



Exercise Intervention Modulates Synaptic Plasticity by Inhibiting Excessive Microglial Activation via Exosomes

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Background: Exosomes can activate microglia to modulate neural activity and synaptic plasticity by phagocytosis of neural spines or synapses. Our previous research found that an early 4-week exercise intervention in middle cerebral artery occlusion (MCAO) rats can promote the release of exosomes and protect the brain. This study intended to further explore the intrinsic mechanism of neuroprotection by exosome release after exercise.

Methods: Rats were randomly divided into four groups: the sham operation (SHAM), middle cerebral artery occlusion (MCAO) with sedentary intervention (SED-MCAO), MCAO with exercise intervention (EX-MCAO), and MCAO with exercise intervention and exosome injection (EX-MCAO-EXO). Modified neurological severity score (mNSS), cerebral infarction volume ratio, microglial activation, dendritic complexity, and expression of synaptophysin (Syn) and postsynaptic density protein 95 (PSD-95) were detected after 28 days of intervention.

Results: (1) The exercise improved body weight and mNSS score, and the survival state of the rats after exosome infusion was better. (2) Compared with the SED-MCAO group, the EX-MCAO ($P = 0.039$) and EX-MCAO-EXO groups ($P = 0.002$) had significantly lower cerebral infarct volume ratios ($P < 0.05$), among which the EX-MCAO-EXO group had the lowest ($P = 0.031$). (3) Compared with the SED-MCAO group, the EX-MCAO and EX-MCAO-EXO groups had a significantly decreased number of microglia ($P < 0.001$) and significantly increased process length/cell ($P < 0.01$) and end point/cell ($P < 0.01$) values, with the EX-MCAO-EXO group having the lowest number of microglia ($P = 0.036$) and most significantly increased end point/cell value ($P = 0.027$). (4) Compared with the SED-MCAO group, the total number of intersections and branches of the apical and basal dendrites in the EX-MCAO and EX-MCAO-EXO groups was increased significantly ($P < 0.05$), and the increase was more significant in the EX-MCAO-EXO group ($P < 0.05$). (5) The expression levels of Syn and PSD-95 in the EX-MCAO ($P_{\text{Syn}} = 0.043$, $P_{\text{PSD-95}} = 0.047$) and EX-MCAO-EXO groups were significantly higher than those in the SED-MCAO

group ($P < 0.05$), and the expression levels in the EX-MCAO-EXO group were significantly higher than those in the EX-MCAO group ($P < 0.05$).

Conclusion: Early exercise intervention after stroke can inhibit the excessive activation of microglia and regulate synaptic plasticity by exosome release.

Keywords: MCAO, exercise intervention, exosomes, synaptic plasticity, microglial

INTRODUCTION

Stroke is one of the common causes of disability and death worldwide (Yang et al., 2021). Ischemic stroke can lead to interruption of brain energy supply, resulting in nerve cell damage and inflammatory cascade, and finally in neurological dysfunction (del Zoppo, 2009). The ischemic penumbra is activated within a few days of cerebral ischemia (Moskowitz et al., 2010), which is an important time window for cerebral protection. Studies have found that 3 days after ischemia, the brain releases signals of growth factors promoting synaptogenesis, such as growth-associated protein 43 and synaptophysin (Stroemer et al., 1995), which are important for neurological recovery (Coleman et al., 2017).

Exercise is an effective rehabilitation strategy after stroke (Xing and Bai, 2020). After 7 days of grasping training immediately after cerebral ischemia, grasping ability can be restored to the level before injury (Zeiler et al., 2013). The exercise intervention was performed 1 day and 1 week after cerebral ischemia in rats, and it was found that exercise training had the best effect on reducing motor dysfunction after 1 day (Shi et al., 2020).

Exercise promotes exosome release into the circulation (Fruhbeis et al., 2015; Whitham et al., 2018; Brahmer et al., 2019). Both acute (Fruhbeis et al., 2015) and long-term exercises can increase serum exosome levels (Bertoldi et al., 2018; Ma et al., 2018). A study found that exosomes induce long-term brain protection and promote neural recovery (Otero-Ortega et al., 2018).

A previous study by our team confirmed that exercise intervention in the early stage of cerebral infarction in rats (Shi et al., 2020; Shi and Wan, 2021) improved the concentration of exosomes in blood and brain tissues and that the exosomes in blood were targeted to accumulate in the brain, improving neural plasticity and protecting neural function (Li et al., 2021). However, the mechanism by which exosomes enter and function in the brain has not been thoroughly explored.

Studies have found that exosomes can regulate the activation of microglia (Zagrean et al., 2018) by inhibiting the activation of M1 microglia (Li et al., 2019; Zheng et al., 2019; Duan et al., 2020) and regulating neural plasticity. Exosomes derived from microglia can significantly reduce cerebral infarct volume and dysfunction (Song et al., 2019a). Microglia have a key role in regulating synaptic connections and synaptic remodeling (Frost and Schafer, 2016; Yu et al., 2021). Studies have found that after cerebral ischemia, microglia can directly contact synapses for a long time (Wake et al., 2009), directly phagocytose spines and synapses, and induce synaptic formation to regulate neural

activity and synaptic plasticity (Crapser et al., 2021; Hanslik et al., 2021; Sancho et al., 2021; Wang and Li, 2021). However, whether the exercise-promoted release of exosomes modulates synaptic plasticity *via* microglia is unclear.

In this study, mNSS score, cerebral infarction volume ratio, microglial activation, and synaptic plasticity changes were detected after 4 weeks of intervention in different groups. In this way, regulation of synaptic plasticity by inhibition of excessive activation of microglia by exosomes was found to be the mechanism of early exercise intervention after stroke.

MATERIALS AND METHODS

Experimental Animals and Groups

Forty-eight adult male Sprague–Dawley rats (8–10 weeks old and weighing 280–320 g; Beijing Huafukang Biotechnology Co., Ltd., China) were used in this study. The experimental procedure was performed according to the National Institutes of Health (NIH) Laboratory Care and Use Guidelines to minimize the number of animals used and pain in the animals during the experiment and was approved by the Laboratory Animal Welfare Ethics Committee of Tianjin Medical University General Hospital (IRB2021-DWFL-403).

The rats were randomly divided into four groups: the sham operation (SHAM), MCAO with sedentary intervention (SED-MCAO), MCAO with exercise intervention (EX-MCAO), and MCAO with exercise intervention and exosome injection (EX-MCAO-EXO). The schematic of the protocol is shown in **Figure 1**.

MCAO Model

The modified Longa thread embolization method was used to prepare the MCAO model (Longa et al., 1989). After the rats were anesthetized, the external carotid artery and the proximal end of the common carotid artery were ligated, and a fine silicon-coated surgical nylon monofilament was inserted from the common carotid artery to the internal carotid artery for 60 min (Beijing Xinong Technology Co.; Ltd., 2838-A4, 0.38 ± 0.02 mm). Longa score was determined after 24 h, and those with 1–3 points were included in this experiment.

