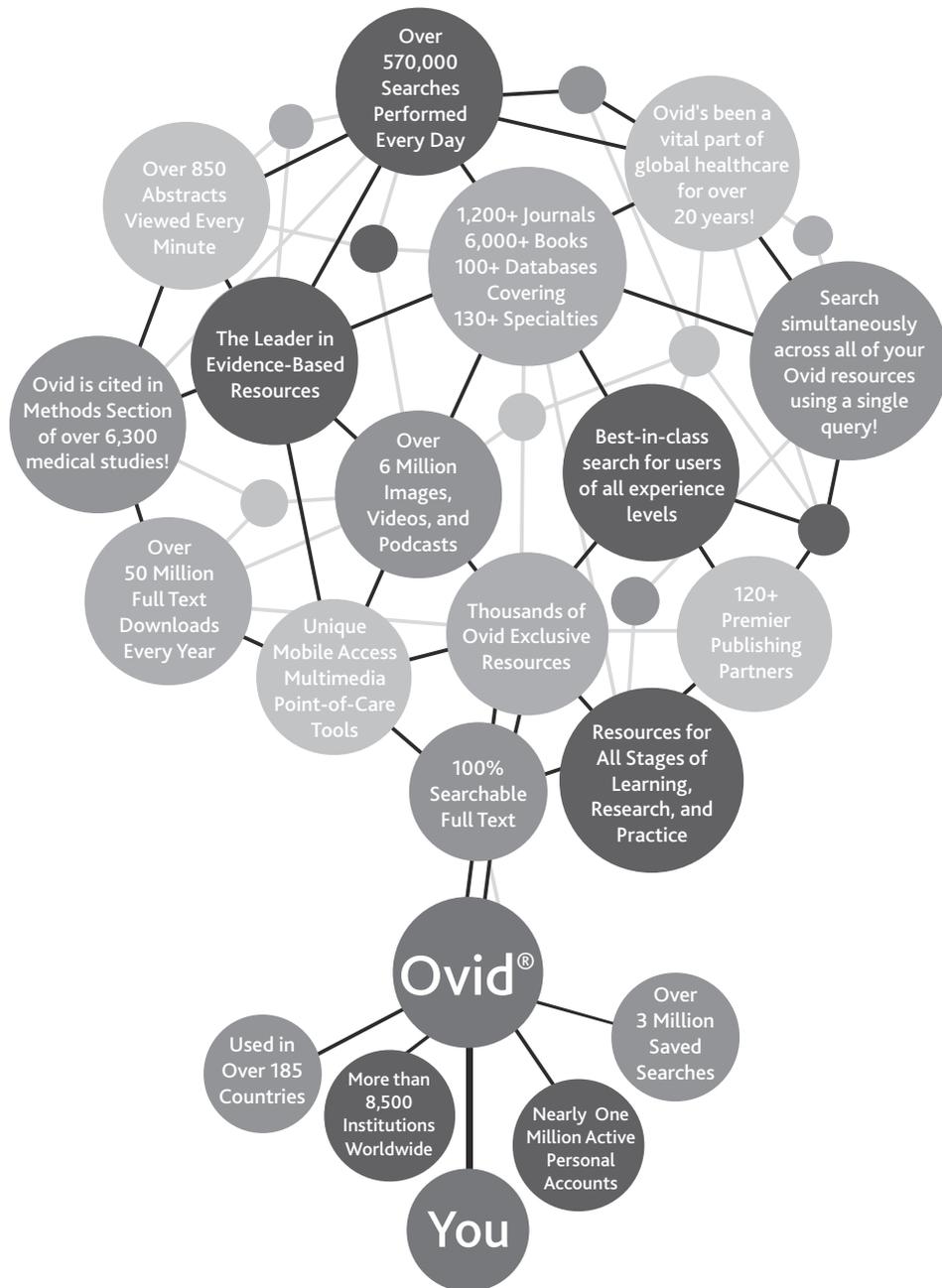
There are no conflicts of interest.

