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Research paper

Swimming exercise reverses CUMS-induced changes in depression-like behaviors and hippocampal plasticity-related proteins

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ABSTRACT

Background: Stress-induced failed resilience of brain plasticity can contribute to the onset and recurrence of depression. Chronic stress has been reported to open windows of epigenetic plasticity in hippocampus. However, how hippocampal plasticity underlies depression-like behaviors and how it adapts in response to stress has not been addressed. The present study aimed to investigate the signaling mechanisms of CUMS affecting hippocampal plasticity-related proteins expression and the regulation of swimming exercise in mice.

Methods: Male C57BL/6 mice were subjected to chronic unpredictable mild stress (CUMS) for 7 weeks. From the 4th week, CUMS mice were trained in a moderate swimming program for a total of 4 weeks. A videocomputerized tracking system was used to record behaviors of animals for a 5-min session. Real-time PCR and Western Blotting were used to examine gene expression in mouse hippocampus.

Results: Our results demonstrated that CUMS induced depression-like behaviors, which were reversed by swimming exercise. Moreover, the behavioral changes induced by CUMS and exercise were correlated with hippocampal plasticity-related proteins expression of growth-associated protein-43 (GAP-43) and synaptophysin (SYN). The molecular mechanisms regulating this plasticity may include SIRT1/mircoRNA, CREB/BDNF, and AKT/GSK-3β signaling pathways.

Limitations: We did not establish a correlation between depression-like behaviors induced by chronic stress and epigenetic changes of hippocampal plasticity, either a causal molecular signaling underling this plasticity.

Conclusions: Our findings have identified swimming exercise effects on CUMS-induced changes in depression-like behaviors and hippocampal plasticity-related proteins, which provide a framework for developing new strategies to treat stress-induced depression.

1. Introduction

The brain possesses remarkable structural and functional plasticity in response to stress (McEwen, 2007), whereas stress-induced failed resilience of brain plasticity can contribute to the onset and recurrence of depression (Southwick and Charney, 2012). Chronic stress associated with the development of depression (Ferrarelli, 2017), has been reported to open windows of epigenetic plasticity in hippocampus (Nasca et al., 2015). Chronic unpredictable mild stress (CUMS) in rodents, a classical animal model of depression, has been linked to hippocampal plasticity (Wu et al., 2007). Most literature on structural and functional plasticity induced by stress in rodents has focused on the hippocampus and prefrontal cortex (Russo and Nestler, 2013). The hippocampus is an especially plastic and vulnerable region of brain and is a target of stress response (McEwen, 1999). However, how hippocampal plasticity underlies depression-like behaviors and how it adapts in response to stress has not been addressed.

Both clinical and animal studies on stress-induced hippocampal plasticity have paid more attention to structural plasticity, including atrophy of dendrites in CA3 region and suppressed neurogenesis of dentate gyrus granule neurons (McEwen, 1999). It has been suggested that molecular mechanisms underlying this plasticity are possible to

Abbreviations: AKT, protein kinase B; BDNF, brain-derived neurotrophic factor; CREB, cAMP response element-binding protein; CUMS, chronic unpredictable mild stress; FST, forced swim test; GAP-43, growth-associated protein-43; GSK-3β, glycogen synthase kinase-3β; OFT, open field test; SIRT1, sirtuin 1; SPT, sucrose preference test; SYN, synaptophysin; TST, tail suspension test

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become 'overwhelmed' in response to stress and consequently promote pathological behaviors (Russo and Nestler, 2013). Hippocampal sirtuin 1 (SIRT1), identified as one of two genome-wide significant loci contributing to depression (Anon, 2015), has been reported to mediate chronic stress-elicited depression-like phenotype and aberrant dendritic atrophy (Abe-Higuchi et al., 2016). The promotion of hippocampal memory and synaptic plasticity has been found to be correlated with significant activation of SIRT1/mircoRNA signaling (Chen et al., 2016). MircoRNA has been reported to regulate SIRT1/cAMP response element-binding protein (CREB) signaling (Zhang et al., 2017). Activating CREB/brain-derived neurotrophic factor (BDNF) pathway in hippocampus can contribute to ameliorative effects of alpha-linolenic acid supplement on cognitive deficits (Gao et al., 2016). In schizophrenia, mircoRNA has also been reported to regulate neuronal level of glycogen synthase kinase-3β (GSK-3β), which acts downstream of BDNF signaling (Thomas et al., 2017). Inhibition of protein kinase B (AKT)/GSK-3β signaling can mediate depression-like behaviors and synaptic plasticity of hippocampal neuron in CUMS rats (Mao et al., 2017). GSK-3β deletion in dentate gyrus has been found to inhibit hippocampal synaptic transmission and reduce protein level of synaptophysin (SYN, a presynaptic marker) (Liu et al., 2017a, 2017b, 2017c). Besides SYN, growth-associated protein-43 (GAP-43, a marker of neuronal structural plasticity) may also play a role in pathophysiology of depression and mechanisms of antidepressants (Iwata et al., 2006).

However, side effects of antidepressants attenuate their efficacy and safety as reliable strategies for anti-depression treatment in clinical practice (Varela and Adan-Manes, 2017). As a non-pharmacological coping strategy, physical exercise as a regular life-style prevents depression relapse much better than antidepressant medication in clinical reports (Strawbridge et al., 2002). Adult hippocampal neurogenesis has been suggested as an important target associated with antidepressant effects of exercise (Sun et al., 2017). It has been reviewed that aerobic exercise contributes to hippocampal plasticity-related proteins expression (Gomes et al., 2013). Treadmill running has been found to activate hippocampal plasticity-related proteins expression of BDNF and SYN in obese rats (Cai et al., 2016). Co-treatment of 7,8-Dihydroxyflavone and voluntary running wheel exercise can ameliorate the reductions in hippocampal levels of SYN and GAP-43 (Krishna et al., 2017). Therefore, the aim of the present study was to investigate the signaling mechanisms of CUMS affecting hippocampal plasticity-related proteins expression and the regulation of swimming exercise in mice.

2. Materials and methods

2.1. Animals and groups

Male C57BL/6 mice (5-week old, 15–20 g) obtained from Shanghai SLAC Experimental Animal Center (Shanghai, China) were housed with a 12-h light:dark cycle under controlled temperature (22 ± 2 °C) and humidity (50 ± 10%), and were given standard diet and water ad libitum. All mice were divided into three groups: control (Con), CUMS, CUMS + Swim; n = 8 per group. All procedures were in accordance with the guidelines for the use of laboratory animals published by the People's Republic of China Ministry of Health (No. 55 order, January 25, 1998) and were approved by the Experimental Animal Care and Use Committee at East China Normal University (ECNU 2006-05).