Brain-Derived Exosome Extraction

Brain tissues were ground into a homogenate and centrifuged at $1,500 \times g$ for 20 min and $13,000 \times g$ for 3 min to thoroughly remove cellular debris. Next, supernatants were filtered through a $22\text{-}\mu\text{m}$ -pore-size filter to exclude particles > 220 nm in diameter. Ultracentrifugation was performed for 2 h at $100,000 \times$

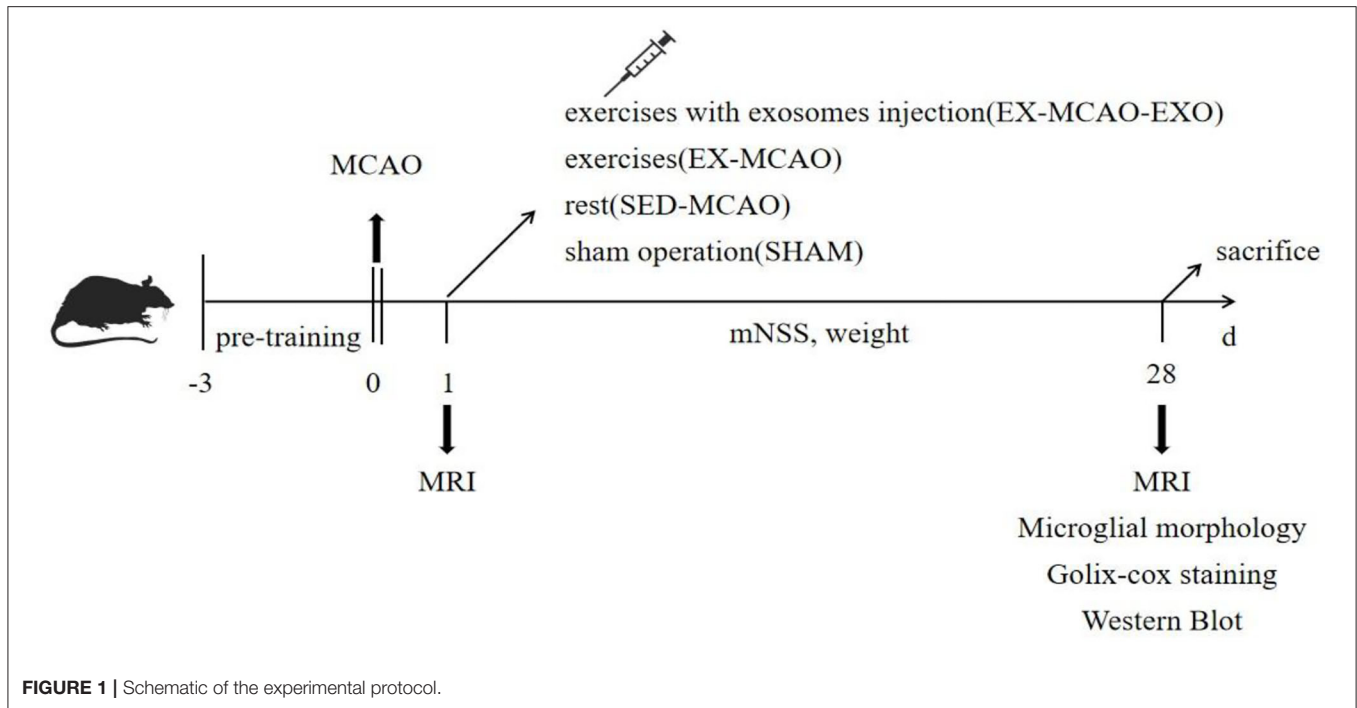


FIGURE 1 | Schematic of the experimental protocol.

g, and precipitates were resuspended in PBS (Ngolab et al., 2017). All centrifugation steps were performed at 4°C.

Exercise Intervention

The exercise program adopts the previous research program of the team (Shi et al., 2020; Li et al., 2021). All the rats were randomly divided into groups after 3 days of treadmill preintervention. The exercise intervention started 1 day after MCAO. The rats were made to exercise on a treadmill (ZS-PT of Zhongshi Dichuang Company, Beijing, China, angle of 0°, speed of 12 m/min) for 30 min each time, 5 times/week, for a total of 4 weeks.

Rats in the EX-MCAO-EXO group received brain-derived exosomes containing 100 µg of protein through tail vein injection 1 d after MCAO (Xin et al., 2013; Zhang et al., 2015; Safakheil and Safakheil, 2020; Li et al., 2021) and then underwent the same exercise intervention as the EX-MCAO group.

Neurological Function Assessment and Weight

Changes in body weight and neurological function of rats in each group were detected 1, 3, 7, 14, 21, and 28 d after MCAO. The modified neurological severity score (mNSS) was used for neurological scoring and ranged from 0 to 18. The mNSS test is a classic method for comprehensively assessing sensory, motor, balance, and reflexes in MCAO rats. The more severe the neurological deficit in rats, the higher the score.

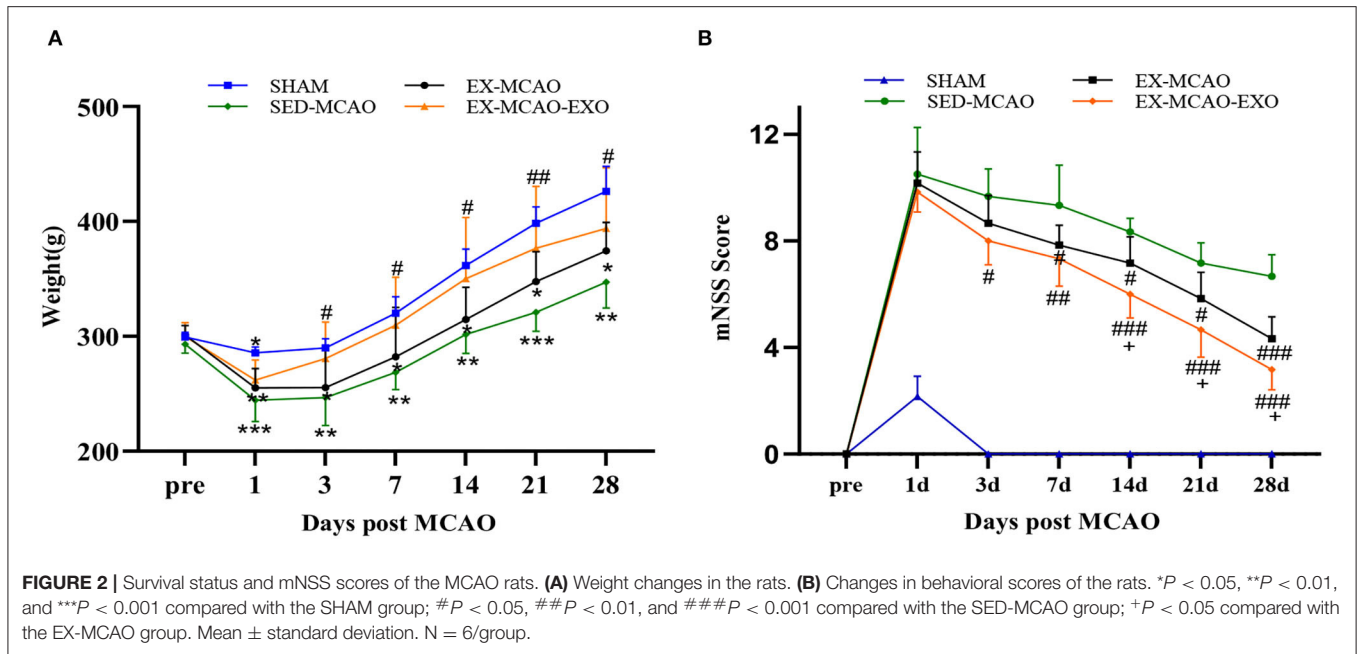
MRI Scan

Before the exercise intervention and after 28 days of intervention, the rats were anesthetized (3% isoflurane, nasal inhalation anesthesia). Magnetic resonance scanning