### References

- Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* 2006; **3**:e442.
- Micheli L, Ceccarelli M, D'Andrea G, Tirone F. Depression and adult neurogenesis: positive effects of the antidepressant fluoxetine and of physical exercise. *Brain Res Bull* 2018; **143**:181–193.
- Choi SH, Bylykbashi E, Chatila ZK, Lee SW, Pulli B, Clemenson GD, et al. Combined adult neurogenesis and BDNF mimic exercise effects on cognition in an Alzheimer's mouse model. *Science* 2018; **361**:eaan8821.
- Imboden C, Gerber M, Beck J, Eckert A, Pühse U, Holsboer-Trachsler E, Hatzinger M. Effects of aerobic exercise as add-on treatment for inpatients with moderate to severe depression on depression severity, sleep, cognition, psychological well-being, and biomarkers: study protocol, description of study population, and manipulation check. *Front Psychiatry* 2019; **10**:262.
- Keshavarzi S, Kermanshahi S, Karami L, Motaghinejad M, Motevalian M, Sadr S. Protective role of metformin against methamphetamine induced anxiety, depression, cognition impairment and neurodegeneration in rat: the role of CREB/BDNF and Akt/GSK3 signaling pathways. *Neurotoxicology* 2019; **72**:74–84.
- Gu Y, Ihara Y. Evidence that collapsin response mediator protein-2 is involved in the dynamics of microtubules. *J Biol Chem* 2000; **275**:17917–17920.
- Vemu A, Atherton J, Spector JO, Moores CA, Roll-Mecak A. Tubulin isoform composition tunes microtubule dynamics. *Mol Biol Cell* 2017; **28**:3564–3572.
- Wu Z, Wang G, Wei Y, Xiao L, Wang H. PI3K/AKT/GSK3 $\beta$ /CRMP-2-mediated neuroplasticity in depression induced by stress. *Neuroreport* 2018; **29**:1256–1263.
- Beasley CL, Pennington K, Behan A, Wait R, Dunn MJ, Cotter D. Proteomic analysis of the anterior cingulate cortex in the major psychiatric disorders: evidence for disease-associated changes. *Proteomics* 2006; **6**:3414–3425.
- Xiang D, Sun S, Wang G, Liu Z. Effects of CRMP2 DNA methylation in the hippocampus on depressive-like behaviors and cytoskeletal proteins in rats. *Front Cell Neurosci* 2021; **15**:644663.
- Stein DJ, Vasconcelos MF, Albrechet-Souza L, Ceresér KMM, de Almeida RMM. Microglial over-activation by social defeat stress contributes to anxiety- and depressive-like behaviors. *Front Behav Neurosci* 2017; **11**:207.
- Kim TK, Kim JE, Choi J, Park JY, Lee JE, Lee EH, et al. Local interleukin-18 system in the basolateral amygdala regulates susceptibility to chronic stress. *Mol Neurobiol* 2017; **54**:5347–5358.
- Ito D, Imai Y, Ohnawa K, Nakajima K, Fukuuchi Y, Kohsaka S. Microglia-specific localisation of a novel calcium binding protein, Iba1. *Brain Res Mol Brain Res* 1998; **57**:1–9.
- Bassett B, Subramaniam S, Fan Y, Varney S, Pan H, Carneiro AMD, Chung CY. Minocycline alleviates depression-like symptoms by rescuing decrease in neurogenesis in dorsal hippocampus via blocking microglia activation/phagocytosis. *Brain Behav Immun* 2021; **91**:519–530.
- Brás JP, Guillot de Suduiraut I, Zanoletti O, Monari S, Meijer M, Grosse J, et al. Stress-induced depressive-like behavior in male rats is associated with microglial activation and inflammation dysregulation in the hippocampus in adulthood. *Brain Behav Immun* 2022; **99**:397–408.
- Jin C, Flavell RA. Molecular mechanism of NLRP3 inflammasome activation. *J Clin Immunol* 2010; **30**:628–631.
- Boucher D, Monteleone M, Coll RC, Chen KW, Ross CM, Teo JL, et al. Caspase-1 self-cleavage is an intrinsic mechanism to terminate inflammasome activity. *J Exp Med* 2018; **215**:827–840.
- Liu C, Yuan D, Zhang C, Tao Y, Meng Y, Jin M, et al. Liquiritin alleviates depression-like behavior in CUMS mice by inhibiting oxidative stress and NLRP3 inflammasome in hippocampus. *Evid Based Complement Alternat Med* 2022; **2022**:7558825.
- Surget A, Wang Y, Leman S, Ibaguen-Vargas Y, Edgar N, Griebel G, et al. Corticolimbic transcriptome changes are state-dependent and region-specific in a rodent model of depression and of antidepressant reversal. *Neuropsychopharmacology* 2009; **34**:1363–1380.
- Liu W, Xue X, Xia J, Liu J, Qi Z. Swimming exercise reverses CUMS-induced changes in depression-like behaviors and hippocampal plasticity-related proteins. *J Affect Disord* 2018; **227**:126–135.
