Voluntary exercise attenuates mice anxiety after traumatic brain injury via inhibiting NLRP3 inflammasome activation

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Abstract

Background: Psychological disorders, particularly the anxiety after traumatic brain injury (TBI) have received great attention both in clinical practice and experimental investigation. New therapeutic strategies are urgently required nowadays to improve mental condition of TBI patients. Neuroinflammation is the typical adaptive response that played important roles in psychiatric illness after brain tissue damage. Therefore, the immune-targeted therapy has aroused attention. Voluntary exercise has been considered as the most essential rehabilitative intervention following TBI. However, there were some experiments showed opposite effects, which varies with different time windows. Whether and when the voluntary exercise should be taken after TBI remain to be defined. The aim of the current study was to elucidate whether in-time voluntary exercise could improve the neurological recovery and reduce the anxiety of TBI mice by inhibiting the neuroinflammation via attenuating the activation of NLRP3 inflammasome.

Methods: Kunming mice (8 weeks) were subjected to TBI and randomly divided into 4 groups One week voluntary wheel running (VWR) was applied to TBI mice soon after trauma - at 2nd day after injury. The neurofunction of TBI mice was detected by neurological severity score (NSS) and beam walking assay. Anxiety behavior was evaluated by open-field (OF) test and elevated plus maze (EPM) test. The cellular inflammatory signal, including IL-12, IFN-γ, CCL2, IL-10 and TGF-β, as well as the activation of microglia and NLRP3 inflammasome in the perilesional cortex were investigated.

Results: After one week VWR, neurological function recovery was enhanced while the anxiety behavior of TBI mice was significantly eased. Accompanied with this, anti-inflammatory signaling, IL-10 in particular, greatly elevated. NLRP3 expression, caspase-1 activation and pro-inflammatory cytokines IL-1β/ IL-18 secretion were significantly inhibited. All these alterations were consistent with reduced microglia activation in the perilesional site and positively correlated with the weaken of anxiety behavior.

Conclusion: In-time VWR could be a high potential therapeutic strategy for neuropsychiatric disorders following TBI by targeting neuroinflammation.

Introduction

Traumatic brain injury (TBI) is one of the major health problem with high rates of mortality and disability [1]. Approximately 20 millions of people worldwide are affected by TBI and about 2.5 to 6.5 millions of patients suffering from the complications of TBI [2–4]. In the past decades, the mental and psychological disorders, particularly the anxiety had received great attention during trauma treatment and rehabilitation [4, 5]. According to the Centers for Disease Control and Prevention (CDC), about 3–28% of TBI patients in American suffered from anxiety and the outcomes were not ideal, either [5]. The anxiety behavior after TBI was not consistently invariable. It exhibited a time-dependent increase up to 3 weeks [6, 7]. Therefore, deeper investigations and the proper intervention measures towards the anxiety behavior after TBI are urgently required.

Neuroinflammation is a central pathophysiological feature of TBI. Previous study has demonstrated that suppressing the ongoing inflammatory response after TBI could be the key point for treatment of post-trauma anxiety [8–10]. As we know that NLR family pyrin domain- containing protein 3 (NLRP3) was involved in the neural inflammatory response to brain tissue damage. The complex NLRP3 inflammasome, which formed with NLRP3, caspase-1 and adaptor protein ASC, acted as the necessary component of the innate immune system. It has been confirmed that the activation of the NLRP3 inflammasome and the releasing of pro-inflammatory cytokines were greatly up-regulated following TBI [11–14]. Consequently, NLRP3 inflammasome has been considered as a potential therapeutic target for the management of neuroinflammation after TBI [15]. The evidence in rodents study has confirmed that suppressing NLRP3 inflammasome activation could produce obvious improvement of neurofunctional outcomes following TBI, both in molecular biological and pharmacological aspects [12, 16–20].

Voluntary exercise, as one of the essential rehabilitative intervention, has been proved that could attenuate neuronal loss, reduce neuroinflammation, improve mood and then facilitate recovery after TBI [21–23]. Existed data showed that voluntary exercise or voluntary exercise pre-conditioning facilitated neuroplasticity after TBI, through reducing the level of inflammatory factors and elevating the level of brain-derived neurotrophic factor (BDNF) [24–28]. However, there were some other experiments showed opposite effects, depending on the time windows [24–26, 29].

Since there is a heightened stress response after a mild TBI during the first 2 post-injury weeks [24], whether and when the voluntary exercise should be taken after TBI remain to be defined. In the current study, voluntary exercise was performed on the 2nd day post-TBI and lasted for one-week. Then, both of the neurofunctional recovery and the neuroinflammation, including the motor coordination and anxiety behavior improvement, levels of cellular inflammatory signals and NLRP3 inflammasome activation were investigated. Our results will offer strong supports and great hope for outcome improvement following TBI.

Materials And Methods

Construction of mouse model of TBI

Kunming mice (8 weeks old, weight 28 g ~ 32 g) were obtained from Medical Animal Center of Xi’an Jiaotong University and housed in a humidity- and temperature-controlled (40% ~ 60%, 20°C ~ 24°C) environment with a 12 h/12 h light/dark cycle. All animal experimental procedures were conducted in accordance with guidelines established by the Animal Committee of Xi’an Jiaotong University Health Science Center. Three mice were caged together and free access to food and water for the duration of experiments.