2.2. Chronic unpredictable mild stress procedure

The CUMS procedure was performed as described (Surget et al., 2009) with a slight modification. Mice in CUMS group were subjected to different stressors: cage tilting for 24 h (45°), wet bedding for 24 h (200 ml of water per cage), cold swimming for 5 min (at 10 °C), swimming in hot water for 5 min (at 40 °C), fasting for 48 h, water deprivation for 24 h, level shaking for 10 min, tail nip for 1 min (1 cm from the end of the tail), and inversion of the light/dark cycle for 24 h. It should be pointed out that cold water and hot water stressors are used broadly in CUMS regimen (Hu et al., 2017). In view of the innate ability to swim in rodents, cold water and hot water stressors in CUMS should be considered as temperature stress rather than water stress. These stressors were applied for 49 days, during which each stressor was applied 5–6 times. Mice received one of these stressors at different time every day and the same stressor was not applied consecutively over two days so that animals could not predict the occurrence of stimulation. Control group was undisturbed except for necessary procedures such as routine cage cleaning.

2.3. Exercise protocol

As an innate ability of rodents, swimming exercise presents advantages over treadmill running; moreover, swimming requires an unelaborate device relative to treadmill running and spontaneous wheel exercise (Seo et al., 2014). Moreover, studies using this model revealed similarities in the adaptations to the exercise in relation to those observed in humans (Gobatto et al., 2001; Voltarelli et al., 2002). Thus, swimming is the most used in exercise physiology studies and induces various changes in the functions of the brain (Ra et al., 2002). Mice were trained in a moderate swimming program with no weight loading in free style, the antidepressant effects of which have been validated by our previous study (Liu et al., 2017a, 2017b, 2017c) and other report (Jiang et al., 2014). Daily swimming exercise was performed in a large glass water tank (100 cm(L) × 60 cm(W) × 80 cm(H)) at 32 ± 1 °C, a thermostat being used to maintain water temperature and an aquarium thermometer being stuck on the glass to present real-time temperature. The water depth was 60 cm so that the mice could not support themselves by touching the bottom with their feet; additionally, liquid soap was added to reduce surface tension and to abolish floating behavior (Mazzardo-Martins et al., 2010). The swimming was continuously supervised. The animals were swum as a group of six to eight mice, because it has been demonstrated that the intensity of swimming exercise was significantly raised by interaction among the animals (Iemitsu et al., 2004). The swimming program 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. The adaptation was aimed at reducing the water-induced stress without promoting physiological alterations in relation to the physical training (Contarteze et al., 2008). Then, the training period began from the 4th week during CUMS exposure, with intensity of 60 min/day, 5d/week, for a total of 4 weeks. Generally, swimming 1 h a day for 5 days a week is considered as moderate exercise, while swimming more than that is classified as strenuous exercise (Seo et al., 2014). Damghani et al. have suggested that only 14 days of swimming exercise (45 min/day, five days per week) is sufficient to reduce depression in rats (Damghani et al., 2016). Exercise was performed at the same time every day (between 9:00 and 11:00 a.m.). After swimming, mice were toweled dry and kept warm by electric heater.

2.4. Behavioral testing

Except sucrose preference, a videocomputerized tracking system (DigBehav, Jiliang Co. Ltd., Shanghai, China) was used to record the behaviors of the animals. All testing equipment was thoroughly cleaned between each session.

2.4.1. Sucrose preference test (SPT)

The procedure was performed as described previously (Willner et al., 1987). Briefly, 72 h before the test mice were trained to adapt 1% sucrose solution (w/v): two bottles of 1% sucrose solution were placed in each cage, and 24 h later 1% sucrose in one bottle was replaced with tap water for 24 h. After adaptation, mice were deprived of water and food for 24 h, followed by the sucrose preference test, in which mice housed in individual cages had free access to two bottles containing

200 ml of sucrose solution (1% w/v) and 200 ml of water, respectively. At the end of 24 h, the sucrose preference was calculated as a percentage of the consumed 1% sucrose solution relative to the total number of liquid intake.

2.4.2. Forced swim test (FST)

Among all animal models, the forced swim test (FST) remains one of the most used tools for screening antidepressants (Petit-Demouliere et al., 2005), since it has good predictive validity and allows rapid and economical detection of substances with potential antidepressant-like activity (Castagne et al., 2011). According to published protocol (Porsolt et al., 1977), the swimming sessions were conducted by placing the mice in cylinders (30 cm height × 10 cm diameters) containing 25 °C water 20 cm deep so that the mice could not support themselves by touching the bottom with their feet. The FST was conducted for 5 min and immobility time was recorded. Floating in the water without struggling and only making movements necessary to keep its head above the water were regarded as immobility. Here, it should be pointed out that the FST differs from swimming exercise protocol. Firstly, the water temperature is more comfortable to swim in training than in FST. Secondly, swimming exercise was performed in a large glass water tank to ensure enough secured space for each subject to freely swim, whereas the FST was conducted in a small cylinder. Thirdly, mice swam as a group during exercise training to allow interaction among animals, but the FST was only available for one mouse at a time. Based on the above-mentioned three aspects, the FST seems to induce stress similar to cold water challenge, confined space hazard, and separation stress. Thus, the FST is used to assess a classical depressive behavior - despair behavior in mice.

2.4.3. Tail suspension test (TST)

The test was performed as described by Guo and Lu (2014). Mice were individually suspended by the tail to a vertical bar on the top of a box (30 × 30 × 30 cm), with adhesive medical tape affixed 2 cm from the tip of the tail. The immobility time was recorded for a 5-min test session. In TST, immobility was defined as the absence of any limb or body movements except those caused by respiration.

2.4.4. Open field test (OFT)

The open field test (OFT) is routinely used to study anxiety-like behaviors in mouse (Carola et al., 2002). In addition, the OFT is also employed to evaluate the effects of antidepressant treatment (Shyong et al., 2017). This study aimed to examine antidepressant effect of swimming exercise, so the OFT was used to measure exploratory behavior and general activity in mice. The test was performed as described previously (Zheng et al., 2006) with minor modifications. Each mouse was placed in the center of the open field (30 × 30 × 30 cm chamber, with 16 holes in its floor) for 5 min in a quiet room after weighed. Parameters assessed were the number of poking into holes and rearing, and the distance traveled in the center.