- Antoniuk S, Bijata M, Ponimaskin E, Wlodarczyk J. Chronic unpredictable mild stress for modeling depression in rodents: meta-analysis of model reliability. *Neurosci Biobehav Rev* 2019; **99**:101–116.
- Ma H, Li C, Wang J, Zhang X, Li M, Zhang R, et al. Amygdala-hippocampal innervation modulates stress-induced depressive-like behaviors through AMPA receptors. *Proc Natl Acad Sci U S A* 2021; **118**:e2019409118.
- Nebiker L, Lichtenstein E, Minghetti A, Zahner L, Gerber M, Faude O, Donath L. Moderating effects of exercise duration and intensity in neuromuscular vs. endurance exercise interventions for the treatment of depression: a meta-analytical review. *Front Psychiatry* 2018; **9**:305.
- Tang J, Liang X, Zhang Y, Chen L, Wang F, Tan C, et al. The effects of running exercise on oligodendrocytes in the hippocampus of rats with depression induced by chronic unpredictable stress. *Brain Res Bull* 2019; **149**:1–10.
- Xiao Q, Wang F, Luo Y, Chen L, Chao F, Tan C, et al. Exercise protects myelinated fibers of white matter in a rat model of depression. *J Comp Neurol* 2018; **526**:537–549.
- Liu W, Wang H, Xue X, Xia J, Liu J, Qi Z, Ji L. OGT-related mitochondrial motility is associated with sex differences and exercise effects in depression induced by prenatal exposure to glucocorticoids. *J Affect Disord* 2018; **226**:203–215.
- Zhang H, Kang E, Wang Y, Yang C, Yu H, Wang Q, et al. Publisher correction: brain-specific Crmp2 deletion leads to neuronal development deficits and behavioural impairments in mice. *Nat Commun* 2018; **9**:16229.
- Zhang JN, Koch JC. Collapsin response mediator protein-2 plays a major protective role in acute axonal degeneration. *Neural Regen Res* 2017; **12**:692–695.
- Quach TT, Honnorat J, Kolattukudy PE, Khanna R, Duchemin AM. CRMPs: critical molecules for neurite morphogenesis and neuropsychiatric diseases. *Mol Psychiatry* 2015; **20**:1037–1045.
- Wang H, Xiao L, Wang H, Wang G. Involvement of chronic unpredictable mild stress-induced hippocampal LRP1 up-regulation in microtubule instability and depressive-like behavior in a depressive-like adult male rat model. *Physiol Behav* 2020; **215**:112749.
- Yang C, Wang G, Wang H, Liu Z, Wang X. Cytoskeletal alterations in rat hippocampus following chronic unpredictable mild stress and re-exposure to acute and chronic unpredictable mild stress. *Behav Brain Res* 2009; **205**:518–524.
- Yoshimura T, Kawano Y, Arimura N, Kawabata S, Kikuchi A, Kaibuchi K. GSK-3beta regulates phosphorylation of CRMP-2 and neuronal polarity. *Cell* 2005; **120**:137–149.
- Arimura N, Ménager C, Kawano Y, Yoshimura T, Kawabata S, Hattori A, et al. Phosphorylation by Rho kinase regulates CRMP-2 activity in growth cones. *Mol Cell Biol* 2005; **25**:9973–9984.
- Zheng Y, Sethi R, Mangala LS, Taylor C, Goldsmith J, Wang M, et al. Tuning microtubule dynamics to enhance cancer therapy by modulating FER-mediated CRMP2 phosphorylation. *Nat Commun* 2018; **9**:476.
- Wilson SM, Moutal A, Melemedjian OK, Wang Y, Ju W, François-Moutal L, et al. The functionalized amino acid (S)-lacosamide subverts CRMP2-mediated tubulin polymerization to prevent constitutive and activity-dependent increase in neurite outgrowth. *Front Cell Neurosci* 2014; **8**:196.
- Uchida Y, Ohshima T, Sasaki Y, Suzuki H, Yanai S, Yamashita N, et al. Semaphorin3A signalling is mediated via sequential Cdk5 and GSK3beta phosphorylation of CRMP2: implication of common phosphorylation mechanism underlying axon guidance and Alzheimer's disease. *Genes Cells* 2005; **10**:165–179.
- Feng X, Zhao Y, Yang T, Song M, Wang C, Yao Y, Fan H. Glucocorticoid-driven NLRP3 inflammasome activation in hippocampal microglia mediates chronic stress-induced depressive-like behaviors. *Front Mol Neurosci* 2019; **12**:210.
- Yue N, Huang H, Zhu X, Han Q, Wang Y, Li B, et al. Activation of P2X7 receptor and NLRP3 inflammasome assembly in hippocampal glial cells mediates chronic stress-induced depressive-like behaviors. *J Neuroinflammation* 2017; **14**:102.
- Reddy VS, Harskamp RE, van Ginkel MW, Calhoun J, Baisden CE, Kim IS, et al. Interleukin-18 stimulates fibronectin expression in primary human cardiac fibroblasts via PI3K-Akt-dependent NF-kappaB activation. *J Cell Physiol* 2008; **215**:697–707.
- Xie Y, Wu Z, Sun L, Zhou L, Wang G, Xiao L, et al. The effects and mechanisms of exercise on the treatment of depression. *Front Psychiatry* 2021; **12**:705559.