Feeney's free-fall epidural impact method was used to construct TBI model. Briefly, mice were anesthetized with pentobarbital sodium (10 mg/kg) via intraperitoneal injection and placed into a stereotaxic frame. Then a 4-mm-diameter craniotomy was performed at a point that was centered 1 mm posterior and 2 mm lateral to the right of bregma. The skull cap was carefully removed without damaging

of leptomeninges. The hammer in 80 g dropped from 25 cm high was used to induce craniocerebral injury. After TBI, all mice were allowed to recover on a heat pad to maintain core body temperature at 36°C ~ 37°C. Mice in control group (named as sham) underwent the same surgical procedure but without TBI.

Voluntary exercise strategy

Voluntary wheel running (VWR) was used as the voluntary physical activity. A total number of 32 mice were divided into 4 groups (8 mice for each group) and named as Sham group, TBI group, Sham + VWR group and TBI + VWR group, respectively. Two days before TBI, mice in VWR group were placed in a clean polycarbonate cage with stainless-steel running wheel (12.7 cm in diameter, Lafayette Instrument, USA) individually, to adapt to the environment. Exercise (in Sham + VWR group and TBI + VWR group) was carried out at 2nd day after TBI (Fig. 2A). Running activity was monitored daily and lasted for one week. The running distance and the total number of wheel's rotation were measured by Scurry VitalView Acquisition and Analysis Software (version 1.1). Mice in other two groups were housed in standard cages without running wheel.

Neurological severity score (NSS) test

To assess the motor, sensory, and reflex of mice after TBI and to evaluate the neurofunctional recovery with exercise intervention, NSS test was applied as previously described [30]. The test that was blinded to the two observers was carried out at 2 days after TBI and 7 days after VWR, respectively. The scale ranges from 0 to 10 (normal: score 0; maximal deficit: score 10) and 1 point was awarded for the lack of reflex or for the inability to perform the task. A maximal of 10 points indicated the most severe neurological dysfunction with failure of all tasks.

Motor coordination and balance test

The motor coordination and balance were assessed by the beam walking assay once daily until 9 days after TBI. Blinded evaluation was performed to lessen observer bias during the whole process. Before TBI, all mice were pre-trained until the proficiency to walk across a wooden beam without pausing was achieved. The beam walking test apparatus consists of a flat bench (70 cm x 35 cm) with a wooden beam (100 cm long and 1 cm wide) located 20 cm above the bench and held to it on two posts. The mouse was placed in the same starting position. The experimenter observed the site of falling off and recorded the distance between starting and dropping position.

Behavioral tests for anxiety

Mice were subjected to a series of behavioral tests following VWR. All tests were performed according to the standard procedure and optimized in our lab [31]. All data were analyzed in a double-blind manner by using automated video tracking software (Smart 3.0 video tracking system, USA). Anxiety behavior was evaluated by open-field (OF) test and elevated plus maze (EPM) test.

OF test Each mouse was placed in an OF environment for acclimation and baseline measurements. The mice were individually placed into the center of the apparatus (50 cm × 50 cm) to explore for 5 min. The movements of the animals were recorded by an overhead video camcorder. The traveling time in the software-defined 25 cm × 25 cm center region of the apparatus was recorded for each animal. The time spent in the center region was measured and the total distance traveled were calculated.

EPM test The EPM apparatus consisted of two enclosed arms and two open arms (35 cm in length × 6 cm in width × 15 cm in height; Harvard Apparatus, Holliston, MA) at 90^{o} angle to each other with all arm platforms elevated 50 cm from the floor. At the start of a trial, the mouse was placed in the center with its nose directed toward the same closed arm and allowed to explore the maze freely for 5 min. The total time spent, total distance covered, and distance in and entries into each arm and the center were digitally recorded. The times entered and the duration in the open arms were calculated, showed as a percentage of the total number of entries (sum of open and closed arms entries) and total time (sum of duration in both arms). The anxiolytic effect was represented by an increase in the percentage of the enter times and the time spent in the open arms and the total number of entries and the total time spent remained unchanged [32].

Histology and immunofluorescence staining

Mouse was perfused transcardially with 0.01 M phosphate buffer saline (PBS) followed by 4% paraformaldehyde (PFA) in 0.1 M PBS (pH 7.4) after anesthetization at 1.5 h post-TBI or behavioral tests. Whole brain was rapidly dissected and post-fixed in PFA for 24 h at 4°C. After gradient dehydration with shaking overnight, the brain tissues were embedded with OCT and coronal sectioned into slices (10 μm thick) in a cryostat microtome (CM1950; Leica Microsystems, Wetzlar, Germany). Hematoxylin and eosin (H&E) staining was performed, following the standard procedures, to assess the degree of brain tissue damage. For immunofluorescence staining, brain sections were firstly treated with antigen retrieval solution (C1035, Solarbio, China) for 15 min and washed with 0.01 M PBS for 5 min. Then blocked with 5% bovine serum albumin (BSA) in 0.3% Triton X-100 for 2 h at room temperature (RT). The sections were then incubated over night at 4°C with primary antibodies, including rabbit polyclonal anti-Ionized calcium binding adaptor molecule 1 (Iba1, 019-19741, 1:500, Wako, Japan), rat monoclonal anti-CD68 (ab53444, 1:100, abcam, USA), mouse monoclonal anti-NLRP3 (AG-20B-0014, 1:50, Whatman, UK), rabbit polyclonal anti-c-fos (26192-1-AP, 1:50, Proteintech, Wuhan, China). On the following day, sections were incubated at RT in the dark for 2 h with secondary antibodies, including Alexa Fluor 488 and 594 conjugated Donkey anti-rabbit/Donkey anti-mouse (1:600, Invitrogen, USA) and Goat anti-rat (1:300, abcam, USA). After thoroughly wash, the brain sections were then incubated with 4',6-Diamidino-2-phenylindole (DAPI) for 5 min to label the cell nuclei and mounted with Antifade Mounting Medium (Sigma, USA). All immunofluorescence images were captured under fluorescent microscope (BX57, Olympus Corporation, Japan).