2.5. Tissue collection

Mice were decapitated and hippocampus was rapidly and carefully isolated on ice-plate. Tissues were frozen in liquid nitrogen and then stored at − 80 °C until assays.

2.6. Real-time PCR

Total RNA was prepared from frozen tissues using TRIzol (Invitrogen, Chromos, Singapore) and purified according to the instructions included. Double-stranded cDNA was synthesized from ~ 1 μg of total RNA using ReverTra Ace® qPCR RT Kit (TOYOBO, Osaka, Japan). Real-time PCR reactions were set up using the SYBR-Green PCR kit (TOYOBO, Osaka, Japan) and were cycled in StepOne™ Real-Time PCR System (Applied Biosystems, CA, USA). PCR was performed in a fluorescence temperature cycler containing 4pmol of each primer, 2.0 × Master SYBR Green I (contains Taq DNA polymerase, reaction buffer, dNTP mix, SYBR Green I dye, and 10 mM $MgCl_2$), and 2.0 ul template in a total volume of 20 ul. The amplification occurred in a three-step cycle (denaturation at 95 °C for 15 s, annealing at 61 °C for 30 s, extension and data collection at 72 °C for 45 s) for 40 cycles. The threshold cycle (CT) of each target product was determined and normalized to internal standard β-actin. Fold changes in the expression of genes of interest were calculated using the $2^{-\Delta\Delta Ct}$ method. PCR primers were as follows: SIRT1 (sense 5′-GCGCTTTG TTTGCTGGGAAT-3′; antisense 5′-CTCACAGCATGCACAACTGTC-3′), miR-124 (sense 5′-AC ACTCCAGCTGGGcgtgttcacagcggac-3′; antisense 5′-TGGTGTCGTGG A GTCG-3′), miR-134 (sense 5′-ACACTCCAGCTGGGtgtgactggttgacca-3′; antisense 5′-TGGTGTCGTGGAGTCG-3′), miR-138 (sense 5′-ACAC TCCAGCTGGGagctggtg ttgtgaatc-3′; antisense 5′-TGGTGTCGTGG AGTCG-3′), U6 (sense 5′-CTCGCTTCG GCAGCACA-3′; antisense 5′-AAC GCTTCACGAATTTGCGT-3′), and GAPDH (sense 5′-AATGTGTCCG TCGTGGATCTGA-3′; antisense 5′-AGTGTAGCCCAAG ATGCCCTTC-3′).

2.7. Western blotting

The total protein concentration was assessed using the Bradford assay and run on an SDS-PAGE gel. Western blot analysis was performed using the primary antibody (anti-SIRT1, #wl00599, 1:1000, Wanleibio; anti-CREB, #wl02835, 1:1000, Wanleibio; anti-BDNF, #ab203573, 1:1000, Abcam; anti-AKT, #4685, 1:1000, Cell Signaling; anti-phosphorylated ATK on Ser473, #9270S, 1:1000, Cell Signaling; anti-GSK-3β, #9315S, 1:1000, Cell Signaling; anti-phosphorylated GSK-3β on Ser9, #9322S, 1:1000, Cell Signaling; anti-phosphorylated GSK-3β on Tyr216, #ABIN753532, 1:200, Antibody-online; anti-GAP43, #wl00641, 1:1000, Wanleibio; anti-SYN, #ab32127, 1:1000, Abcam) in TBST overnight at 4 °C. Monoclonal anti-β-Tubulin (#sc-9104, 1:1000, Santa Cruz) was used as an internal control. 30 μl samples were electrophoresed and transferred to PVDF membranes. After blocking, membranes were incubated with different antibodies. The blots were incubated HRP-conjugated secondary antibodies and signals detected by enhanced chemiluminescence (ECL) WB detection reagents.

2.8. Statistical analysis

Data are presented as mean ± SEM. Analyses were performed using GraphPad Prism, version 6.01 (GraphPad Software). Body weight was analyzed by the means of a repeated measurement ANOVA. Differences among experimental groups were determined by one-way ANOVA followed by Bonferroni test for *post hoc* comparisons. Correlations were calculated by Pearson's correlation. Statistical significance was set at $P < 0.05$ or 0.01.

3. Results

3.1. Effects of CUMS and swimming exercise on depression-like behaviors

With respect to effects of CUMS and swimming exercise on body weight (Fig. 1A), a repeated measurement ANOVA yield a main effect of time ($F_{(8,63)} = 72.276$, $P < 0.01$), a main effect of group ($F_{(2,21)} = 93.498$, $P < 0.01$) and significant group by time interaction ($F_{(16,168)} = 5.568$, $P < 0.01$). No significant differences were found in the baseline body weight of the three groups, but the body weight of the CUMS group (from the 2nd week till the 7th week except the 3rd week) was significantly lower than that of the control group. This catch-up growth is consistent with the study of Drake et al., in which prenatal dexamethasone-exposed rats have shown catch-up growth by 7 weeks (Drake et al., 2010). There were no significant differences of body weight in CUMS mice with swimming or not over the 7-week period, except that swim group was lighter than no swim group in CUMS mice at the end of the 8th week. In addition, swimming exercise did not