(9.4T, Bruker BioSpec94/30 UER+PET insert, Germany) was conducted to collect T2-weighted images (T2WI, SE sequence, FOV = 35 × 35 mm, matrix = 256 × 256 mm, TR = 2,500 ms, TE = 33 ms, thickness = 0.8 mm, and slices = 20). Infarct volume ratio = (total volume of contralateral – uninjured volume of ipsilateral)/total volume of contralateral.

Microglial Immunofluorescence Staining

Brain tissue was soaked in 4% paraformaldehyde for 12 h and then removed, placed in 15 and 30% sucrose solutions for complete dehydration, rinsed with PBS, and blotted dry. Brain tissue sections were prepared at a thickness of 10 µm on a cryostat (Leica CM1860, Germany) at -22 °C. The frozen sections were rewarmed for 20 min, washed thrice in PBS, ruptured with 0.3% Triton X-100 (Solarbio, China) for 30 min, and blocked with 3% Albumin Bovine V (Solarbio, China) for 1 h. Then, the sections were incubated with the primary antibody against Iba1 (1:500; Abcam, United Kingdom) diluted with Antibody Diluent (Solarbio, China) at 4°C overnight. A goat anti-rabbit Alexa Fluor 488 secondary antibody (1:200; Invitrogen, United States) was added to the sections, and they were incubated for 1 h at room temperature. DAPI (Abcam, United Kingdom) was added dropwise for staining and mounting. Images of the peri-infarct area were taken using an inverted fluorescence microscope (Olympus IX73, Japan). Microglia were skeletonized using the method of Young and Morrison; that is, immunofluorescence images were background-removed, binarized, and skeletonized using the ImageJ software. Then, the skeletonized microglial morphology was analyzed (Young and Morrison, 2018).



Golgi-Cox Staining

Golgi-Cox staining (FD Rapid Golgi Staining Kit; FD Neuro Technologies, United States) was performed to observe changes in dendrites in the infarcted penumbra cortex (Hu et al., 2020). Dendritic structures were analyzed by laser confocal scanning microscopy (Olympus FV1000, Japan). The Fiji software (<https://imagej.net/Fiji>) was used for neuron tracking. Sholl analysis was conducted to analyze trajectory, automatically drawing a concentric circle with the cell body as the center, with a step of 10 μm . The complexity of dendrites was quantified by the number of intersections and the number of neuron branches.

Western Blot Analysis

Total protein was extracted from peri-infarcted brain tissues, and total protein concentration was determined with a BCA kit (Solarbio, China). Proteins were separated by polyacrylamide gel electrophoresis (SDS-PAGE) and blotted on polyvinylidene fluoride (PVDF) membranes. The membranes were incubated with 5% non-fat milk for 1 h at room temperature and with anti-Syn (1:2,000; Abcam, United Kingdom), PSD-95 (1:1,000; Affinity, China), and β -tubulin (1:3,000; Solarbio, China) antibodies at 4°C overnight. Then, the cells were incubated with the IgG antibody (1:1,000; Cell Signaling Technology, Danvers, MA, United States) for 1 h at room temperature. Visualization was performed on a gel electrophoresis imager using an ECL hypersensitive chemiluminescent solution (Millipore, Germany).

Statistical Analysis

Statistical software SPSS 25.0 (SPSS Inc., Armonk, NY, United States) and GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, United States) were used. Data are presented as means \pm standard deviations (SD). The Shapiro–Wilks test was

performed to verify the normal distribution of the data. A one-way analysis of variance (ANOVA) was carried out to compare multiple groups, and an LSD t -test was performed for multiple comparisons between pairs. $P < 0.05$ was considered significant.

RESULTS

Body Weight and mNSS Score of Rats

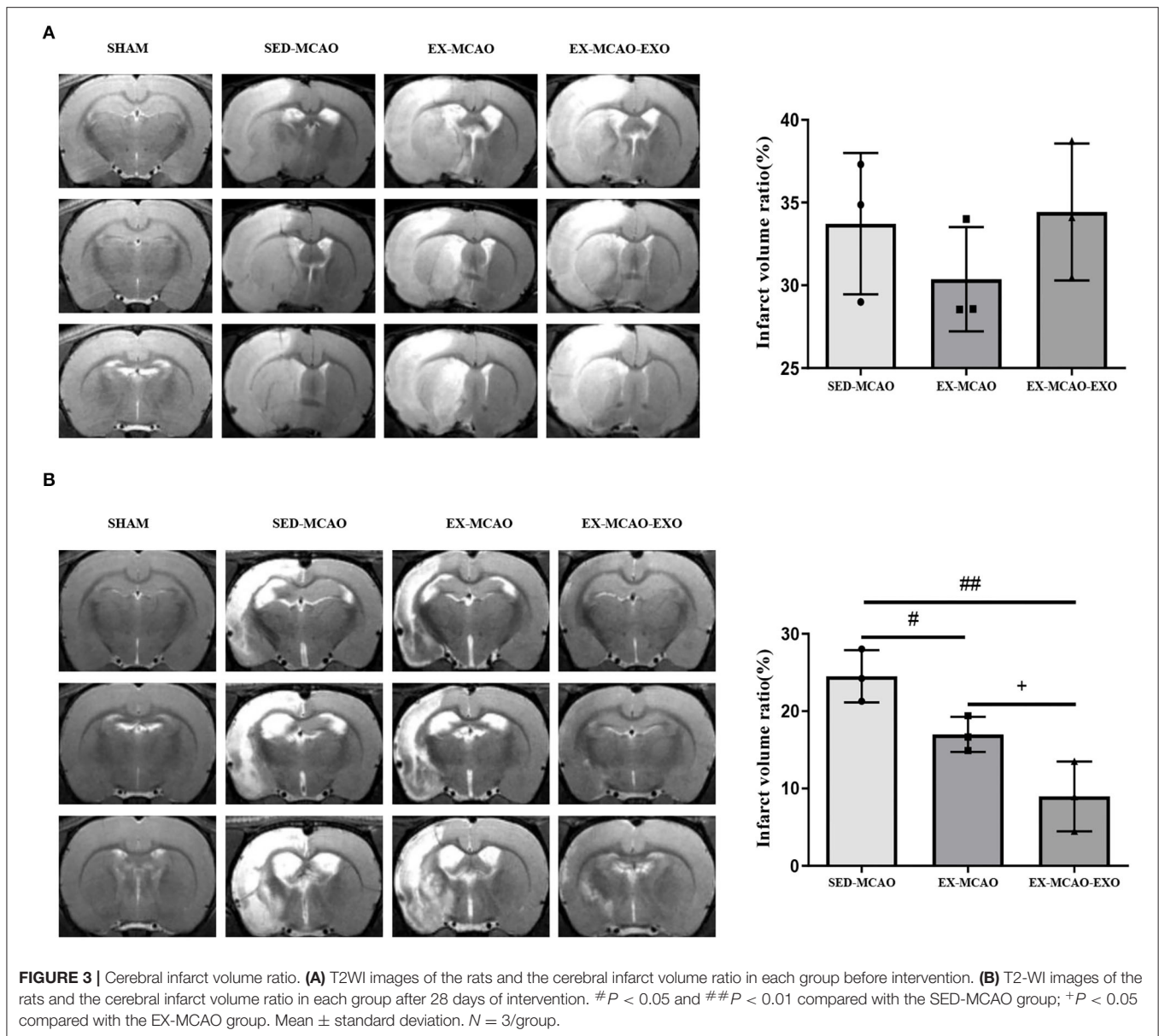
The body weight and neurological score of each group were detected 1, 3, 7, 14, 21, and 28 d after the MCAO.