Western blot assay

Molecules related to inflammatory cell signaling pathway were detected by Western blot assay. Protein was extracted from perilesional cortex using the RIPA lysate buffer (PE01, Pioneer, China). The protein was then separated on 10% SDS-PAGE gel and transferred to polyvinylidene fluoride membrane (IPVH00010, Millipore, Billerica, MA, USA). After blocking with 5% (m/v) skim milk (232100, BD Biosciences, USA.) for 3 h at RT, the membrane was incubated with primary antibodies overnight at 4°C. Following antibodies that diluted in skim milk at 1:1000 were used. Rabbit polyclonal anti-caspase-1 (ab179515, 1:1000, abcam, USA), rabbit polyclonal anti-IL-1β (ab283818, 1:1000, abcam, USA), rabbit polyclonal anti-IL-18 (ab207323, 1:1000, abcam, USA) and mouse monoclonal anti-GAPDH (60004-1-Ig, 1:10000, Proteintech, China). On the following day, after washing with Tris-buffered saline-Tween 20 (TBST) buffer for 3 times, the membranes were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit or goat anti-mouse (1:10000, Proteintech, China) secondary antibody for 2 h at RT. The membranes were then washed with TBST and visualized using an enhanced chemiluminescence kit (WBKLS0100, Millipore, USA). Images were digitally scanned and quantified by densitometry using Imaging J software (US NIH).

Quantitative real-time PCR analysis

Quantitative real-time PCR (qRT-PCR) was used to analyze the relative mRNA expression of genes that related to the pro- or anti-inflammatory factors and NLRP3 inflammasome. Total RNA of perilesional cortex was extracted by the Trizol reagent (Takara, Dalian, China) and transcribed into cDNA using reverse transcription kit (K1622, Thermo Scientific, USA). Then qPCR was performed using SYBR green dye with gene specific primer sets and iQ5 PCR thermal cycler (Bio-Rad, Hercules, USA) with following cycle parameters: 95°C for 3 min, 40 cycles at 95°C for 10 s, 60°C for 30 s and 72°C for 30 s. The mRNA expression of target genes were normalized to GAPDH and quantified by the $2^{-\Delta\Delta}$CT method. The primer sequences used were as follows: Pro-inflammatory factors: IL-12: sense primer 5′-GTCCTCAGAAGCTAACCATCTCC − 3′, antisense primer 5′- CCAGAGCCTATGACTCCATGTC − 3′; IFN-γ: sense primer 5′- GAACTGGCAAAAGGATGGTGA − 3′, antisense primer 5′- TGTGGGTTGTTGACCTCAAAC-3′; CCL2: sense primer 5′- TTAAAAACCTGGATCGGAACCAA − 3′, antisense primer 5′-GCATTAGCTTCAGATTTACGGGT − 3′. Anti-inflammatory factors: IL-10: sense primer 5′-GGCAGAGAAGCATGGCCCAGAA − 3′, antisense primer 5′- AATCGATGACAGCGCCTCAGCC-3′; TGF-β: sense primer 5′- CTCCCGTGGCTTCTAGTGC − 3′, antisense primer 5′- GCCTTAGTTTGGACAGGATCTG − 3′. Activation of NLRP3 inflammasome: IL-1β: sense primer 5′- TGGGAAACAACAGTGGTCAGG − 3′, antisense primer 5′- CCATCAGAGGCAAGGAGGAA − 3′; NLRP3: sense primer 5′- ATTACCCGCCCGAGAAAGG − 3′, antisense primer 5′- CATGAGTGTGGCTAGATCCAAG − 3′. Housekeeping gene: GAPDH: sense primer 5′-GCCAAGGCTGTGGGCAAGGT − 3′, antisense primer 5′- TCTCCAGGCGGCACGTCAGA − 3′.

Data collection and statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). In each experimental group, at least three samples were selected. Four sections from each brain and three randomly selected fields were imaged and counted. For Western blot assay and qRT-PCR, data from four samples were used. All data

were analyzed by using Prism 8 (GraphPad Software, Inc., San Diego, USA) with $p \leq 0.05$ considered as statistically significant. Differences between two groups were analyzed by unpaired Student's t test. Relationship between NLRP3 inflammasome activation and anxiety-like behavior was analyzed by two-tailed Mann-Whitney U-test.

Results

VWR improved neurological recovery of TBI mice

Damage of brain tissue and motor coordination of TBI mice were investigated. Obvious tissue loss with increased number of c-fos positive neurons were observed in the perilesional cortex of TBI mice (Fig. 1A, B). Accompany with these, neurological function impairment that detected by NSS was noticed (Fig. 1C). At 2nd day after TBI, VWR was carried out and lasted for 7 days (Fig. 2A). No significant difference in daily and total running wheel revolutions was found between sham and TBI groups (Fig. 2B, C), suggesting the willingness and ability of exercise of the mice in two groups were comparable. As we expected, the motor coordination of TBI mice was significantly improved after VWR, showed by the reduced score in NSS (Fig. 2D) and the fast increase of walking distance across wooden beam (Fig. 2E).