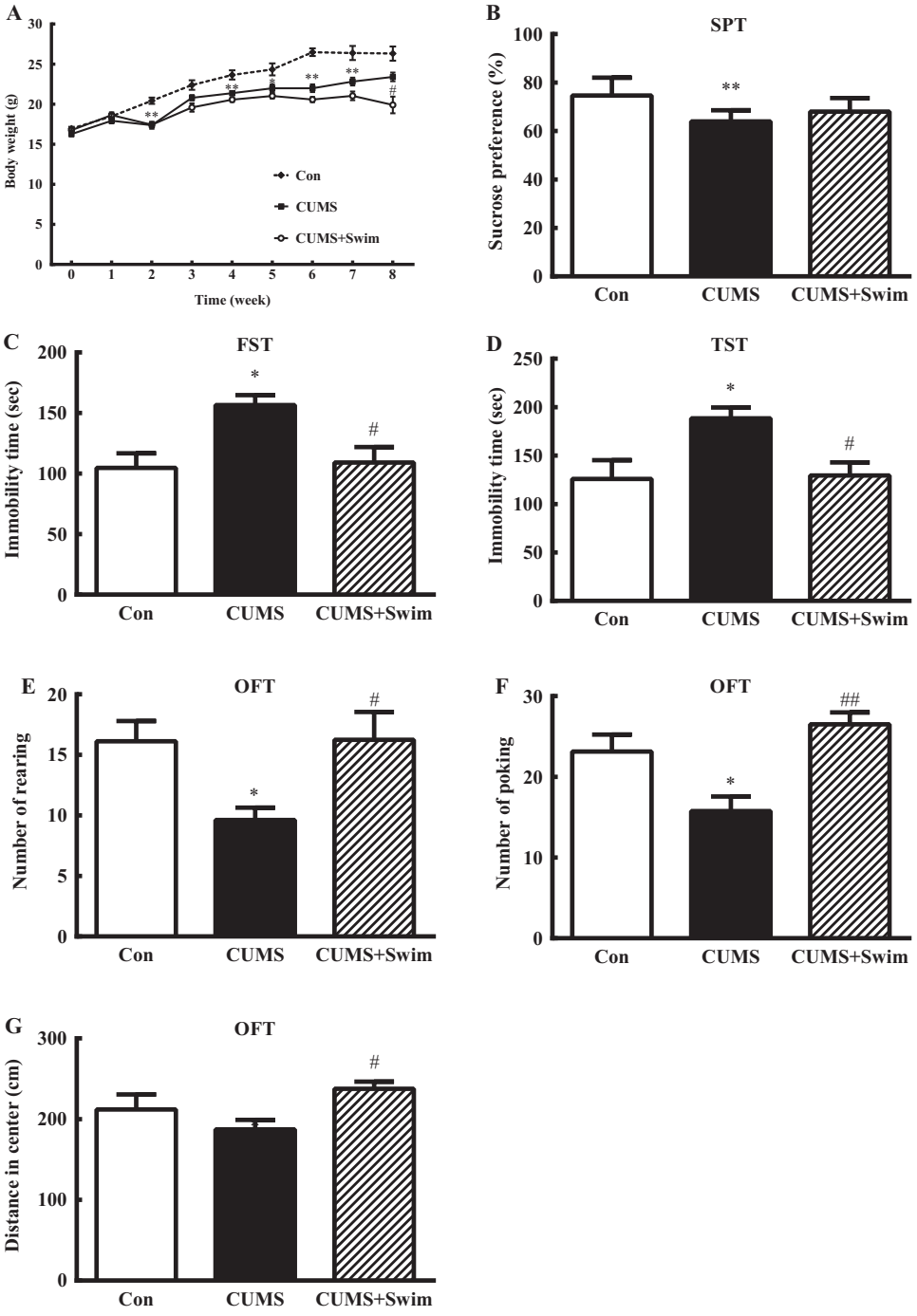

Fig. 1. Effects of CUMS and swimming exercise on depression-like behaviors. (A) Changes in body weight during 8-week treatment, (B) Sucrose preference in sucrose preference test (SPT), (C) Immobility time in forced swim test (FST), (D) Immobility time in tail suspension test (TST), (E) Number of rearing in open field test (OFT), (F) Number of poking in OFT, (G) Distance in center in OFT. Data are presented as means ± SEM (n = 8 per group). $*p < 0.05$, $**p < 0.01$ versus control (Con); $\#p < 0.05$, $\#\#p < 0.01$ versus CUMS.

block CUMS-induced body weight loss. Similarly, it has been reported that swimming training decrease body weight significantly compared to sedentary mice (Kim et al., 2014). In our previous study, CUMS rats with treadmill preconditioning have also been found to gain less weight and growth rate than sedentary CUMS rats (Liu and Zhou, 2012). The reason for these results may be that physical activities consume more energy with no difference in levels of food intake (Kim et al., 2014).

Then, we examined the effects of CUMS and swimming exercise on depression-like behaviors in mice. As shown in Fig. 1, one-way ANOVA revealed a significant effect of treatment on sucrose preference test ($F_{(2,21)} = 6.474$, $p < 0.01$); forced swim test ($F_{(2,21)} = 6.632$, $p < 0.01$); tail suspension test ($F_{(2,21)} = 5.377$, $p < 0.05$); open field test ($F_{(2,21)} = 4.765$, $p < 0.05$ for rearing; $F_{(2,21)} = 9.126$, $p < 0.01$ for poking; $F_{(2,21)} = 3.398$, $p < 0.05$ for distance). Post hoc analysis indicated that CUMS induced depression-like behaviors, including reduced percentage of sucrose preference ($p < 0.01$, Fig. 1B) in SPT and increased immobility time in FST ($p < 0.05$, Fig. 1C) and TST ($p < 0.05$, Fig. 1D), as well as reduced number of rearing ($p < 0.05$, Fig. 1E) and poking ($p < 0.05$, Fig. 1F) in OFT, compared to the control. Swimming exercise ameliorated depression-like behaviors induced by CUMS, including decreased immobility time in FST ($p < 0.05$, Fig. 1C) and TST ($p < 0.05$, Fig. 1D), as well as increased number of rearing ($p < 0.05$, Fig. 1E) and poking ($p < 0.01$, Fig. 1F), and the distance traveled in center ($p < 0.05$, Fig. 1G) in OFT. There was no significant difference in depression-like behaviors between control and CUMS + Swim groups, suggesting that swimming exercise can reverse depression-like behaviors in CUMS mice.