In the comparison of body weight (Figure 2A), the body weight of rats in each group increased with time throughout the experiment, and the body weights of all of the MCAO groups were lower than that of the SHAM group ($P < 0.05$). Compared with those in the SED-MCAO group, the body weights in the EX-MCAO and EX-MCAO-EXO groups increased, and the body weight in the EX-MCAO-EXO group increased significantly on the 3rd day (280.5 ± 12.97 vs. 246.67 ± 9.91 , $P < 0.05$).

The comparison of mNSS scores (Figure 2B) showed that compared with that of the SED-MCAO group, the mNSS of the EX-MCAO group decreased significantly on day 7 (7.83 ± 0.31 vs. 9.33 ± 0.62 , $P < 0.05$) and the mNSS of the EX-MCAO-EXO group decreased significantly on day 3 (8 ± 0.37 vs. 9.67 ± 0.42 , $P < 0.05$). The score of the EX-MCAO-EXO group was significantly lower than that of the EX-MCAO group on day 14 (6 ± 0.37 vs. 7.17 ± 0.4 , $P < 0.05$).

Cerebral Infarction Volume Ratio

There was no significant difference in the cerebral infarction volume ratio between the experimental groups before the intervention ($P > 0.05$, Figure 3A). After 28 days of the intervention (Figure 3B), compared with the SED-MCAO group, the EX-MCAO (16.99 ± 1.31 vs. $24.52 \pm 1.95\%$, $P < 0.05$) and



EX-MCAO-EXO groups had a significantly decreased cerebral infarction volume ratio (8.98 ± 2.6 vs. $24.52 \pm 1.95\%$, $P < 0.01$). Compared with the EX-MCAO group, the cerebral infarct volume ratio of the EX-MCAO-EXO group was significantly decreased ($P = 0.031$).

Microglia Activation

After 28 days of the intervention (**Figure 4**), the results of fluorescent staining of microglia in the peri-infarcted area of the brain showed that the number of microglia in the SED-MCAO (106.89 ± 7.89 vs. 9.11 ± 1.31 , $P < 0.001$) and EX-MCAO (45.22 ± 5.86 vs. 9.11 ± 1.31 , $P < 0.01$) groups was increased significantly when compared with the SHAM group, and there was no significant change in the EX-MCAO-EXO group (26.22 ± 4.01 vs. 9.11 ± 1.31 , $P = 0.053$). Compared with the SED-MCAO

group, the number of microglia in the EX-MCAO and EX-MCAO-EXO groups was significantly decreased ($P < 0.001$), and the EX-MCAO-EXO group had the lowest number ($P = 0.036$).

The morphological characteristics of microglia in the peri-infarcted area of the brain after 28 days of the intervention are shown in **Figure 5A**.

The process length/cell assessment is shown in **Figure 5B**. Compared with the SHAM group, the process length/cell in the SED-MCAO (238.64 ± 3.64 vs. $1,183.91 \pm 125.63$, $P < 0.001$) and EX-MCAO (766.49 ± 68.61 vs. $1,183.91 \pm 125.63$, $P < 0.05$) groups was significantly decreased, and the EX-MCAO-EXO group had no significant difference ($1,027.46 \pm 133.36$ vs. $1,183.91 \pm 125.63$, $P = 0.291$). Process length/cell was significantly higher in the EX-MCAO and EX-MCAO-EXO groups than in the SED-MCAO group ($P < 0.01$). There