# Ovid®

The world's most trusted medical research platform.

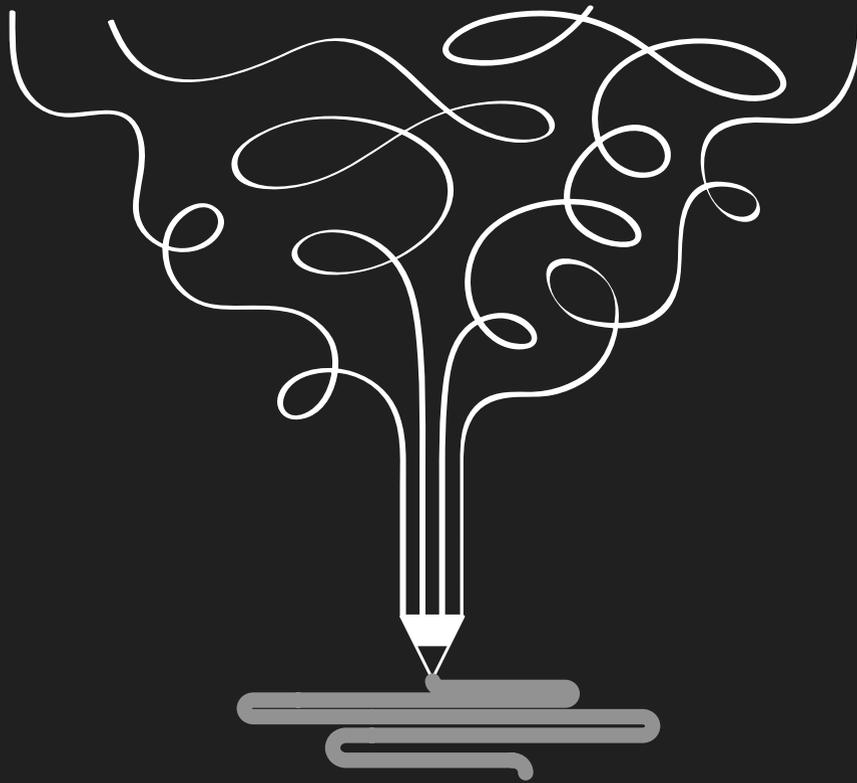


Make Ovid the source for all of your medical, nursing, and healthcare research needs.

VISIT YOUR LIBRARY - ASK A LIBRARIAN - EXPLORE OVID

# Getting Published is a Process.

*We're Here to Help.*



## Get started today!

[authors.lww.com](http://authors.lww.com)



Wolters Kluwer