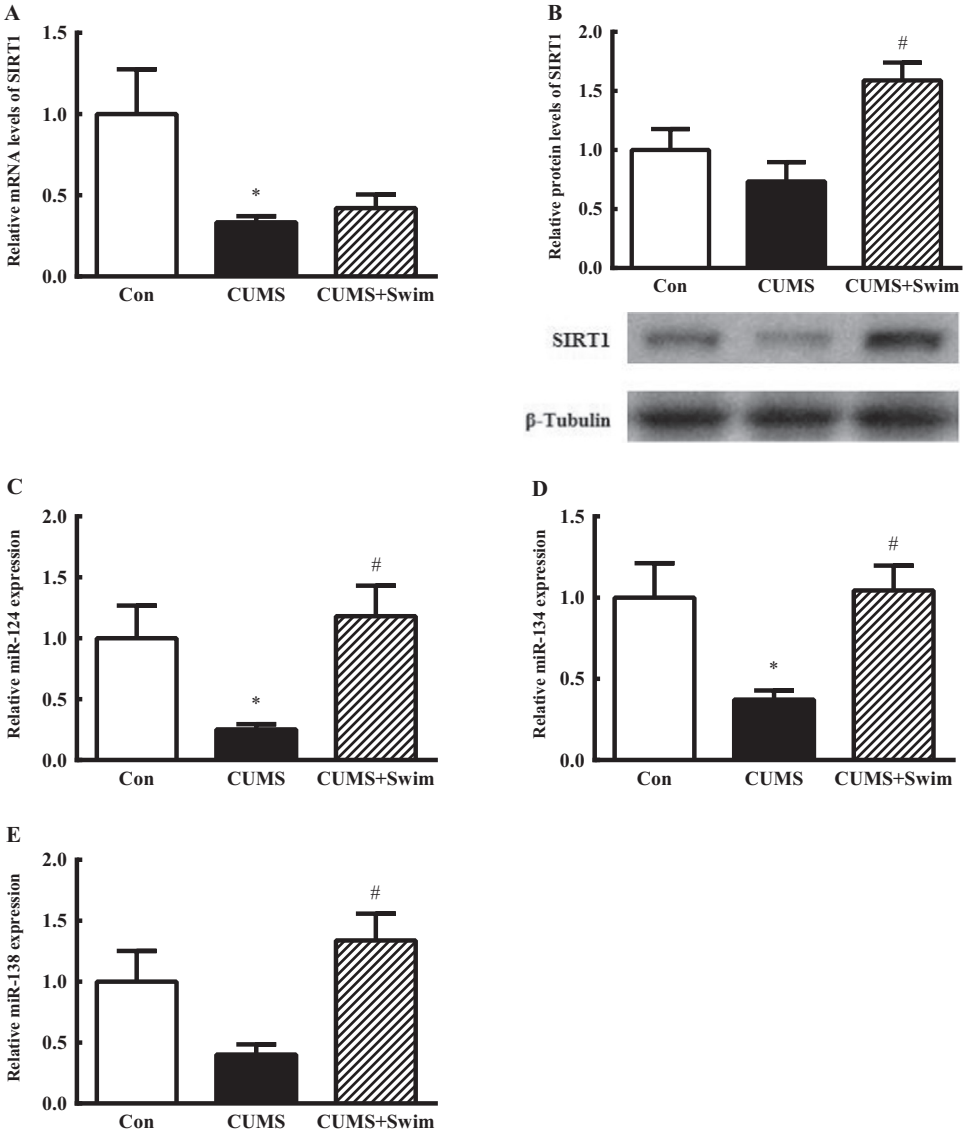

Fig. 2. Effects of CUMS and swimming exercise on SIRT1/mircoRNA signaling. The relative levels of SIRT1 mRNA (A) and SIRT1 protein (B, n = 4–5), and the relative expression of miR-124 (C), miR-134 (D), miR-138 (E) in hippocampus. Data are presented as means ± SEM (n = 7–8 per group). *$p < 0.05$ versus control (Con); #$p < 0.05$ versus CUMS.

3.2. Effects of CUMS and swimming exercise on SIRT1/mircoRNA signaling

To test whether CUMS induces depression-like behaviors via SIRT1/mircoRNA pathway, RT-PCR was used to examine the related gene expression. As shown in Fig. 2, one-way ANOVA revealed a significant effect of treatment on the levels of SIRT1 ($F_{(2,19)} = 4.127$, $p < 0.05$), miR-124 ($F_{(2,19)} = 4.851$, $p < 0.05$), miR-134 ($F_{(2,19)} = 5.275$, $p < 0.05$), and miR-138 ($F_{(2,19)} = 5.111$, $p < 0.01$). Post hoc analysis indicated that CUMS reduced the levels of SIRT1 ($p < 0.05$, Fig. 2A), miR-124 ($p < 0.05$, Fig. 2C), and miR-134 ($p < 0.05$, Fig. 2D) in hippocampus, whereas swimming exercise increased hippocampal expression of miR-124 ($p < 0.05$, Fig. 2C), miR-134 ($p < 0.05$, Fig. 2D), and miR-138 ($p < 0.05$, Fig. 2E) in CUMS mice.

Then, we further examined the effects of CUMS and swimming exercise on SIRT1 protein expression. One-way ANOVA revealed a significant effect of treatment on the level of SIRT1 protein ($F_{(2,10)} = 6.326$, $p < 0.05$). Post hoc analysis indicated that swimming exercise increased hippocampal SIRT1 protein expression ($p < 0.05$, Fig. 2B) in CUMS mice. There was no significant difference in SIRT1/mircoRNA gene expression between control and CUMS + Swim groups, suggesting that the antidepressant effect of swimming exercise may be associated with SIRT1/mircoRNA signaling.

3.3. Effects of CUMS and swimming exercise on CREB/BDNF and AKT/GSK-3β signaling

To determine whether CREB/BDNF and AKT/GSK-3β pathway is involved in the effects of CUMS and swimming exercise on depression-like behaviors, WB was used to examine the related protein expression in hippocampus. As shown in Fig. 3, one-way ANOVA revealed a significant effect of treatment on the protein levels of CREB ($F_{(2,11)} = 5.839$, $p < 0.05$), BDNF ($F_{(2,11)} = 13.180$, $p < 0.01$), AKT ($F_{(2,10)} = 6.071$, $p < 0.05$) and Ser473 phosphorylation ($F_{(2,10)} = 4.327$, $p < 0.05$), GSK-3β ($F_{(2,10)} = 6.110$, $p < 0.05$), phosphorylation of GSK-3β at Ser9 ($F_{(2,10)} = 5.885$, $p < 0.05$) and Tyr216 ($F_{(2,11)} = 6.884$, $p < 0.05$). Post hoc analysis indicated that swimming exercise increased the protein levels of CREB ($p < 0.05$, Fig. 3A), BDNF ($p < 0.01$, Fig. 3B), AKT ($p < 0.05$, Fig. 3C) and Ser473 phosphorylation ($p < 0.05$, Fig. 3D), GSK-3β ($p < 0.05$, Fig. 3E), phosphorylation of GSK-3β at Ser9 ($p < 0.05$, Fig. 3F) and Tyr216 ($p < 0.05$, Fig. 3G) in CUMS mice. Although CUMS only reduced AKT protein level ($p < 0.05$, Fig. 3C) to a significant extent, other proteins expression in CUMS group were lower than those in control group. Except BDNF protein level ($p < 0.01$, Fig. 3B), there was no significant difference in other proteins expression between control and CUMS + Swim groups.