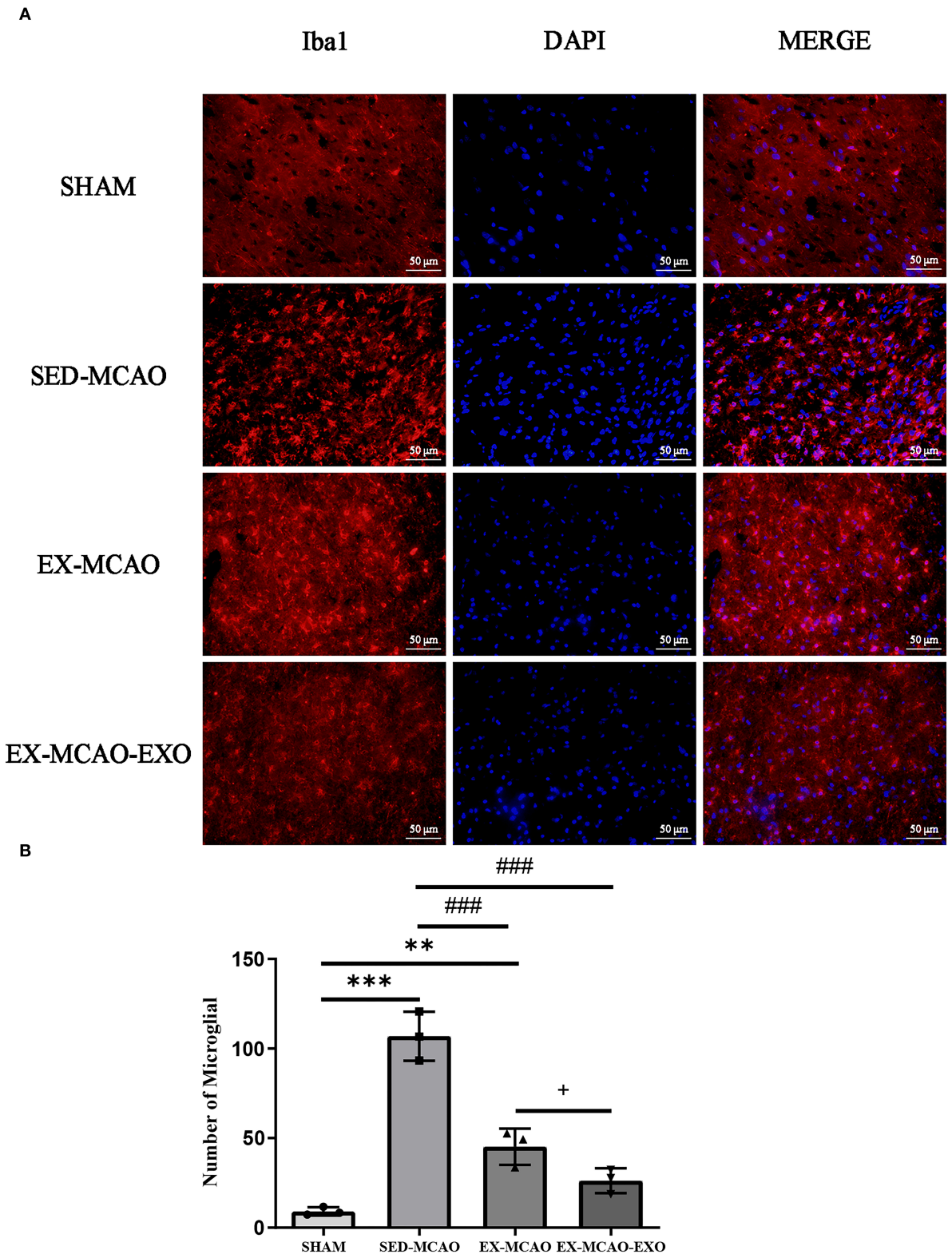
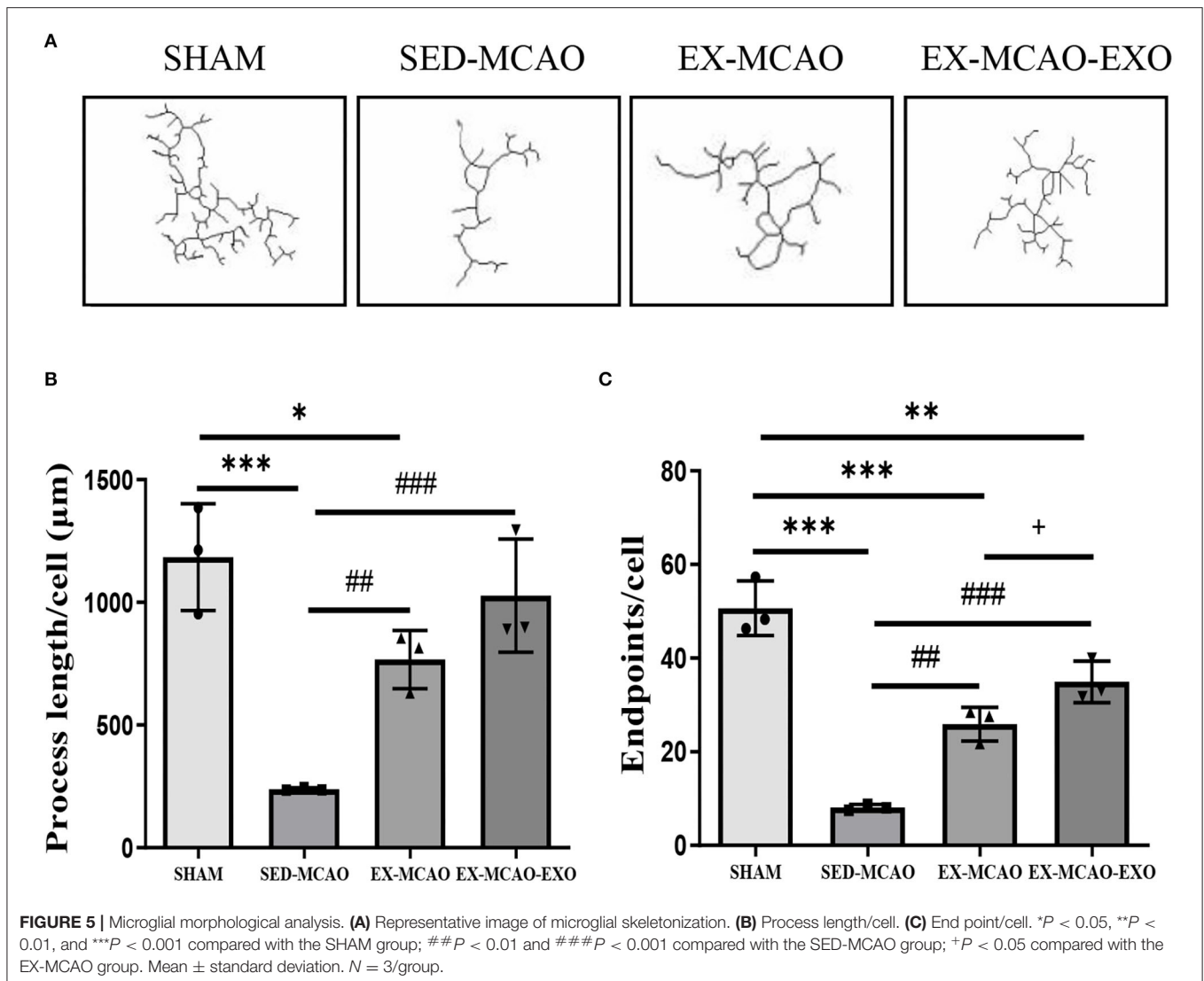


FIGURE 4 | (A) Representative immunofluorescence images of Iba1 staining. Bar = 50 μ m. **(B)** Number of microglia. $**P < 0.01$ and $***P < 0.001$ compared with the SHAM group; $###P < 0.001$ compared with the SED-MCAO group; $+P < 0.05$ compared with the EX-MCAO group. Mean \pm standard deviation. $N = 3$ /group.



was no significant difference between the EX-MCAO-EXO and EX-MCAO groups ($P = 0.096$).

The endpoints/cell assessment is shown in **Figure 5C**. The end point/cell in the MCAO groups was lower than that in the SHAM group ($P < 0.01$). Compared with the SED-MCAO group, end point/cell was significantly increased in the EX-MCAO (25.91 ± 2.08 vs. 8.11 ± 0.37 , $P < 0.01$) and the EX-MCAO-EXO groups (34.95 ± 2.55 vs. 8.11 ± 0.37 , $P < 0.001$), among which the EX-MCAO-EXO group had the highest end point/cell ($P = 0.027$).

Neuronal Dendrite Characteristics

Representative images of neuronal dendrites in the peri-infarct cortex were obtained using by using Golgi staining (**Figure 6**). Sholl analysis of the images (**Figure 7A**) revealed the number of branches and intersections between apical and basal dendrites (**Table 1**).

Further statistics on these parameters found that whether in basal or apical dendrites, the number of branches and intersections in the SED-MCAO and EX-MCAO groups was

significantly lower than that in the SHAM group ($P < 0.05$). Compared with the SED-MCAO group, the number of branches and intersections in the EX-MCAO and EX-MCAO-EXO groups was significantly increased ($P < 0.05$), and the increase was more significant in the EX-MCAO-EXO group ($P < 0.05$).