VWR eased anxiety of TBI mice

Locomotor activity in OF and EPM was assessed after 7 days exercise to evaluated anxiety behavior of mice. No difference in total distance was found between different groups (Fig. 3A, B) in regard to the OF test. Notably, compared with the mice in sham group, TBI mice spent significantly less time in the central area. Nevertheless, after one week exercise, mice spent much longer time in the central area, which was almost identical to that in sham group (Fig. 3A, C). As for EPM test, less numbers of entries into the open arms was observed in TBI group while this phenomenon was completely reversed after one week VWR (Fig. 3D, E). No difference was observed in time spent in open arms between different groups (Fig. 3D, F).

VWR reduced TBI-induced neuroinflammation

To evaluate the effect of VWR on inflammation reaction following TBI, mRNA expression levels of three pro-inflammatory factors and two anti-inflammatory factors were detected by qRT-PCR at day 9 after TBI. After VWR for 7 days, TBI induced inflammation, showed by elevated pro-inflammatory factors including IL-12, IFN-γ and CCL2, was significantly reduced (Fig. 4A-C). In consonance with this, the anti-inflammatory effect of VWR, showed by elevation of IL-10 and TGF-β, was obviously observed (Fig. 4D, E). It is worth noting that the level of IL-10 rose dramatically after VWR, no matter with or without TBI. While the range of VWR induced elevation of IL-10 after TBI was significantly less than that in sham group (Fig. 4D). This indicated that IL-10 might work in a different pattern with TGF-β (Fig. 4E). Accompanying with the alteration of inflammatory factors, the activation of microglia, showed by the $Iba1^+$ and $CD68^+$ cells in the perilesional cortex, was significantly inhibited by VWR (Fig. 4F, G).

VWR eased mice anxiety behavior via inhibiting of NLRP3 activation

To further investigate the possible working pathway of VWR on anti-anxiety following TBI, NLRP3 inflamasome activation and the correlation with anxiety behavior were analyzed. Two important inflammatory mediators, NLRP3 and pro-caspase-1 were detected at day 9 after TBI. Both of the expression of NLRP3 mRNA and pro-caspase-1 significantly elevated after TBI and reduced by VWR (Fig. 5A, B). This led to the synchronous alteration of NLRP3 inflamasome and mature caspase-1 in microglia (Fig. 5C-E). In addition, IL-18 and IL-1β were also detected. A similar alter tendency of IL-18 was noticed (Fig. 5H). However, IL-1β altered in a different pattern, both in mRNA and protein level. It rose significantly after TBI and remarkably dropped down after VWR, while the VWR induced reduction was independent of injury (Fig. 5G). Regarding the pro-IL-1β expression, it elevated by TBI but not affected by VWR (Fig. 5F). Further analyzation showed the positive correlation between reduced NLRP3/ IL-1β mRNA and eased anxiety, both in OF test (Fig. 6A, C) and EPM test (Fig. 6B, D).

Discussion

In the current study, one-week VWR was performed at 2nd day after TBI. The anxiety behavior of mice was significantly eased and accompanied with the dramatic reduction of trauma induced neuroinflammation. The neurological function recovery of TBI mice was enhanced while the NLRP3 activation was reduced.

A clinical event that TBI and concussion substantially increase the risk for developing psychiatric disorders has caused great attentions in the past decade [33]. Both of the clinical cases and epidemiological investigations supported that anxiety were more prevalent in patients with a TBI [33–36]. In the current study, accompanied with the obvious brain tissue damage, the neural functional deficits including motor coordination impairment and anxiety behavior were clearly observed soon after TBI. Mice spent significantly less time in the central area of open field and less entries into the open arms, indicated the strong avoidance behavior.

Previous study showed that the anxiety behavior after TBI exhibited a time-dependent increase [6, 7]. During the first 2 post-injury weeks [24], there is a heightened stress response. Therefore, a timely intervention soon after injury to alleviate anxiety behavior might be more critical. In the current study, a voluntary exercise was carried out at 2nd day after TBI, since the voluntary physical activity and exercise has been considered as the most essential rehabilitative intervention following brain injury [22, 23]. We noticed that, the total running distance and the total number of wheel's rotation of TBI mice, which indicating the willingness and ability of exercise, was comparable with control mice within the period of 7 days. After 7 days VWR, besides improvement of motor coordination, the reduction of anxiety was successfully achieved. Although previous study demonstrated that voluntary exercise facilitates neuroplasticity after TBI when it occurs during a delayed time window [24–26], there was other experiment showed the exercise improved rat memory when it was commenced soon and later after injury [25]. Our results further confirmed that it is possible to use voluntary exercise at early stage (2 days after TBI) to ease the anxiety behavior of TBI mice.

Neuroinflammation is one of the typical responses after brain injury. Immediately after TBI, cellular inflammatory signaling, including pro-inflammatory and anti-inflammatory signaling, is initiated and typically peaks within 7 days after injury [37]. Based on the examining of relationship between inflammation and neuropsychiatric risk after TBI [38, 39], immune-targeted therapies to treat anxiety and mood disorders has been fully considered. Some evidence also showed that anti-inflammatory, whenever at acute or delayed stage, could efficiently decrease the development and maintenance of anxiety behavior after TBI [9, 10, 40]. In keeping with these existed data [41, 42], we found that both of the pro-inflammatory and the anti-inflammatory signals rose up after TBI in different degree. The anti-inflammatory signals, IL-10 in particular, greatly elevated after VWR. The alteration of those cellular signals was consistent with microglia activation in the perilesional site. This demonstrated that VWR at early stage could efficiently reduce the injury induced neuroinflammation (Fig. 7).