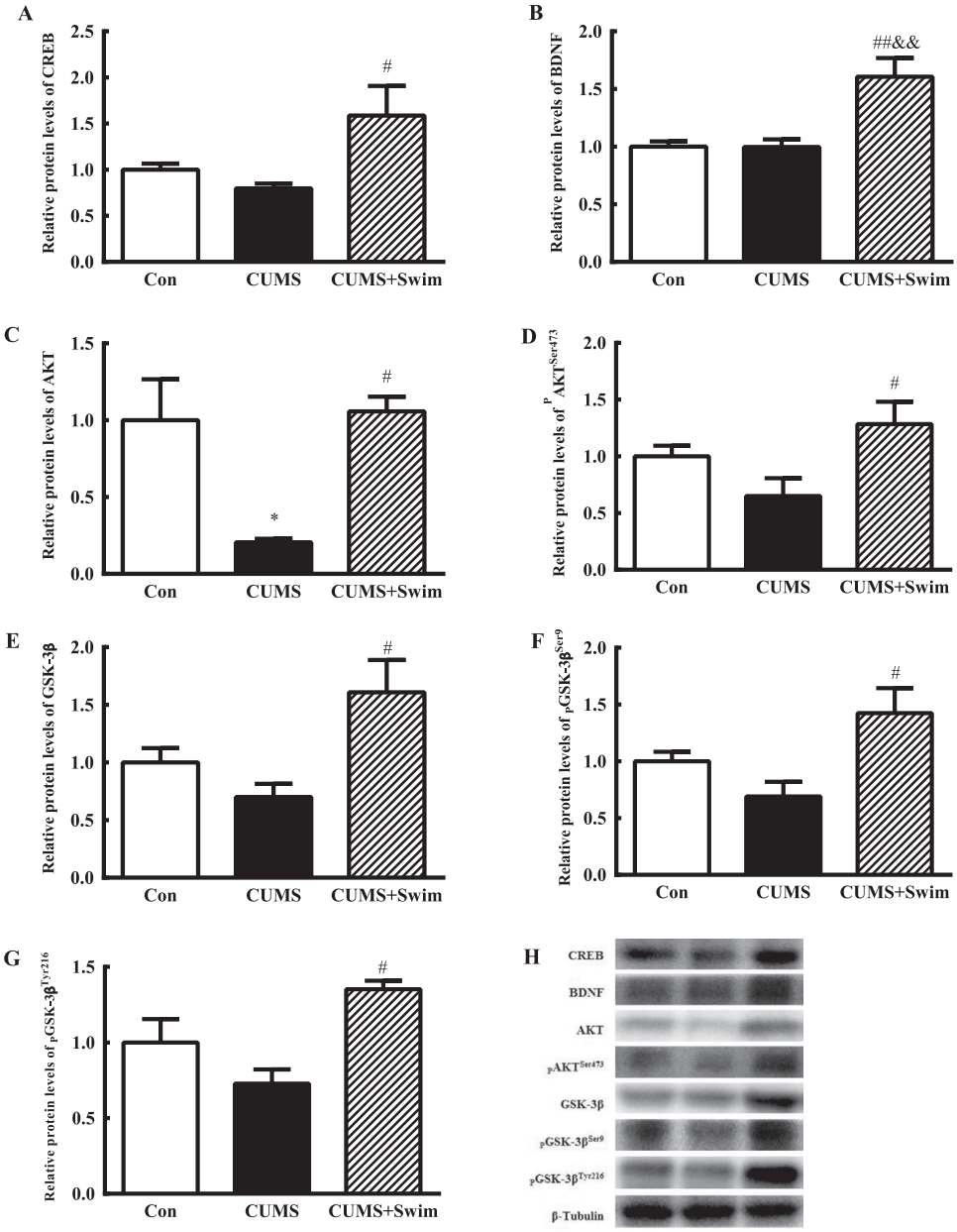

Fig. 3. Effects of CUMS and swimming exercise on CREB/BDNF and AKT/GSK-3β signaling. The relative protein levels of CREB (A), BDNF (B), AKT (C), AKT phosphorylation at Ser473 (D), GSK-3β (E), GSK-3β phosphorylation at Ser9 (F) and Tyr216 (G), and protein bands (H) in hippocampus. Data are presented as means ± SEM (n = 4–5 per group). *$p < 0.05$ versus control (Con); #$p < 0.05$, ##$p < 0.01$ versus CUMS; &&$p < 0.01$ versus Con.

3.4. Effects of CUMS and swimming exercise on plasticity-related proteins expression

To test the effects of CUMS and swimming exercise on hippocampal plasticity, WB was used to examine plasticity-related protein expression in hippocampus. As shown in Fig. 4, one-way ANOVA revealed a significant effect of treatment on the protein levels of GAP-43 ($F_{(2,10)}$ = 6.125, $p < 0.05$) and SYN ($F_{(2,9)}$ = 8.804, $p < 0.01$). Post hoc analysis indicated that CUMS reduced SYN protein level ($p < 0.05$, Fig. 4B), whereas swimming exercise increased the protein levels of GAP-43 ($p < 0.05$, Fig. 4A) and SYN ($p < 0.01$, Fig. 4B) in CUMS mice. In addition, GAP-43 protein expression ($p < 0.05$, Fig. 4A) in CUMS + Swim group was significantly higher than that in control group.

3.5. Correlation between depression-like behaviors and plasticity-related proteins expression

To further verify that depression-like behaviors is linked to hippocampal plasticity in CUMS mice, we assessed the correlation between the protein levels of GAP-43, SYN, and the parameters of depression-like behaviors. There was a positive correlation between GAP-43 level and poking number (R^2 = 0.343, $p < 0.05$; Fig. 5E) and distance in center (R^2 = 0.733, $p < 0.01$; Fig. 5F) in OFT. An inverse correlation between GAP-43 level and immobility time in TST (R^2 = 0.578, $p < 0.01$; Fig. 5C) was found. Similarly, there was a positive correlation between SYN level and sucrose preference in SPT (R^2 = 0.525, $p < 0.01$; Fig. 5A), number of rearing (R^2 = 0.444, $p < 0.05$; Fig. 5D) and poking (R^2 = 0.885, $p < 0.01$; Fig. 5E) and distance in center (R^2 = 0.539, $p < 0.01$; Fig. 5F) in OFT, but a negative correlation between SYN level and immobility time in FST (R^2 = 0.823, $p < 0.01$; Fig. 5B) and in TST (R^2 = 0.769, $p < 0.01$; Fig. 5C). These results suggest that CUMS-induced depression-like behaviors are associated with hippocampal plasticity.

4. Discussion

The results of the present study demonstrated that CUMS induced depression-like behaviors, which were reversed by swimming exercise.