The Expression of Synaptic Plasticity Proteins

After 28 days of the intervention (**Figure 8**), the protein expression levels of Syn and PSD-95 in the SED-MCAO (Syn: 0.73 ± 0.03 vs. 1.12 ± 0.07 , $P < 0.001$; PSD-95: 0.57 ± 0.05 vs. 1.02 ± 0.08 , $P < 0.001$) and EX-MCAO (Syn: 0.91 ± 0.06 vs. 1.12 ± 0.07 , $P < 0.05$; PSD-95: 0.76 ± 0.05 vs. 1.02 ± 0.08 , $P < 0.01$) groups were significantly lower than that in the SHAM group, but there was no significant difference between the EX-MCAO-EXO and the SHAM groups (Syn: 1.1 ± 0.07 vs. 1.12 ± 0.07 , $P > 0.05$; PSD-95: 0.95 ± 0.06 vs. 1.02 ± 0.08 , $P > 0.05$). The protein expression levels of Syn and PSD-95 in the EX-MCAO and EX-MCAO-EXO groups were significantly higher than those in the

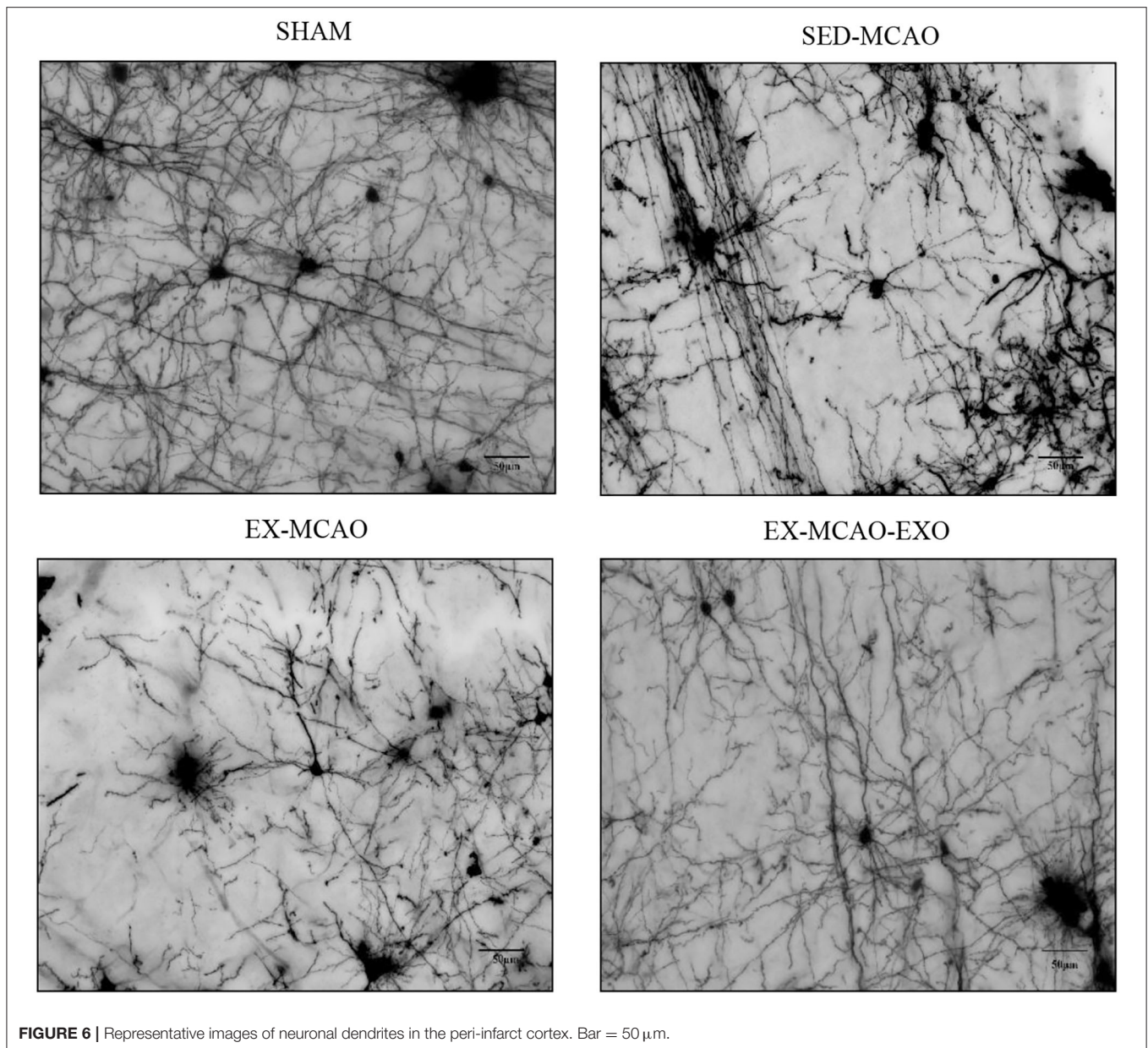


FIGURE 6 | Representative images of neuronal dendrites in the peri-infarct cortex. Bar = 50 μ m.

SED-MCAO group ($P < 0.05$), and the expression level in the EX-MCAO-EXO group was the highest ($P < 0.05$).

DISCUSSION

This study found that after 28 days of exercise intervention in MCAO rats: (1) the rats gained weight and had improved neurological function, (2) the cerebral infarct volume ratio decreased, (3) excessive microglial activation was significantly inhibited, (4) the total number of dendritic intersections and branches in the peripheral area of the infarction was significantly improved, and (5) the protein expression levels of Syn and PSD-95 in the peripheral area of infarction were increased. Compared with the simple exercise group, the exosome infusion

combined with the exercise group showed a further improvement in the above indicators.

Exercise Intervention Improves Body Weight and Neurological Function in MCAO Rats, and Exosomes Are Involved in the Process of Neural Recovery

Stroke often results in neurological deficits and weight loss, negatively affecting prognosis and function (Yang et al., 2019). Numerous studies have shown that exercising early (days to weeks) after stroke improves neurological function in rats (Coleman et al., 2017). Our previous study found that an exercise intervention started 1 day after stroke has the best