In accordance with the above reduction of neuroinflammation, we noticed that NLRP3 inflamasome activation was also inhibited by VWR. Besides of the expression of NLRP3 in microglia, both of the caspase-1 activation and proinflammatory cytokines IL-1β/IL-18 secretion were significantly inhibited. This VWR caused inhibition was positively correlated with the weaken of anxiety behavior of TBI mice. Taken together, it further demonstrated that VWR could be a very useful therapeutic strategy for neuropsychiatric disorders following TBI by targeting neuroinflammation [15].

Conclusion

In conclusion, our data demonstrated that the VWR which carried out soon after TBI could efficiently improve the neurofunctional recovery, including improve the motor coordination and ease the anxiety. It also inhibited the trauma induced neuroinflammation, showed by the elevation of anti-inflammatory signaling and the reduction of NLRP3 activation. This further confirmed that VWR could be a high potential therapeutic strategy for neuropsychiatric disorders following TBI via targeting the neuroinflammation.

Abbreviations

VWR: Voluntary wheel running; TBI: Traumatic brain injury; NLRP3: Nod-like receptor pyrin domain-containing 3; OF: Open field; EPM: Elevated plus maze; PBS: Phosphate-buffered saline; BSA: Bovine serum albumin; RT: Room temperature; Iba1： Ionized calcium binding adaptor molecule 1； IL-1β: Interleukin-1 beta; IL-18: Interleukin-18; TBST: Tris buffered saline-Tween 20; qPCR: Quantitative real time-polymerase chain reaction; SEM: Standard error of the mean.

Declarations

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Author contributions

JA, HL and GW supervised the project. XH and YO conducted the main part of the experiment, analyzed data and prepared the draft of manuscript. JL, MS, QG and ZC performed TBI model, NSS test, beam-walking and behavioral tests. JL and RT performed sample collection and Western Blotting assay. MS, YP and WW contributed to images acquisition and cell counting. QG contributed to immunohistochemical staining and data analysis. JA and GW contributed to data analysis and manuscript prepare. HL made the major contributors in modifying the manuscript. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The raw data supporting the conclusions of this article will be made available by the corresponding authors, on reasonable request.

Declarations

Ethics approval and consent to participate

The animal study was reviewed and approved by the Animal Committee of Xi'an Jiaotong University Health Science Center.