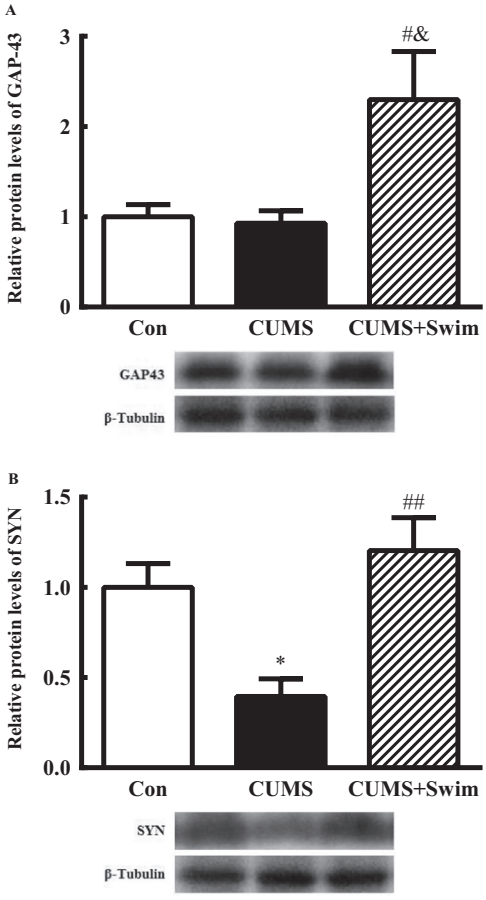

Fig. 4. Effects of CUMS and swimming exercise on plasticity-related proteins expression. The relative protein levels of GAP-43 (A) and SYN (B) in hippocampus. Data are presented as means ± SEM (n = 4–5 per group). *$p < 0.05$ versus control (Con); #$p < 0.05$, ##$p < 0.01$ versus CUMS; &p < 0.05 versus Con.

Moreover, the behavioral changes induced by CUMS and exercise were correlated with hippocampal plasticity-related proteins expression of GAP-43 and SYN. Therefore, our study focused on the molecular mechanisms regulating this plasticity in mice hippocampus.

SIRT1, a mammalian orthologue of the yeast Sir2 histone deacetylase, is a critical epigenetic modulator (Hasegawa et al., 2013), protecting against acute and chronic stress (Bordone and Guarente, 2005; Rodgers et al., 2005). SIRT1 gene has been reported to be associated with depressed population (Kishi et al., 2010). SIRT1 is ubiquitously present in brain areas, especially in the hippocampus, prefrontal cortex and basal ganglia (Zakhary et al., 2010). SIRT1 knockout can induce decreased dendritic branching, branch length, and complexity of neuronal dendritic arbors in hippocampus, as well as altered hippocampal gene expression, which plays an important role in synaptic and structural functions (Michan et al., 2010). These results indicate that SIRT1 regulates hippocampal plasticity. Furthermore, SIRT1 has been found to modulate synaptic plasticity and memory formation via a microRNA-mediated mechanism in mice hippocampus (Gao et al., 2010). SIRT1 normally suppresses expression of miR-124 (Brennan et al., 2016), miR-134 (Lages et al., 2011) and miR-138 (Liu et al., 2013), forming negative feedback loops to regulate this plasticity. Resveratrol-induced improvement of learning and memory is likely to be regulated through reduced levels of miR-124 and miR-134, which may in turn upregulate CREB level to subsequently promote BDNF synthesis (Zhao et al., 2013). Tetrahydroxystilbene glucoside has also been reported to improve hippocampal memory with upregulation of SIRT1 and downregulation of miR-134 (Chen et al., 2016). Contrary to these findings, our study showed that CUMS reduced the levels of both SIRT1 and miR-124 and miR-134 in hippocampus with no effect on miR-138 level, whereas swimming exercise increased hippocampal expression of miR-124/134/138 in CUMS mice. Consistent with our results, chronic immobilization stress has been reported to decrease miR-134 level in rat amygdala and hippocampal CA1 region (Meerson et al., 2010). Chronic restraint stress-induced anhedonia in SPT and immobility in FST has also been found to be accompanied by a reduction in spine number, with no effect in levels of miR-132 or miR-134 in rat hippocampal neurons (Castaneda et al., 2015). Thus, further evidence is necessary to confirm the role of SIRT1/mircoRNA signaling in response to chronic stress.

SIRT1/miR-134 regulates memory and plasticity via CREB/BDNF pathway in mice hippocampus (Gao et al., 2010). Tetrahydroxystilbene glucoside has been found to promote hippocampal memory via upregulating CREB phosphorylation and BDNF expression (Chen et al., 2016). BDNF signaling plays a key role in depression-like behaviors induced by CUMS and alterations of dendritic spines in hippocampal CA1 pyramidal neurons (Qiao et al., 2017), and also plays a role in resilience to stress promoted by isoquinoline in prefrontal cortex of defeated mice (Pesarico et al., 2017). BDNF is critical for stabilizing hippocampal synaptic plasticity and it is a gene target of antidepressant treatment and cAMP-CREB cascade (Manji et al., 2003). CREB-BDNF signaling may contribute to decreased expression of plasticity-related proteins in hippocampus, thus resulting in depression-like behaviors (Bian et al., 2015). Maternal separation can inhibit CREB-BDNF signaling via decreasing the inhibitory phosphorylation of GSK-3β (Bian et al., 2015), whose overexpression in hippocampal dentate gyrus can induce prodepressant-like effects and increase sensitivity to chronic stress (Zhang et al., 2013). Changes to synaptic plasticity in stress and depression may also correlate to AKT/GSK-3β pathway besides CREB-BDNF signaling (Marsden, 2013). CUMS-induced depression-like behaviors and hippocampal plasticity changes has been linked to AKT/GSK-3β signaling (Mao et al., 2017). Although CUMS only reduced AKT protein level to a significant extent in the present study, we found that swimming exercise increased significantly both CREB/BDNF and AKT/GSK-3β signaling in hippocampus of CUMS mice. Adjunctive exercise has been reported to increase serum BDNF concentrations in depressed patients (Kerling et al., 2017). Running exercise-induced BDNF upregulation in mice hippocampus is CREB-dependent (Chen and Russo-Neustadt, 2009). Moreover, voluntary running wheel-induced expression of CREB/BDNF has been reported to be associated with increased expression of AKT/GSK-3β in rat hippocampus (Chen and Russo-Neustadt, 2005). Besides, AKT/GSK-3β pathway has also a part in antidepressant effects of lithium and ketamine (Costemale-Lacoste et al., 2016). It has been reported that AKT/GSK-3β pathway in hippocampus is involved in antidepressant-like effect of atorvastatin in mice (Ludka et al., 2016). The antidepressant effect of ginseng total saponins has been suggested to be mediated through interfering with hippocampal GSK-3β-CREB signaling and reversing decrease of plasticity-related proteins (Chen et al., 2014).