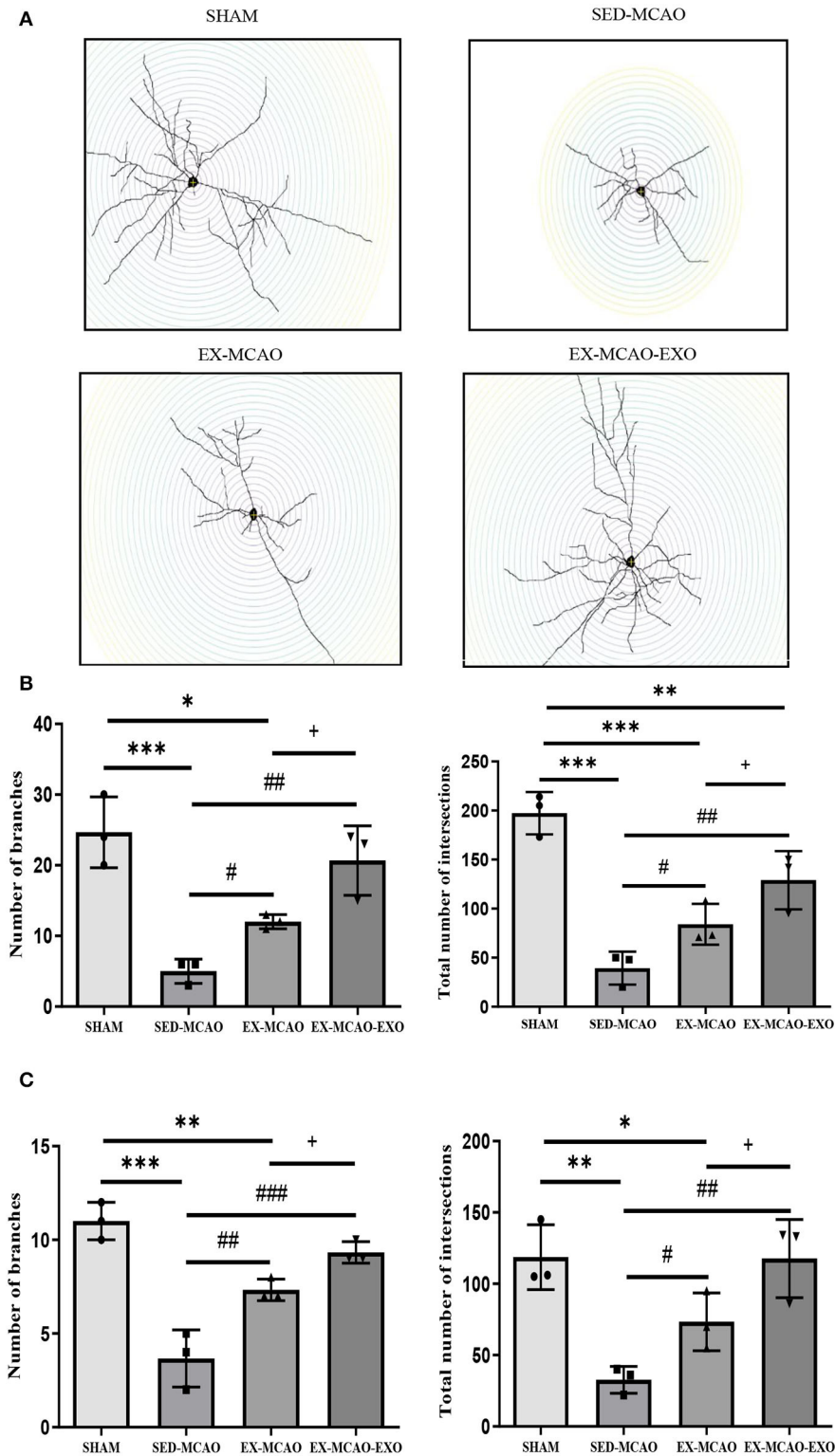
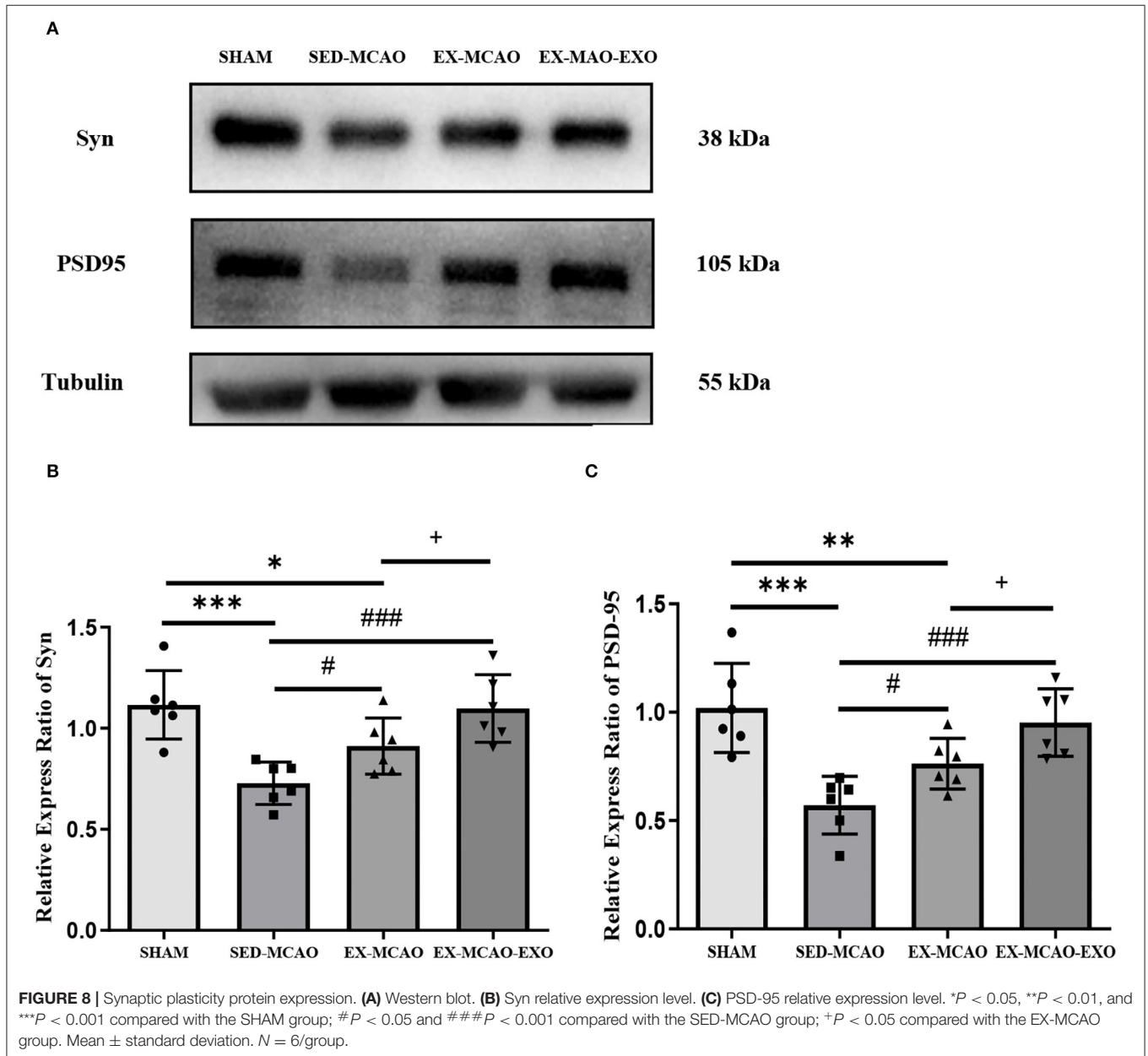


FIGURE 7 | Characteristics of basal and apical dendrites in each group were analyzed by Sholl analysis. **(A)** Representative images of each group for Sholl analysis, with a step of 10 μm . **(B)** Total number of branches and intersections in basal dendrites. **(C)** Total number of branches and intersections in apical dendrites. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared with the SHAM group; # $P < 0.05$, ## $P < 0.01$, and ### $P < 0.001$ compared with the SED-MCAO group; + $P < 0.05$ compared with the EX-MCAO group. Mean \pm standard deviation. $N = 3/\text{group}$.