Consent for publication

Not applicable.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figures

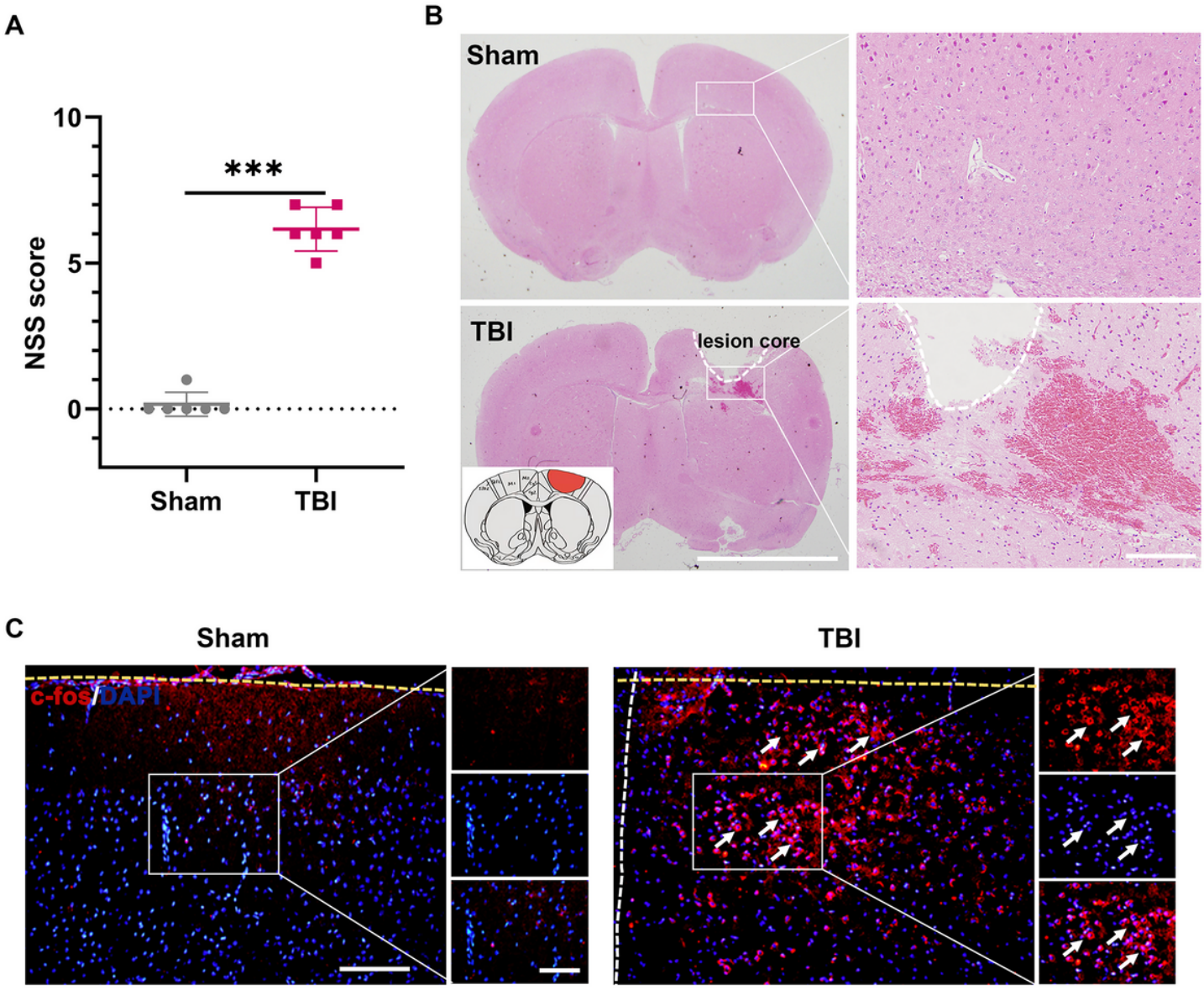

Figure 1

Outcomes of TBI. **(A)** Damage of neurological function and brain tissue Motor coordination of TBI mice was detected by NSS (n = 6). Brain tissue loss **(B)** and damage of neuron **(C)** were observed by H&E staining and c-fos immunostaining. ***p□0.0001 vs. Sham. Unpaired student's t test. c-fos: red; DAPI: blue; scale bar =100 μm.

Figure 2

Effects of VWR on sensorimotor coordination and balance in TBI mice. **(A)** The diagrammatic sketch of experimental design and VWR strategy. Evaluation of daily **(B)** and total **(C)** running wheel revolutions between TBI and Sham mice (n = 8). Assessment of NSS score **(D)** (n = 12) and distance of beam walking **(E)** (n = 6). ***p 0.0001, **p 0.001, *p 0.01, vs. Sham. $^{\&\&}p$ 0.001, vs. TBI. $^{\#\#\#}p$ 0.0001, $^{\#\#}p$ 0.001, $^{\#}p$ 0.01 vs. Sham+VWR. ns indicates no significance. Unpaired student's t test.