Changes of synaptic strength involve both pre- and postsynaptic mechanisms, and downregulation of growth factors and synaptic proteins is a mechanism of synaptic plasticity in hippocampus (Liu et al., 2017a, 2017b, 2017c). Thus, we further examined hippocampal proteins expression of GAP-43 and SYN, two important presynaptic markers. Results indicated that CUMS reduced SYN protein level, whereas swimming exercise increased the protein levels of both GAP-43 and SYN in CUMS mice. Moreover, CUMS-induced depression-like behaviors were associated with hippocampal expression of these two presynaptic proteins. Consistent with our findings, footshock stress can decrease GAP-43 protein level in rat prefrontal cortex (Kavushansky et al., 2009). Adult female offspring prenatally exposed to ethanol can display behavioral abnormalities and decreased hippocampal GAP-43 mRNA level, which can be normalized by thyroxine treatment to the alcohol-consuming mother (Wilcoxon et al., 2005). The antidepressants desipramine and tranylcypromine have been revealed to increase GAP-43 mRNA and protein expression in dentate gyrus (Chen et al., 2003). In addition,

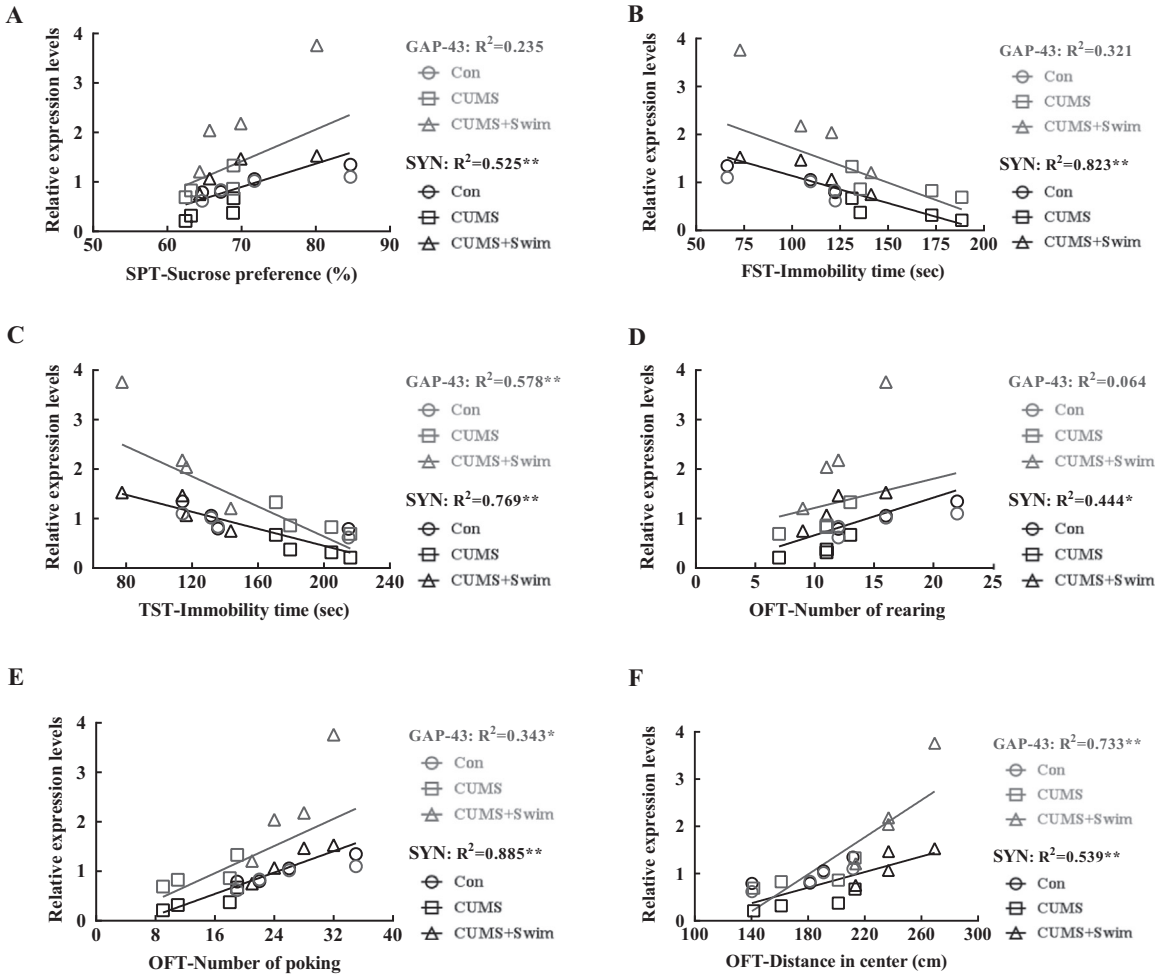

Fig. 5. Correlation between depression-like behaviors and plasticity-related proteins expression. (A) Sucrose preference in SPT, (B) Immobility time in FST, (C) Immobility time in TST, (D) Number of rearing in OFT, (E) Number of poking in OFT, (F) Distance in center in OFT. R: Pearson's correlation coefficient; n = 4 per group: Con, CUMS, CUMS + Swim.

spontaneous and forced exercise can promote cognitive function and GAP-43 expression in aged rat hippocampus (Cheon and Koo, 2013). Similarly, learned helplessness has been reported to induce depression-like behaviors and decrease SYN immunostaining in hippocampal CA3 region, whereas fluoxetine treatment can fully recover SYN labeling to control values (Reines et al., 2008). Hydrogen sulfide can also increase the fluorescence of SYN in hippocampus of CUMS rats (Hou et al., 2017). Administration of granulocyte colony-stimulating factor has been found to significantly reverse CUMS-induced depression-like behaviors and upregulate SYN expression in rat hippocampus (Li et al., 2016). Voluntary running can also improve depressive phenotype and SYN protein level in the hippocampus of corticosterone-exposed rats (Yau et al., 2014).

5. Conclusion

In conclusion, our results revealed the signaling mechanisms of chronic stress affecting hippocampal plasticity-related proteins expression and the regulation of swimming exercise. Further study is needed to establish a correlation between depression-like behaviors induced by chronic stress and epigenetic changes of hippocampal plasticity, and also a causal molecular signaling underling this plasticity.

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