TABLE 1 | Characteristics of basal and apical dendrites in each group.

		SHAM	SED-MCAO	EX-MCAO	EX-MCAO-EXO
Basal dendrites	Number of branches	24.67 ± 2.91	5.00 ± 9.68	12.00 ± 0.58	20.67 ± 2.85
	Total number of intersections	197.33 ± 12.44	39.33 ± 9.68	84.00 ± 12.01	129.00 ± 17.16
Apical dendrites	Number of branches	11.00 ± 0.58	3.67 ± 0.88	7.33 ± 0.33	9.33 ± 0.33
	Total number of intersections	118.67 ± 13.17	32.67 ± 5.46	73.33 ± 11.67	117.67 ± 15.84



improvement effect (Shi et al., 2020); exercise can promote the release of circulating exosomes that enter ischemic brain tissues and promote brain tissue recruitment of more exosomes (Li et al., 2021). In this study, compared with the SED-MCAO group,

mNSS was decreased significantly in the EX-MCAO group from the 7th day and decreased significantly in the EX-MCAO-EXO group from the 3rd day, and mNSS in the EX-MCAO-EXO group was significantly lower than that in the EX-MCAO group from

the 14th day. Therefore, we speculate that exosomes play a role in improving neurological function and participate in the process of neural repair caused by exercise.

Exercise Intervention Improves the Cerebral Infarction Volume Ratio and Exosomes Participate in the Process of Brain Structure Repair

Before the intervention, there was no significant difference in the ratio of cerebral infarct volume among the groups. The exercise intervention can reduce the volume of cerebral infarction. Two weeks of moderate-intensity treadmill exercise can significantly reduce the volume of cerebral infarction (Pan et al., 2021). After 24 h of ischemia–reperfusion, both mild and severe exercises can significantly reduce the volume of cerebral infarction (Li et al., 2020). Cheng et al. found that treadmill exercise promotes neurogenesis, enhances myelin recovery, and reduces cerebral infarct volume (Cheng et al., 2020). Zheng et al. showed that exosomes can significantly reduce the infarction volume ratios of ischemic rats 6 h after MCAO (Zheng et al., 2019) and have neuroprotective effects (Zhou et al., 2020). Our study found that infarct volume could be reduced 28 days after the intervention and that infarct volume was further reduced after exosome infusion, which further indicated the special role of exosomes in an exercise intervention.

Exercise Intervention Inhibits the Excessive Activation of Microglia, and Exosomes Are Involved in the Inhibitory Process

Microglia play a dual role in ischemic brain injury (Ran et al., 2021). On the one hand, after cerebral ischemia, microglia respond rapidly to the microenvironment of the brain (Dong et al., 2021), change from a resting state (“branching” with pruning synaptic function) to an activated state (“amoeba” with phagocytic function), and migrate to the ischemic region to protect neurons by forming phagocytic fragments (Shi and Pamer, 2011). On the other hand, excessive activation of microglia releases inflammatory factors and cytotoxic substances that exacerbate brain damage (Ma et al., 2017; Xing and Bai, 2020).

Exercise inhibits microglial activation (Liu et al., 2022). Liu C et al. found that activation of microglia was inhibited after swimming training (Liu et al., 2021a). Kodali et al. found that intermittent running could reverse the excessive activation of microglia and increase their branches (Kodali et al., 2021). This study found that after MCAO, the number of microglia significantly increased, and process length/cell and end point/cell values decreased, suggesting that microglia were activated and transformed into “amoeba.” After exercise intervention, microglial activation was inhibited, and microglial activation was further inhibited by exosome input.

Studies have found that exosomes can inhibit microglial activation. Moss L et al. found that intranasal exosomes inhibit microglial activation (Moss et al., 2021). Plasma exosomes inhibit LPS-induced microglial inflammatory response (Wang et al.,

2021). Bone marrow mesenchymal stem cell-derived exosomes alleviate neuroinflammation by modulating microglial activation (Liu et al., 2021b).

A previous study by our team confirmed that exercise can promote the release and migration of exosomes into the brain (Li et al., 2021), so we speculate that exosomes are involved in the process of exercise intervention inhibiting the activation of microglia.

Inhibition of Microglial Activation Promotes Synaptic Remodeling, and Exosomes Regulate Synaptic Plasticity

Resting microglia can participate in the formation and pruning of neuronal synapses (Peferoen et al., 2014). Crapser J et al. suggested that microglia may prune synapses by engulfing the extracellular matrix surrounding the synapses (Crapser et al., 2021). Microglia have been found to mediate changes in synaptic plasticity (Innes et al., 2019) through the processes of neurogenesis and axogenesis (Yu et al., 2021). Activation of M2 microglia enhances neuroplasticity and angiogenesis and promotes functional recovery (Song et al., 2019b). Inhibition of M1 microglial activation enhances synaptic plasticity (Sun et al., 2020). Leech et al. found that inhibition of excessive microglial activation increases dendritic spine density (Leech et al., 2020).

Improving synaptic connections between neurons is particularly important for recovery after ischemic brain injury (Dabrowski et al., 2019). Approximately 90% of excitatory synapses are located on dendrites, and the morphological structure and complexity of dendrites affect the connection between synapses and the efficiency of signal transmission (Nguyen et al., 2021). This study found that dendritic complexity was reduced and that the expression levels of Syn and PSD-95 were reduced after MCAO, suggesting that synaptic structure was disrupted and synaptic function was compromised. The exercise intervention increased the expression of dendritic complexity and synaptic plasticity-related proteins, and synaptic plasticity was further enhanced after exosome input.

Exosomes can regulate synaptic plasticity through microglia. Studies have shown that exosomes can regulate neuroplasticity (Xin et al., 2013) and neuronal development and maintain myelin and synaptic function in peripheral brain infarcts by enhancing neurite remodeling (Domingues et al., 2020), thus affecting the morphology and function of microglia (Vogel et al., 2018; Garcia et al., 2022) and the density of dendritic spines (Sobue et al., 2018). Combined with our previous studies, we speculate that exercise can regulate synaptic plasticity by inhibiting the excessive activation of microglia after exosomes enter the brain.

LIMITATIONS OF THE STUDY

More time points were not tested to observe the dynamic changes in the experimental indicators. We have not performed related

experiments with exosome inhibitors, and this will be further explored in future studies.

SUMMARY

Early exercise intervention after stroke can inhibit the excessive activation of microglia by exosomes, thereby regulating synaptic plasticity and protecting neural function.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Laboratory Animal Welfare Ethics Committee of Tianjin Medical University General Hospital (IRB2021-DWFL-403).

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AUTHOR CONTRIBUTIONS

CL, JH, WL, CK, CH, YB, BP, and JW performed the experiments. CL wrote the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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