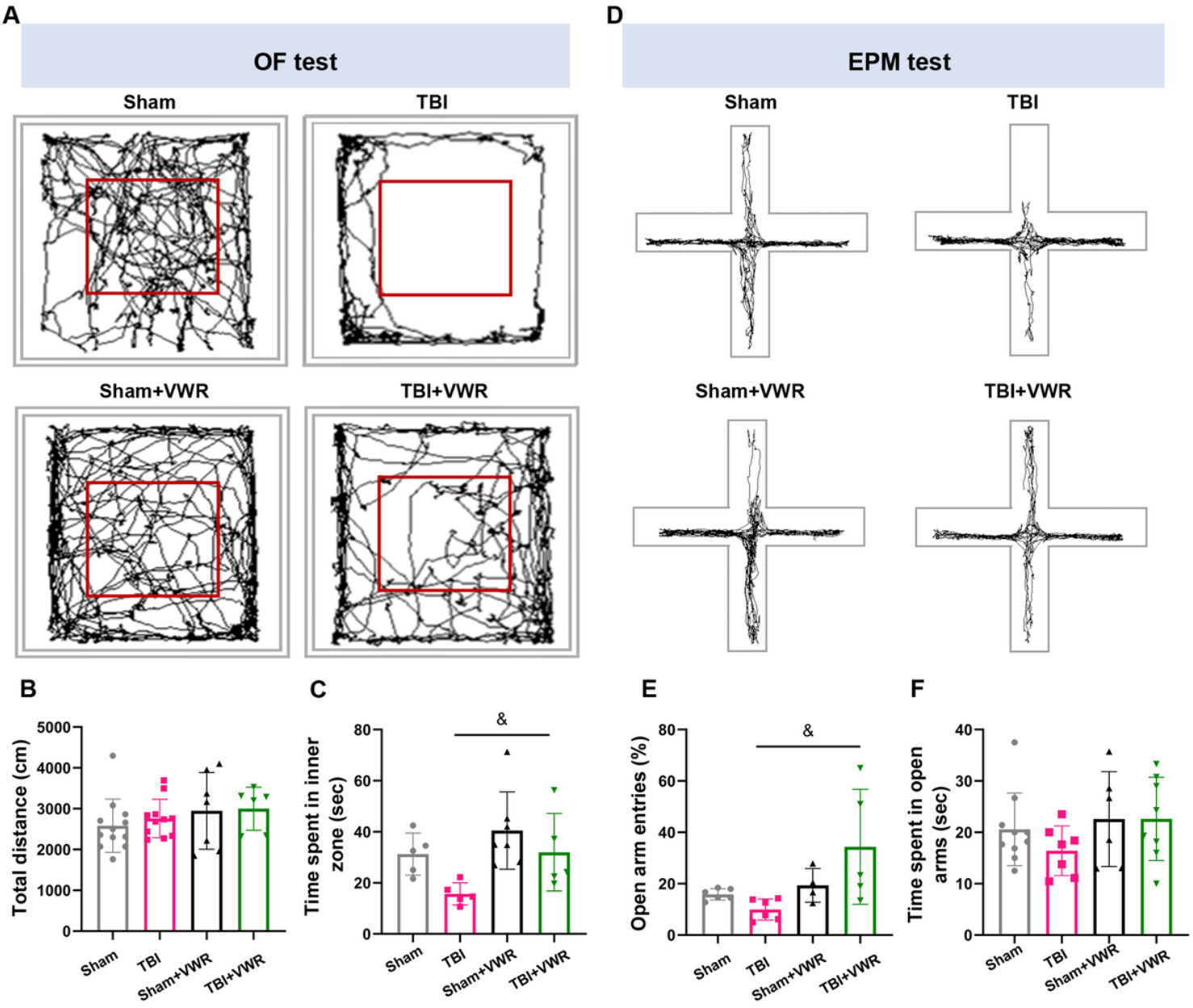

Figure 3

Effects of VWR on anxiety-like behavior of TBI mice. Traces of locomotor activity in the OF test **(A)** and EPM **(D)** (n = 8). Total distance **(B)** and time spent in central area **(C)** in OF examination. Open arm entries **(E)** and time spent in open arms **(F)** in the EPM examination. $^{\&}p$ 0.05, vs. TBI. Unpaired student's t test.

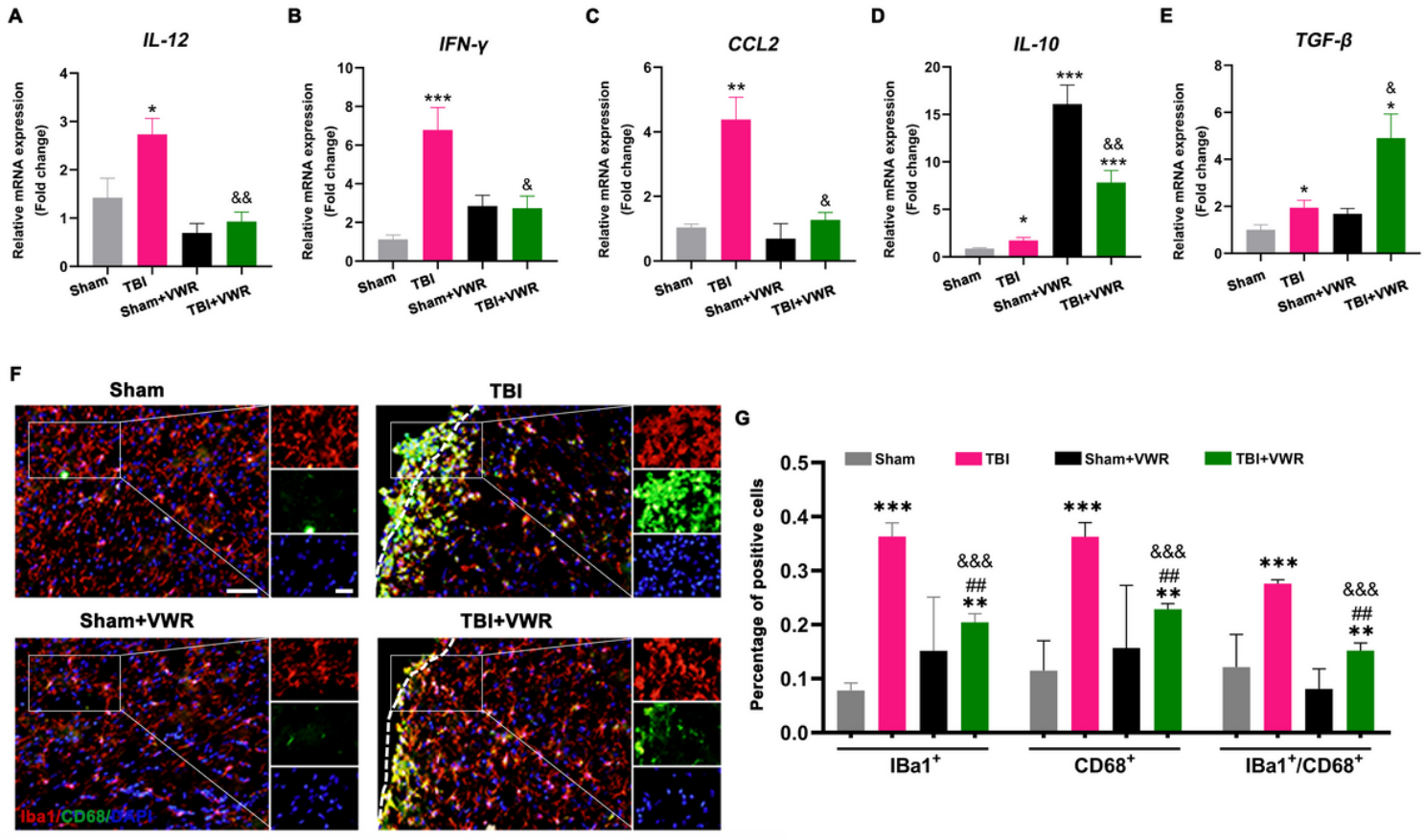

Figure 4

Effects of VWR on inflammatory response after TBI. Expression of IL-12 **(A)**, IFN-γ **(B)**, CCL2 **(C)**, IL-10 **(D)** and TGF-β **(E)** mRNA in perilesional cortex, detected by qRT-PCR (n = 6). **(F, G)** Quantification of Iba1$^+$ and CD68$^+$ microglia in perilesional cortex, detected by immunostaining. ***p 0.0001, **p 0.001, *p 0.05, vs. Sham. $^{&&&}p$ 0.0001, $^{&&}p$ 0.001, $^{&}p$ 0.05, vs. TBI. $^{\#\#}p$ 0.001 vs. Sham +VWR. Unpaired student's t test. Iba1: red; CD68: green; DAPI: blue; scale bar =100 μm.

Figure 5

Effects of VWR on NLRP3 activation. Expression of NLRP3 mRNA **(A)** and pro-caspase-1 **(B)** and caspase-1 **(C)** in perilesional cortex were detected by qRT-PCR and Western blot assay (n = 5). NLRP3 inflammasome (NLRP3$^+$, green) in microglia (Iba1$^+$, red) was detected by immunofluorescent staining **(D)** and quantified **(E)** (n = 4). Expression of IL-1β **(F)**, pro-IL-1β **(G)** and IL-18 **(H)** in the perilesional cortex were detected by Western blot assay (n = 4). ***p 0.001, **p 0.01, *p 0.05 vs. Sham, $^{&&&}p$ 0.0001, $^{&&}p$ 0.01, $^{&}p$ 0.05 vs. TBI. Unpaired student's t test. Iba1: red; NLRP3: green; DAPI: blue; scale bar =100 μm.

Figure 6

Correlation analysis between anxiety behavior and inflammasome activation. Positive correlation between reduced NLRP3 mRNA and eased anxiety behavior in OF test **(A)** and EPM test **(B)** (n = 5). Positively correlation between decreased IL-1β mRNA and alleviated anxiety behavior in OF test **(C)** and EPM test **(D)** (n = 5). Two-tailed Mann–Whitney U-test.

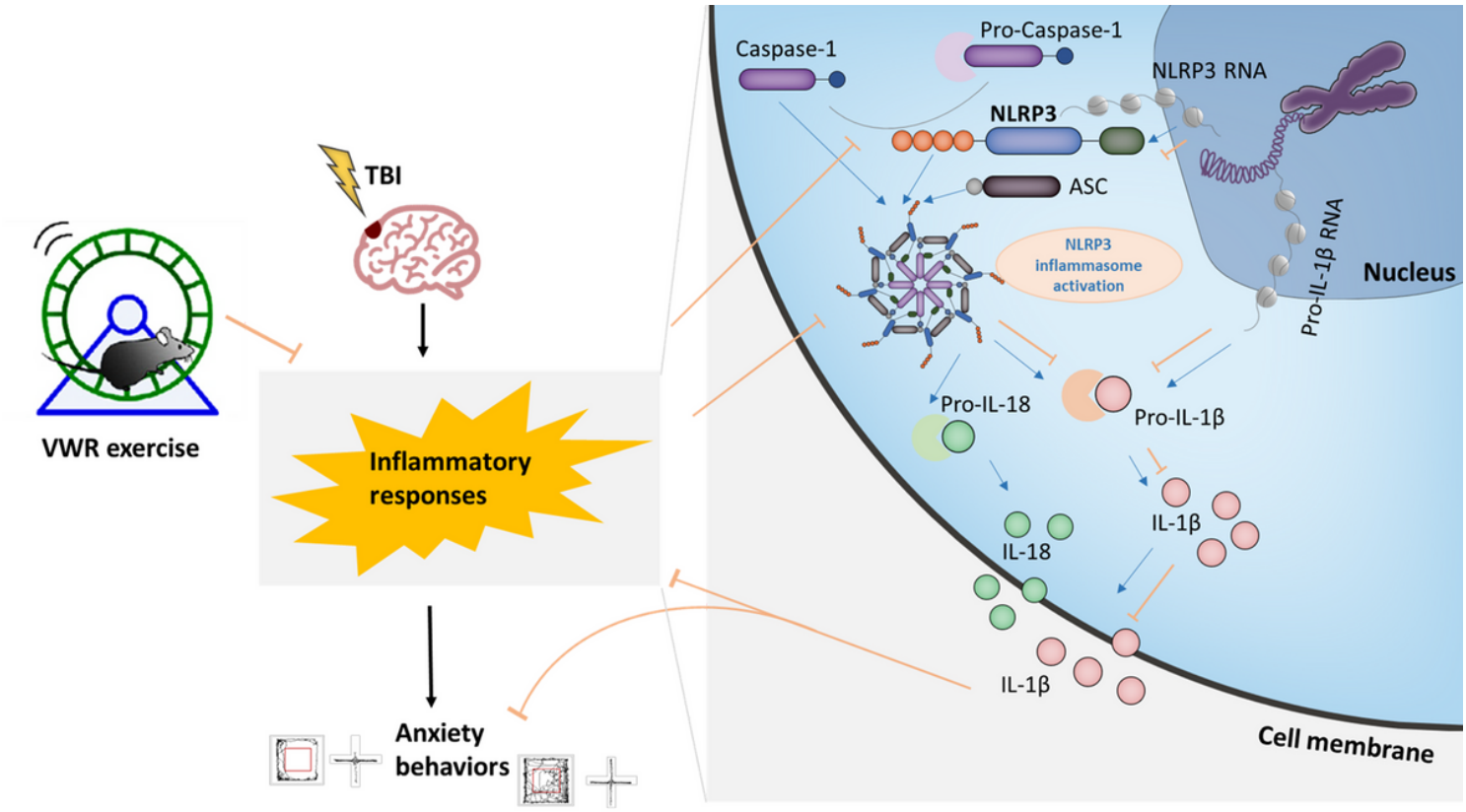

Figure 7

Schematic illustration of VWR exercise improves TBI-induced anxiety behaviors via inhibiting NLRP3 inflammasome.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- IL1mRNAexpression.tif
- WBbands.tif